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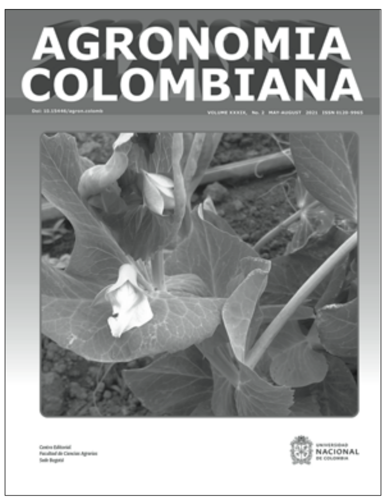

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Interaction of triacontanol with other plant growth regulators on morphology and yield of field pea (*Pisum sativum* L.)
Article on pages: 187-195.

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STATEMENT OF RETRACTION - SCIENTIFIC ARTICLE

The Journal Agronomía Colombiana publicly announces the retraction of the following scientific article because its publication violated the author's rights and intellectual property rules:

"Growth of three color hybrids of sweet paprika under greenhouse conditions - Crecimiento de tres híbridos de pimentón de colores en condiciones de invernadero" with Doi: https://doi.org/10.15446/agron.colomb.v33n2.49667 published in 2015 in Agronomía Colombiana 33(2) pages 139-146, with authors appearing as Fernando Javier Peña Baracaldo and Ingeborg Zenner de Polanía.

Consequently, we inform the academic community that the above-cited article has to be retracted. The Journal Agronomía Colombiana strongly disapproves of any activity that leads to violation of the author's rights and always encourages its users to do the same.

RETRACTACIÓN DE ARTÍCULO CIENTÍFICO

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A basic scheme of soybean transformation for glyphosate tolerance using *Agrobacterium tumefaciens* through an approximation of patents: a review

Un esquema básico de transformación de soya para tolerancia a glifosato usando *Agrobacterium tumefaciens* a través de una aproximación de patentes: una revisión

Adriana Carolina Rojas Arias[1,2], Alejandro Chaparro-Giraldo (R.I.P.)[1], and Silvio Alejandro López-Pazos[3*]

ABSTRACT

Concern has been expressed on the control of agricultural biotechnology through patents that may adversely affect the development of competing crops. Soybean is one of the most important crops around the world (~287 million t per year), above potatoes (45 million t per year), tomatoes (23 million t per year), or wheat (116 million t per year), with prices for American producers ranging between USD 278.8 and USD 650.3 t^{-1}. Soybean belongs to the Fabaceae family and has been genetically modified (GM) to improve its tolerance to herbicides, including glyphosate, its resistance to insect pests, and the quality of soy oil. Glyphosate-tolerant soybean has received a gene coding for the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). There are a number of variables that contribute to the development of a GM soybean event. Such variables include tissue culture, selection methods, cloning vectors, and *Agrobacterium* strains that affect transformation efficiency and can be associated with patents. Chlorine gas disinfection is the most appropriate technique for plant material. Production of explants with shoots and molecular and phenotypic features (e.g., antibiotic susceptibility) of bacterial strain must be assessed. A long-term glyphosate selection arrangement is the most suitable and a consistent approach for the selection of events of GM soybean with tolerance to glyphosate. Freedom-to-operate evaluation must be carried out to find the specific elements neccesary for GM plant development that do not infringe the rights of third parties. These rights come into effect from the patent application date for a definite geographical region involving construct design and its synthesis, transformation vector, bacterial strain, methods, or reporter gene. In this review, the protocols relating to experiments for the development of GM soybean using an *epsps* gene are included, and considerations relating to intellectual property rights are involved. The major elements associated with each stage of the development of patents are described including the following: the soybean genotype, seed disinfection, genetic construct design and its synthesis, tissue culture protocols, selection strategy without gene reporter, and *Agrobacterium* strain. This review is a guide for carrying out technical procedures when the desired product is the off-patent GM soybean with tolerance to glyphosate.

Key words: *Glycine max*, N-(phosphonomethyl)glycine), genetically modified crop, 5-enolpyruvylshikimate-3-phosphate synthase, intellectual property rights.

RESUMEN

Se ha expresado preocupación por el control de la biotecnología agrícola a través de patentes que pueden afectar negativamente el desarrollo de cultivos competitivos. La soya es uno de los cultivos productivos más importantes alrededor del mundo (~287 millones t/año) por encima de la papa (45 millones t/año), el tomate (23 millones t/año) o el trigo (116 millones t/año), con precios para los productores estadounidenses que oscilan entre USD 278.8 y USD 650.3 t^{-1}. La soya pertenece a la familia de las Fabaceae y ha sido modificada genéticamente (MG) para mejorar su tolerancia a herbicidas, incluyendo el glifosato, su resistencia a insectos plaga y la calidad del aceite de soya. La soya tolerante al glifosato ha recibido un gen que codifica para la 5-enolpiruvilshikimato-3-fosfato sintasa (EPSPS). Hay una serie de variables que contribuyen al desarrollo de un evento de soya transgénica. Estas variables incluyen el cultivo de tejidos, los métodos de selección, los vectores de transformación y las cepas de *Agrobacterium* que afectan la eficiencia de transformación y que pueden estar asociadas a patentes. La desinfección con cloro gaseoso es la técnica más adecuada para el material vegetal. Se debe evaluar la producción de explantes con brotes y características moleculares y fenotípicas (por ejemplo, susceptibilidad a los antibióticos) de la cepa bacteriana. Una estrategia de selección de glifosato a largo plazo es el enfoque más adecuado y consistente para la selección de eventos MG de soya tolerante a glifosato. Se debe realizar una evaluación de la libertad de operación para encontrar elementos específicos necesarios para el desarrollo de plantas MG que no infrinjan los derechos de terceros. Estos derechos tienen efecto a partir de la fecha de solicitud de la patente para una región geográfica definida que involucra principalmente el diseño del constructo y su síntesis, el vector de transformación, la cepa bacteriana, los métodos, o el gen reportero. En esta revisión se incluyen protocolos relacionados con experimentos para el desarrollo de soya MG utilizando un gen *epsps*, y consideraciones relacionadas con los derechos de propiedad intelectual involucrados. Se describen los principales elementos asociados a cada etapa del desarrollo de patentes: el genotipo de soya, la desinfección de semillas, el diseño de constructo genético y su síntesis, los protocolos de cultivo de tejidos, la estrategia de selección sin gen reportero, y la cepa de *Agrobacterium*. Esta revisión es una guía para llevar a cabo procedimientos técnicos cuando el producto deseado es la soya MG con tolerancia al glifosato libre de patentes.

Palabras clave: *Glycine max*, N-(fosfonometil)glicina), cultivo genéticamente modificado, 5-enolpiruvilshikimato-3-fosfato sintasa, derechos de propiedad intelectual.

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Introduction

Soybean (*Glycine max,* Fabaceae) is a relevant protein source (~16 g proteins/100 g of soybean, or 36-56% dry weight of soybean) that contains all the indispensable amino acids for humans; therefore, it is comparable to chicken or eggs. Soybean seeds contain around 20% lipids and are used as a source of protein in the diet of animals, such as poultry, pigs, or cattle. Their uses in human food include cooked seeds as part of sauces and potages, soy milk, soy flour for cakes, cookies and other baked goods. Soybean is a substitute for meat in vegetarian foods and lecithin is also extracted from its oil. At industrial plants, it is used in the manufacture of metalworking fluids, plastics, surfactants, solvents, and disinfectants. Other parts of the plant are also used as animal feed or green manures (Lusas & Riaz, 1995; Mateos-Aparicio *et al*., 2008; United Soybean Board, 2017).

The plant possesses sugars, insoluble and soluble fiber, vitamins, and minerals. The primary use of soybean meal is as animal feed (~98%) or as industrial substrate (Widholm *et al.,* 2010). Genetically modified (GM) soybean is the most important transgenic crop representing ~91 million ha (50% of global area). A world production of soybean of 349 million t was registered in 2019 (FAO, n.d.). GM soybean represents 75% of the global soybean cultivation. In 2019, The United States (the first producer of GM soybean since 1996) produced 96 million t (31 million ha), and Brazil produced 114 million t (31 million ha) (Lee *et al.,* 2013; FAO, n.d.; ISAAA, n.d.) (Tab. 1).

Glyphosate (N-(phosphonomethyl) glycine, ($C_3H_8NO_5P$)) is a systemic phosphonate herbicide. It is a crystalline powder with a density of 1.704 g cm^{-3}, molecular mass of 169.1 g mol^{-1}, and solubility in water of 1.01 g/100 ml (20°C) that decomposes at 187°C. Glyphosate is an aminophosphonic equivalent of the glycine that acts as a systemic herbicide absorbed through plant leaves. This herbicide is used on commercial crops to control weeds and has been available in the market since 1974 as Roundup®. Glyphosate blocks the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in plant cells responsible for triggering the synthesis of aromatic acids (phenylalanine, tyrosine, and tryptophan), catalyzing the reaction of shikimate-3-phosphate and phosphoenolpyruvate to form 5-enolpyruvyl-shikimate-3-phosphate as product. Since 1996, GM soybean expressing glyphosate tolerance has been marketed in the United States (Duke & Powles, 2008; Duke & Cerdeira, 2010).

Agrobacterium tumefaciens is a plant pathogen that is widely used on GM crops, as it can transfer a fragment of its 200 kb tumor-inducing (Ti) plasmid (T-DNA) as a vector of specific transgenes that is expressed in the plant (Bourras *et al.,* 2015). Soybean is transformed via organogenic and embryogenic methods (e.g., protoplast, cell, tissue, and organ culture, and subsequent regeneration of plants). An efficient regeneration and *Agrobacterium*-based gene transfer procedure established on cotyledonary node explants is applied in soybean (Paz *et al.,* 2004; Jamsheed *et al.,* 2013; Lee *et al.,* 2013).

The commercial use of GM soybean is limited by patents protecting each element used in a biotech process. This process involves DNA sequences comprising coding regions (e.g., event MON87751 that contains specific sequences for insect resistance), regulatory regions (e.g., event dp-305423-1 that possesses an acetolactate synthase

TABLE 1. Brief overview of GM technologies in commercial soybean. Thirty-nine events have been developed for herbicide tolerance, oil quality and insect resistance according to ISAAA (n.d.).

General trait	Specific trait	Technology	Company
Herbicide tolerance	Glyphosate tolerance	Roundup Ready	Monsanto/Bayer, Pioneer-Dupont, Dow AgroSciences, Syngenta
		Genuity	Monsanto/Bayer, Pioneer-Dupont, Syngenta
	Glufosinate-ammonium tolerance	LibertyLink	Bayer, Pioneer-Dupont
	Tolerance to sulfonylurea herbicides	STS	Pioneer-Dupont, Dow AgroSciences, Syngenta
	Tolerance to imidazolinone herbicides	Clearfield	BASF
	Tolerance to glyphosate + tolerance to herbicides via inhibition to acetolactate synthase (ALS)	Optimum GAT	Pioneer-Dupont
Oil quality	Production of oleic acid (low in saturated fat)	Plenish	Pioneer-Dupont
		Vistive Gold	Monsanto/Bayer
	Production of stearidonic acid (a class of omega-3)	SDA Omega-3	Monsanto/Bayer
Pest resistance	Resistance to lepidopteran pests (all of which add up to glyphosate tolerance)	INTACTA RR2 PRO	Monsanto/Bayer

gene conferring tolerance to applications of the herbicide sulfonylurea), vectors (e.g., patent KR102054567B1 that describes the recombinant clone vector DBN01-T), bacterial strains (e.g., patent US20200385746A1 that protects *Rhizobium*-mediated transformation in soybean), or tissue culture protocols (e.g., patent AU691423B2 that protects a protocol to regenerate soybean plants from cotyledonary nodes). This leads to unfortunate decisions that directly influence scientific developments. Two specific cases can be cited: i) the North American Strawberry Growers Association decided to suspend research focused on the development of GM strawberry with resistance to fungus because of patent complexity; ii) the University of Michigan was forced by a judicial decision to destroy GM lines of turfgrass due to the legal action between two companies for patents related to a coding gene and a promoter used in the approach (Thomas, 2005).

Golden rice is a GM crop that produces beta carotene, a precursor of vitamin A. There are advantages for the Golden rice project such as health benefits and low-cost release in emerging nations. Currently, Golden rice is far from being released to developing countries, as there are 40 patents involved in the project that represent a negative factor in the adoption of this technology (Kowalski *et al.*, 2002). For Colombia, 59 patents are involved in the possible development of a GM rice line containing a *cry1Ac* gene (Diazgranados *et al.*, 2016). Five corporations possess a great number of patents of GM crops. The following are a few examples of the events by corporation (the complete list of the GM events is available in the GM approval database (ISAAA, n.d.)): Monsanto/Bayer (alfalfa KK179 x J101, canola GT200 (RT200), cotton MON1076, corn GA21, potato BT10, soybean MON87705, tomato FLAVR SAVR, wheat MON71800); Dupont (canola 73496, cotton 19-51a, corn 4114, soybean DP305423); Syngenta (cotton COT102 (IR102), corn 3272); Bayer (canola HCN92 (Topas 19/2), cotton GHB614, corn T14, rice LLRICE06, soybean A2704-21, sugar beet T120-7), and Dow Agrosciences (cotton 281-24-236, corn DAS40278, soybean DAS68416-4) (Wright & Pardey, 2006) including exclusive cross-licenses (Pisano, 2006). Monsanto was purchased by Bayer in 2016, and Syngenta was acquired by ChemChina in 2017. If an independent GM seed producer wants to generate and/or commercialize GM varieties, the seed company must consider licensing charges. However, intellectual property rights (IPR) of the elements used in a technology do not necessarily prevent its commercial use. Patents are limited to national jurisdiction and to a specific time (20 years) accepting exceptions according to the local regulation (Baker, 2019). By the end of this period, the patent enters the public domain which means that it is open for everyone to use. It is known that once a medical corporation, or similar organization, generates a new pharmacological compound to be used for a disease, it is covered under a patent contract conferred for around 20 years. When the patent time has run out, the substance can be manufactured and marketed by other companies and the pharmacological compound is termed as "generic". This also applies when the company that owned the patents declares them to be inapplicable, invalid, or abandoned, or the market region for the drug has no patent protection application. As a result, the control of the patent is removed, causing a considerable drop in costs that may be more acceptable to society (Grushkin, 2012). Therefore, it is very important to develop a well-documented and specific freedom to operate (FTO) analysis for a GM technology and a specific country (Hincapié Rojas & Chaparro-Giraldo, 2013; Lamprea Bermúdez & Salazar López, 2013). This article provides a step-by-step guide on how to develop a particular glyphosate-tolerant GM soybean event, based on experience gathered from the application of revised protocols supported by the literature, the use by public institutions, and patents related to transformation. An *epsps* gene and *A. tumefaciens* were used in an FTO basis to evaluate whether the GM soybean associate IPRs have expired or are about to expire, and to determine if they have been settled in the region of interest.

Freedom to operate study

Farmers from the United States and Argentina began to plant GM soybean around 1996 after it started to be marketed under the Roundup Ready trademark in the USA. GM soybean occupies around 95.9 million ha in the world (almost 50% of the GM crops worldwide). In 2018, the GM soybean area planted in the USA was 34.08 million ha. Brazil occupied the second position in the world with a production of ~34.86 million ha. GM soybean cultivation has expanded throughout Argentina with 18 million ha in 2018. Monsanto (now Bayer) was not capable of achieving patent protection for GM soybean with tolerance for glyphosate (Roundup Ready soybean seed) in Argentina. The company had requested the IPRs it held in Europe, but the Supreme Court of Argentina rejected that request based on the national patent law that regulates the patent duration of 20 years. Monsanto has registered some GM soybean events related to glyphosate tolerance in Argentina, (e.g., MON87701 x MON89788 and MON89788), perhaps using plant breeder rights (Brookes & Barfoot, 2018; Fries *et al.*, 2019; ISAAA, n.d.).

An FTO study that considers the importance of generating GM soybean with marketing possibilities, before the laboratory activities and at the start of the biotechnological process, should be developed to avoid, as far as possible, the violation of third-party rights. Initially, a list of elements (materials and protocols) to be used in the development of a GM line must be in place. Then, a specific country search must be carried out based on the requested patents/current patents, including plant breeder's rights or "plant patents" (regarding the plant varieties) for each element. Data can be recovered from the United States patent office database (USPTO) (https://www.uspto.gov/patents/search), the European patent office database (ESPACENET) (https://worldwide.espacenet.com/), the Lens suite (https://www.lens.org/lens/), PATENTSCOPE from the World Intellectual Property Organization (https://patentscope.wipo.int/search/es/search.jsf), and Google Patents (https://patents.google.com/). In each of these websites, a regional patent database needs to be requested during the planning stage. Regional patent databases should be studied. Also, biological specialized databases can be reviewed, such as Nucleotide from the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/nucleotide/), or Phytozome (https://phytozome-next.jgi.doe.gov/), to determine the origin of the selective biological information (Wyse & Luria, 2021). Data about patents related to glyphosate tolerant soybean should be obtained during the period from 1996 to the present day (Jefferson, Graff, *et al.,* 2015; Goforth, 2017). Therefore, there is a growing need for competent lawyers specialized on GM intellectual property at this stage.

Patent data should focus especially on claims and year of application without forgetting the importance of the overall description of the invention. An FTO analysis involves the search of patents and their claims because they allow an understanding of the set of approaches that explain the developmental process of a GM crop: cloning vectors, DNA sequences, *Agrobacterium* strains, methods, transfer material, or confidential agreements (Chi-Ham *et al*., 2012; Miralpeix *et al*., 2014; Zanga *et al*., 2015). At the same time, an FTO analysis provides a parallel strategy to develop the same product in a structured way with a focus on commercialization without requirements for additional payments from licenses (Sommer, 2012). A GM soybean project based on FTO requires legal assistance from a lawyer, at least in its first instance of design. One of the first things that must be identified to achieve the goal of a GM soybean event is the soybean variety. Some countries have patent systems that prevent patent protection of certain products, such as germplasm for research and breeding (Correa, 2014). Likewise, if native genetic material is relevant in a biotechnological project, a contractual instrument for the appropriate access and utilization of biological and genetic resources respectful of traditional knowledge may be necessary (Deplazes-Zemp, 2019; Heinrich & Hesketh, 2019). A large number of countries have achieved significant progress in research and development on new soybean varieties in the light of local conditions (Tab. 2) (Cober *et al*., 2009). Just in Africa, there are 13 countries developing local soybean varieties (Benin, Cameroon, Ethiopia, Ghana, Kenya, Mali, Mozambique, Nigeria, Rwanda, Sudan, Uganda, Zambia, and Zimbabwe) (Santos, 2019). There is a program that

TABLE 2. Standard soybean genotype collections. Transgenic processes can be influenced by virulence of *Agrobacterium* strains, plant species or plant varieties with predisposition to being infected (genetic background), and the design of expression cassettes.

Characteristics of Williams 82 soybean genotype (owner: University of Illinois)		Soybean collections	
Phenotypic characteristic*		Country	Accessions
Altitude (m a.s.l.)	~450	China	23578
Harvest (d)	120-132		
Height (m)	1.09-1.23		
Flower color	White	USA	18000
Pubescence color	Brown		
Grain color	Yellow		
Hilum color	Black	Taiwan (Asian Vegetable Research and Development Center)	12508
100 seed weight (g)	14.4-17.9		
Oil (%)	19.5-20.8		
Protein (%)	39.5-42.6	Colombia (Colombian Corporation for Agricultural Research -AGROSAVIA)	1237
Sowing density (plants ha^{-1})	280000		
Yield (kg ha^{-1})	3460-3850		

*Soybean genotypes adapted to various thermal zones (≥24°C, humidity ~80%), with productivity of ~3500 kg ha^{-1}, a vegetative period of ~100 d, and highly sensitive to the photoperiod.

involves the development of a genetic improvement of soybean for the tropical Colombian environment since 1960 (Tab. 2) (S. Caicedo, personal communication, 12th May 2017). Therefore, it is best to have a local soybean variety adapted to the environmental conditions of the region of interest, if possible.

Our experience in the FTO analysis of GM soybean shows that patent application protects the following: i) DNA promoters or termination regions (CaMV35S, Gmubi, Nos) including their use in vectors for genetic transformation of mono- and dicotyledonous plants; ii) *epsps* gene and its variants and possible uses for genetic transformation of plants; iii) transit peptide (e.g., *Petunia* hybrid); iv) vectors (pCAMBIA vector); v) transformation and regeneration methods, and vi) plant varieties through a special protection known as "breeder's right" (1978 Act and 1991 Act on Common Provisions for the Protection of the Rights of Breeders of Plant Varieties - International Union for the Protection of New Varieties of Plants - UPOV) (Brenner, 1998; Dattée, 2009). According to international guidelines, a natural gene sequence cannot be patented; however, in some countries, patenting a gene sequence isolated from the environment is allowed if it is cloned in a vector (Jefferson, Köllhofer, *et al.,* 2015). The pCAMBIA series is a set of protected plant molecular biology vectors with complete access for research and used for developing new varieties of plants (Jefferson, 2008).

A difficulty in this respect is that several methods are covered by patents that sometimes are not taken into consideration for the development of GM soybean for glyphosate tolerance. On some occasions, it is said that these techniques are being used by the public sector without giving further details. The following section describes our specific methods used when processing GM soybean for glyphosate tolerance and some frequently related patents.

Laboratory procedures and IPR

This section provides a technical guide framework relevant to the design and development of soybean transformation for glyphosate tolerance, based on our experience and perception of IPRs trends. According to Nottenburg and Roa Rodríguez (2008), those elements that have been patented are transformation vectors, vector genes, transgenes, vector design, methods for making recombinant *Agrobacterium* with an engineered vector, recombinant *Agrobacterium* incorporating engineered vectors, improved *Agrobacterium* strains for transformation, methods of preparing plant tissue for transformation, methods of transforming specific plants, and transformed plants and plant cells.

First, the available soybean seeds must be germinated and regenerated. Soybean seeds must be surface sterilized for 16 h using chlorine gas (4.1 ml of 10 N HCl, 100 ml 5% NaClO). Sterilized seeds are germinated in 0.7% agar medium, pH 5.8, for 5 d (27°C, 16/8 h photoperiod). Then, seed regeneration is monitored using a culture medium containing 1X Gamborg vitamins, 1X B5 salts, 30 g L^{-1} sucrose, 1.67 mg L^{-1} BAP, 3 mM MES, and 0.7% agar at pH 5.7. Regeneration containers are incubated for a 16/8 h photoperiod at 27°C. Five days later, the number of germinating seedlings must be determined. Regeneration is continuously monitored for four weeks. Regeneration rates are calculated based on the number of explants with shoots and the number of shoots by explant. Some patents cover similar but non-identical protocols enabling seed regeneration (e.g., EP1517991A4 (application June 22, 2002, and withdrawn status) and US5824877A (application July 22, 1988, and expired status)).

The next step is to design an expression cassette that must be introduced into a transformation vector. We prefer pCAMBIA vectors whose principal characteristics involve high copy numbers, 35S promoter, kanamycin or hygromycin B as selection markers, and GUS as screening marker. Also, pCAMBIA vectors have an FTO with availability for academic research without charges, and licenses for profit companies (for more details please visit https://cambia.org/). Ideally, an expression cassette should be designed in such a way that all the elements are stable with the help of molecular biology tools (PCR, enzyme digestion, cloning and ligation, etc.). This expression cassette can also be designed with an aggregation of genetic elements on a modularized principle through bioinformatics for chemical synthesis. When the expression cassette is obtained from a specialized company, it is important for the agreement to have an unrestricted right of utilization, and to avoid clauses as "only for research". The expression cassette includes a promoter, a sequence of chloroplast transit peptide (e.g., CTP from petunia), a CP4-*epsps* gene (from *Agrobacterium* sp. strain CP4), and a terminator sequence. Expression of transgenes is a random process caused by the codon usage or RNA regulation. According to the plant codon usage associated with transcriptional regulation and translational efficiency, mRNA stability, splicing at the mRNA level, use of special promoters, removal of polyadenylation signals and cryptic splice sites, and elimination of any potential RNA secondary structure adjacent to the translational start codon, the use of modified prokaryotic genes has allowed reaching levels of expression up to 100 times greater than transgenes without modifications (Jackson *et al.,* 2014). A search of patent databases shows that the patents covering the CP4-*epsps* gene expired in 2014.

Once the designed expression cassette is synthetized according to the instructions given in the preceding paragraph, it must be introduced into *A. tumefaciens*. The recombinant strain can be maintained on a Luria-Bertani (LB) medium (50 mg L^{-1} kanamycin). A hypervirulent strain of *A. tumefaciens,* ATCC53213, carrying a pMON546 vector with a petunia *epsps* gene has been described by Kishore and Shah (1988) and Shah *et al.* (1986) in the patent US4971908A (application April 22, 1988, and expired status) and the patent US4940835A (application July 7,1986, and expired status), respectively. *Agrobacterium* strains must be characterized using PCR: i) Ach5FtsZ primers (F: 5'-GAACTTACAGGCGGGCTGGGT-3', R: 5'-CGCCGTCTTCAGGGCACTTTCA-3', product: 369 pb) are specific to *A. tumefaciens* LBA4404; ii) C58GlyA primers (F: 5'-CCACCACCACGACGCACAAGTCT-3', R: 5'- TGCCGAGACGGACACCCGAC-3', product: 423 pb) are useful for the detection of C58C1, EHA101, EHA105, and GV3101 strains; iii) pTiBo542 primers (5'- CCCGCTGAGAATGACGCCAA-3', R: 5'- CCTGCGACACATCGTTGCTGA-3', product: 766 pb) are specific to EHA101 and EHA105 (differentiated from C58C1, LBA4404 and GV3101 strains), and iv) nptI primers (F: 5'- CTGCGATTCCGACTCGTCCA -3', R: 5'- CGGGCAATCAGGTGCGACA-3', product: 572 pb) are special for EHA101 only (Deeba *et al.*, 2014). In our experience, *A. tumefaciens* strains EHA101 and EHA105 are useful for soybean transformation, probably because of their virulence. We tested another bacterium, *Sinorhizobium meliloti* (see https://cambia.org/), to see if we could recreate the soybean infection; however, the results were not encouraging. *Agrobacterium* cultures (OD650 = 0.6-1.0 at 28°C, 250 rpm) must be used for infection of explants (Guo *et al.*, 2020). A bacterial pellet obtained by culture spinning (8000 rpm, 4 min) must be resuspended in cocultivation media (1X Gamborg vitamins, 0.1X B5 salts, 1.67 mg L^{-1} BAP, 0.25 mg L^{-1} GA_3, 3% sucrose, 20 mM MES, 200 μM acetosyringone, pH 5.7).

Cotyledons must be excised at the junction between the hypocotyl and the half-way point of the cotyledon, five mm below the cotyledonary node, and a cut is performed to divide the cotyledonary explants. The plumule is eliminated and two small incisions are made on the cotyledonary node. Explants are infected with recombinant *A. tumefaciens* (containing a designed expression cassette as described above) in coculture broth (30 min), and then inoculated on coculture solid medium (1X Gamborg vitamins, 0.1X B5 salts, 1.67 mg L^{-1} BAP, 0.25 mg L^{-1} GA_3, 3% sucrose, 3.9 g L^{-1} MES, 200 μM acetosyringone, 0.7% agar, and pH 5.7) with the adaxial side down. The co-cultivation plates are incubated in the dark for 3 d at 28°C. The explants are then rinsed in sterile water twice. After the last rinse (using 350 mg L^{-1} cefotaxime), explants are transferred into shoot induction medium (1X Gamborg vitamins, 1X B5 salts, 30 g L^{-1} sucrose, 1.67 mg L^{-1} BAP, 3 mM MES, 0.7% agar, pH 5.7 containing 100 mg L^{-1} timentin, and 350 mg L^{-1} cefotaxime) for two weeks. The shoot induction medium must be refreshed for an additional period of two weeks (Paz *et al*., 2004; Song *et al*., 2013).

Our experience implies a longer-term evaluation of tolerance by using lower concentrations of glyphosate of 0 mg L^{-1} for shoot initiation 1 (regeneration 1), then a glyphosate concentration of 25 mg L^{-1} for shoot initiation 2 (regeneration 2), and then a concentration of glyphosate reduced to 6 mg L^{-1} (shoot elongation) and 0 mg L^{-1} (rooting), respectively. Herbicide selection includes shoot induction without glyphosate (0 μM) (the first two weeks) and 148 μM (subsequent two weeks). After four weeks in shoot induction medium, explants are transferred into a shoot elongation medium (1X MS salts, 1X Gamborg vitamins, 0.5 mg L^{-1} GA3, 0.1 mg L^{-1} IAA, 0.7 mg L^{-1} BAP, 30 g L^{-1} sucrose, 3 mM MES, 50 mg L^{-1} asparagine, 50 mg L^{-1} glutamine, 70 mg L^{-1} vancomycin, 350 mg L^{-1} cefotaxime, 35 μM glyphosate, 0.7% agar, and pH 5.7). A new culture medium must be prepared every two weeks for six weeks (16/8 h photoperiod, 26°C). Every shoot from the elongation step that has at least 2 cm is moved to the rooting medium (0.66X MS salts, 1X Gamborg vitamins, 3 mM MES, 30 g L^{-1} sucrose, 0.7% agar, and pH 5.7). The whole process is summarized in Figure 1. Some alternative selection strategies have been described (Clemente *et al.,* 2000; Monsanto Technology, 2011; Dow AgroSciences, 2014) (Tab. 3). We have used a strategy for the transformation and selection of GM events that is consistent with the strategic FTO. Therefore, the reporter genes from pCAMBIA vectors are removed using restriction enzymes and, in this way, the CP4 *epsps* gene is both a desired trait and gene reporter avoiding unjustified additional IPRs. The use of low concentrations for glyphosate selection in the regeneration media could increase *in vitro* escapes (non-germline transformation or chimerism in primary transformants). It is for this reason that a selection marker is important (e.g., antibiotic resistance or green fluorescent protein genes) (Miki & McHugh, 2004). Selection markers have been at the center of controversy about biosafety concerns; therefore, new approaches to produce marker-free GM plants were developed for novel events (Darbani *et al.*, 2007; Tuteja *et al.*, 2012). Since the glyphosate-tolerant GM soybean development has an FTO focus related to the CP4 *epsps* gene (as mentioned above), the transformation of a high number of explants is essential. The efficiency of soybean transformation can be explained by plant defense mechanisms such as mitogen-activated protein kinases, defense proteins, reactive oxygen species, or hormones (Imam *et al.*, 2016). Soybean transformation has a very narrow efficiency (glyphosate-tolerant events vs. infected explants) of less than ~6% using *Agrobacterium* (Hinchee *et al.*, 1988), probably since *A. tumefaciens* has low infective capacity on soybean tissues. Additionally, it is dependent on the soybean genotype and *A. tumefaciens* strain (Song *et al.*, 2013). These reasons are enough to strongly support the evaluation of a great set of explants, considering the additional loss of material as a result of contamination, seed quality, or genotype resistance.

Transformants that survive long term glyphosate-selection must be transferred to the soil and subjected to leaf painting with glyphosate at 2300 g acid equivalent (ae) h^{-1} and the tolerance behavior could be scored one week after the treatment. ELISA and herbicide painting analysis must also be conducted in T0 plants (hemizygous dominant) (Passricha *et al.*, 2016), and the phenotypic response must be correlated with molecular analysis. With these new materials, it is possible to obtain a high number of homozygous individuals for the trait. Possible GM lines must be evaluated using PCR (transgene presence), ELISA (EPSPS protein), Southern blot (transgene copy number), and real time PCR (transgene expression). The rooted primary transformants must be individualized and propagated in soil for a period of 30 d. The plantlets could be screened with ~0.2% glyphosate (~300 µg per plant) to assess their survival rate 10 d after aspersion (Guo *et al.*, 2020).

The T-DNA and Vir proteins form a complex that controls the supply of a single T-DNA strand (T-strand) into the plant chromosomes. The assimilation of the T-strand is, probably, due to homology repair, or to non-homologous, or perhaps microhomology-mediated end joining that repairs DNA double-strand breaks. The host DNA structure, tissue expression, and soybean genotypes play a relevant role in transformation efficiency (Hintz *et al.*, 1992; Cober *et al.*, 2009; Jamsheed *et al.*, 2013) (Tab. 2). The presence of 5-25 bp of homology between RB/LB of the T-DNA and the plant genome, the frequent insertion of the T-DNA in promoter regions and gene rich regions, and the correspondence of T-DNA tag density and gene density between GC/AT content provide support for the hypothesis of microhomology-facilitated end joining mechanism, which explains why the T-DNA insertion occurs in several locations (introns, terminators, telomeres, or repetitive sequences) (Jamsheed *et al.*, 2013; Bourras *et al.*, 2015). In the present case, there are several patents that include new approaches to the molecular mechanisms used by *A. tumefaciens* during

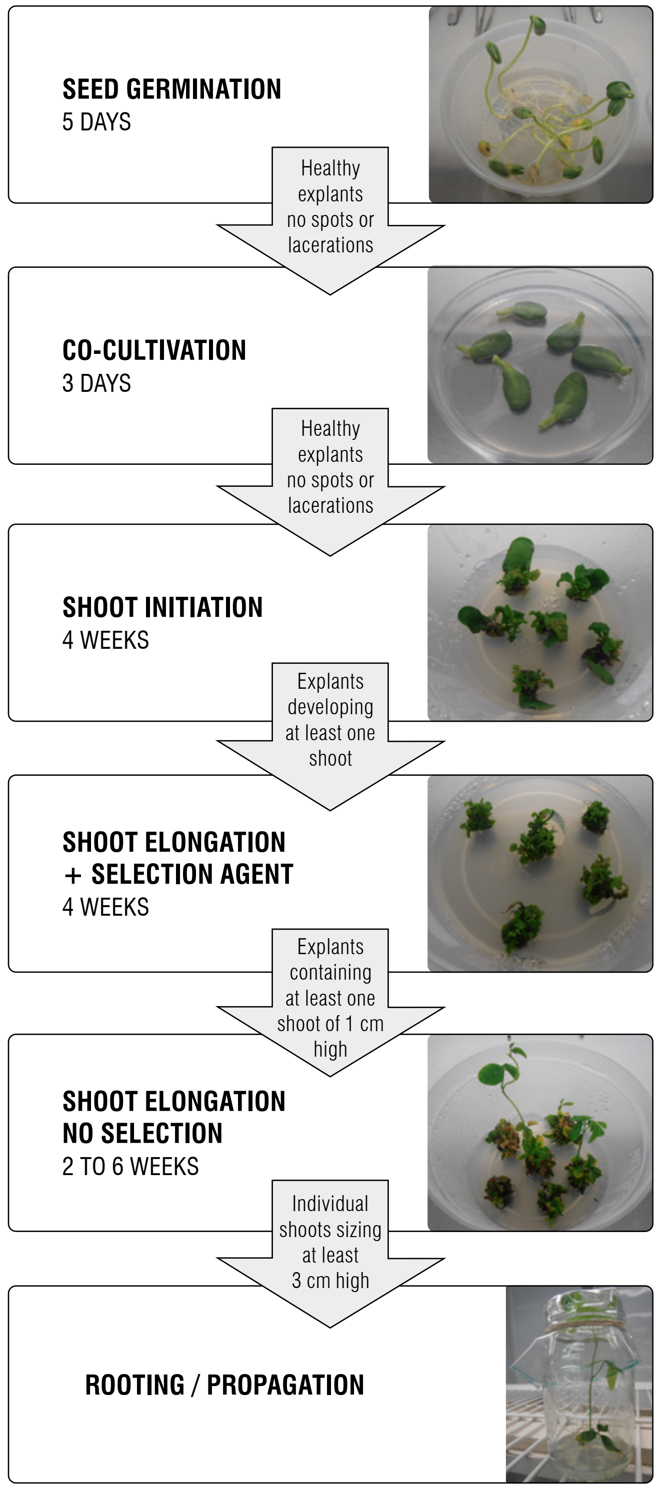

FIGURE 1. Steps in the transformation of soybean by *A. tumefaciens*.

TABLE 3. Selection strategies in GM soybean for glyphosate tolerance.

Explant	Shoot induction		Shoot elongation		Rooting
	Time	Selection	Time	Selection	
Cotyledonary node	4 d	Shoot induction medium without glyphosate	4-10 weeks	Shoot elongation containing glyphosate (50-25 μM)	No selection
	4 weeks	Shoot induction medium with glyphosate (150- 75 μM)			
Seed axis	3 d	OR liquid medium containing glyphosate 200 μM (darkness)	5-6 weeks	WPM medium containing glyphosate 75 μM	No selection
	7 d	MSR medium containing glyphosate 200 μM (darkness)			
Half seed	2 weeks	Shoot induction medium without glyphosate	6-10 weeks + Selection 0-4 weeks	Shoot elongation containing glyphosate 25 μM	No selection
	2 weeks	Shoot induction medium with glyphosate (25-100 μM			

plant transformation: WO2007132193A1 (entitled: modified vird2 protein and its use in improved gene transfer), WO2004035731A2 (entitled: increasing host plant susceptibility to *Agrobacterium* infection by overexpression of the *Arabidopsis vip1* gene), US20150267213A1 (entitled: strains of *Agrobacterium* modified to increase plant transformation frequency), WO2016125078A1 (entitled: *Agrobacterium*-mediated genome modification without T-DNA integration), WO2002052026A2 (describes methods to DNA integration through homologous recombination pathway), and US6800791B1 (an engineered *A. tumefaciens* strain to transfer proteins to plant cell). This is just a small window on the global patents currently taking place about *Agrobacterium* mechanisms, and several of these patents are still valid.

Final considerations and conclusion

The adoption of GM herbicide tolerant crops has positive impacts on agriculture, such as an increase in weed control to herbicide management, compared to traditional systems. These GM crops support crop management with a reduction of the impact on the environment and human health due to a lower use of herbicides in the post-emergent phase with beneficial effects on the soil ecosystem. GM crops also favor the adoption of agricultural conservation practices such as the minimum tillage system that reduces soil erosion, improving water quality and soil degradation (Owen, 2010; Green, 2012). Glyphosate is an herbicide widely used; it is of slow action, a fact that facilitates its translocation from leaves to meristematic tissues and makes it environmentally safe. Glyphosate shows slow mobility in the soil, which reduces the likelihood of contamination of local waterbodies, and a relatively short half-life in the soil. Prior to the introduction of GM crops exhibiting tolerance to glyphosate, this herbicide could only be used in areas where no plant growth is desirable, or with methods avoiding contact with commercial crops. This way, GM crops opened up the possibility for direct use by farmers (Duke & Cerdeira, 2010). GM glyphosate-tolerant soybeans simplify weed management. The farmer may control weeds with just a few applications (one or two) of herbicide during the growth cycle instead of using complicated strategies that include different herbicides incorporated into the soil and/or foliar application. The herbicide could only be applied when there is the presence of weeds because of its post-emergent action, promoting the limited use of these kind of products (using less toxic alternatives). Plowing can be reduced or even eliminated, which lowers expenditure on fossil fuels or equipment with a significant reduction in CO_2 emissions and soil disturbance (Green, 2012).

A patent protects an invention for 20 years; since 2014, plant patents are to expire shortly, and the appreciation of a new FTO framework is one of the most interesting matters in GM soybean programs towards “generic” events. The implementation of an FTO analysis must consider the Cartagena Protocol on Biological Diversity that was adopted on September 11, 2003, and the Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety that was effective on October 15, 2010 (Keiper & Atanassova, 2020). These protocols established that a GM variety should demonstrate biological efficacy, agronomic efficiency, no side-effects on non-target populations, absence of gene flow, and safety for the consumers (Castaño Hernández, 2013; Chaparro-Giraldo, 2013). When it comes to advancing technology with a long history of safe use in different national jurisdictions, there is still a large legacy of scientific articles or official documents that are important for business (McHughen, 2012). This is the case of the Monsanto/Bayer event GTS 40-3-2 that has been released in 27 countries since 1994. The agro-generics strategy is a model than could be used for bolstering administrative efficiency and reducing costs. According to McDougall (2011), based on company data (BASF, Bayer, Dow, Dupont, Monsanto/Bayer, and Syngenta), approximately 13 years and USD 136 million are spent to develop a GM variety: USD 31 million (23%) on gene discovery, USD 69.9 million (51%) on product development, and USD 35.1 million (26%) on regulation and registration. However, Schiek *et al.* (2016) report expenditures of USD 1.6 million and eight years for the development of a potato GM variety by public institutions in underdeveloped countries.

This article offers an approach that is not only limited to the experimental area but also provides a view for an FTO application based on how the development of a GM crop might be understood and planned to increase the likelihood of achieving the desired market result. However, in some cases, it is also possible that legal support might be needed to negotiate some licenses. The soybean genotype, cloning vectors, genetic construct, *A. tumefaciens* strain, selection strategy, reporter gene usage, or *in vitro* protocols were identified as key elements for attaining the objectives defined for the GM development concerned. At the end of this process, it was possible to notice that Google patents has become an important tool for enhancing patent revision for this topic.

In summary, the GM soybean for glyphosate tolerance experimentation needs to be carefully planned and prioritized, and all procedures and conditions must be optimized to ensure quality efficiency. Patents that could prevent access to this technology need to be identified and negotiations with relevant parties regarding a GM soybean for glyphosate tolerance project should be carried out on a case-by-case basis depending on the experimental phases. Finally, it is necessary to consider legislation on regulatory aspects of biosecurity.

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

ACRA, ACG and SALP formulated the research goals, developed the methodology, performed the experiments, collected data, and prepared and wrote the paper.

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Agro-morphological characterization of yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener) reveals elite genotypes for a breeding program in Colombia

Caracterización agro-morfológica del maracuyá amarillo (*Passiflora edulis* f. *flavicarpa* Degener) revela genotipos élite para un programa de mejoramiento en Colombia

John Ocampo[1*], Viviana Marín[2], and Ramiro Urrea[2]

ABSTRACT

Yellow passion fruit cultivation in Colombia lacks detailed genetic studies that allow the establishment of intraspecific variability as a basis for a breeding program. The aim of this research was to establish the genetic relationships among accessions of different geographic origins through an agro-morphological characterization. This research was carried out in the municipality of Palestina (Caldas) at the Luker farm located at 1050 m a.s.l. Fifty-two accessions from Colombia (47), Ecuador (3), Brazil (1), and Costa Rica (1) were characterized with 45 agro-morphological descriptors complemented with phytosanitary evaluations. The quantitative descriptors (25) were analyzed by variance decomposition and principal components (PCA), and the qualitative descriptors (18) were analyzed with the multiple correspondence factor analysis (MCFA). The classification analysis by the neighbor-joining (NJ) method was used for both types of descriptors. PCA results showed six components explaining 71.6% of the total variance that are mainly associated with descriptors related to the size of the flower (tube, nectar camera, and operculum) and weight of the fruit (pulp, shell, and juice content). Thirteen qualitative descriptors were selected, and four factors (44.38% inertia) were identified, mainly associated with pubescence and anthocyanins in the bract, petiole, leaf, and ripe fruit color. The NJ classification analysis showed no relationship between accessions by geographic origin, and the distance between individuals of the same accession was higher than among accessions for both types of descriptors. Thrips (*Neohydatothrips signifier*) and scab (*Cladosporium cladosporioides*) were the pests with the highest incidence in the accessions. Six elite accessions were identified according to the selection index with outstanding fruit quality characteristics, yield, and tolerance to phytosanitary problems. The high intra-accession variability and traits of certain accessions are the basis for future breeding programs to obtain more productive and tolerant cultivars.

Key words: collection, diversity, plant breeding, tropical fruit, Passifloraceae.

RESUMEN

El cultivo del maracuyá amarillo en Colombia carece de estudios genéticos detallados que permitan establecer la variabilidad intraespecífica como base para un programa de fitomejoramiento. El objetivo de esta investigación fue establecer las relaciones genéticas entre accesiones de diferentes orígenes geográficos con base en una caracterización agromorfológica. Esta investigación se realizó en el municipio de Palestina (Caldas), en la granja Luker localizada a 1050 m s.n.m. Se caracterizaron 52 accesiones provenientes de Colombia (47), Ecuador (3), Brasil (1) y Costa Rica (1) con 45 descriptores agromorfológicos complementados con evaluaciones fitosanitarias. Los descriptores cuantitativos (25) fueron sometidos a análisis de descomposición de la varianza y de componentes principales (ACP), y con los cualitativos (18) se realizó un análisis factorial de correspondencias múltiples (AFCM). El análisis de clasificación por el método del vecino más próximo (*neighbor-joining* – NJ) fue empleado para ambos tipos de descriptores. Los resultados del ACP muestran seis componentes que explican el 71.6% de la varianza total que están asociados principalmente con descriptores relacionados con las dimensiones de la flor (tubo, cámara nectarífera y opérculo) y peso del fruto (pulpa, cáscara y contenido de jugo). Se seleccionaron trece descriptores cualitativos, y se identificaron cuatro factores (44.38% inercia) asociados principalmente con la pubescencia y antocianinas en la bráctea, el peciolo y la hoja, y el color del fruto maduro. El análisis de clasificación NJ mostró que no existe relación entre las accesiones por origen geográfico, y la distancia entre individuos de una misma accesión fue superior que entre accesiones para ambos tipos de descriptores. Los trips (*Neohydatothrips signifier*) y la roña (*Cladosporium cladosporioides*) fueron las plagas que presentaron mayor incidencia en las accesiones. Seis accesiones elite fueron identificadas de acuerdo con el índice de selección por sus características sobresalientes de calidad del fruto, rendimiento y tolerancia a problemas fitosanitarios. La alta variabilidad intra-accesión y los atributos de ciertas accesiones son la base para la obtención de cultivares más productivos y tolerantes.

Palabras clave: colección, diversidad, fitomejoramiento, fruta tropical, Passifloraceae.

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Introduction

Yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) is one of the leading fruit species of the Neotropics due to its high nutritional content and economic potential as fresh or processed fruit (Ocampo Pérez *et al*., 2021). This species originated in Brazil and is currently cultivated in tropical areas on four continents from sea level to mountain areas up to 1,500 m a.s.l. (Faleiro *et al*., 2020). The flower is hermaphrodite with a high degree of self-incompatibility (93%) and is cross-pollinated (allogamy) mainly by insects of the genus *Xylocopa* spp. (González *et al*., 2009; Arias-Suárez *et al*., 2014).

Yellow passion fruit was introduced to Colombia from Hawaii (USA) through Venezuela at the experimental station of the Colombian Agricultural Institute (ICA) in Valle del Cauca in the early 1960s (Morton, 1967). Colombian growers have contributed considerably to its domestication process by implementing new agronomic practices for better yield, such as pruning, fertilizer application, and the use of irrigation systems (Ocampo, Urrea, *et al*., 2013). There are currently about 8,165 ha cultivated with yellow passion fruit, with an average annual production of 137,622 t (Agronet, 2019). The departments of Antioquia, Huila, Meta, and Valle del Cauca concentrate the highest production with about 7,115 ha and an average yield that varies between 15 and 21 t ha^{-1} per year. Most of the production (60%) is destined for export, and national consumption is essentially supplied as fresh fruit, ice cream, desserts, and homemade juices. This production system suffers from multiple phytosanitary problems that prevent better crop development, such as collar rot [*Fusarium solani* f. sp. *passiflorae* (Mart.) Appel & Wollenweber], anthracnose [*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.], Soybean mosaic virus (SMV), Bean dwarf mosaic virus (BDMV), thrips (*Neohydatothrips signifier* Priesner), and lance fly (*Dasiops inedulis* Steyskal) (Hernández *et al*., 2011; Osorio Cardona *et al*., 2020), forcing the application of chemical treatments for crop protection. Additionally, the absence of certified nurseries and the lack of breeding programs have led to a decrease in the useful life of plantations from 36 to 18 months and a reduction in annual average yields to just 17.1 t ha^{-1} in the last decades (Agronet, 2019).

Genetic variability for agronomic traits is the key component of breeding programs for broadening the gene pool of crops to face the risk of epidemic plant diseases, obtain greater adaptability to different ecological zones, and ensure sustainable agriculture (Meletti *et al*., 2005; Freitas *et al*., 2011). In Colombia, some studies have been carried out on the genetic variability in some species of the genus *Passiflora* L. at the intra and interspecific levels using morphological descriptors (Primot *et al*., 2005; Ocampo Pérez *et al*., 2009; Viana *et al*., 2010; Checa Coral *et al*., 2011), molecular markers such as amplified fragment length polymorphism (AFLP) (Segura *et al*., 2002; Ocampo *et al*., 2004), and microsatellites (Ortiz *et al*., 2012; Bernal-Parra *et al*., 2014). These studies have established the distances between cultivated species and their wild relatives as a strategy for the exploration and conservation of genetic resources. They have also allowed the development of further research on interspecific hybridization between the main economically important species of *Passiflora* and their closest wild species (Ocampo *et al*., 2016).

Most of research efforts in yellow passion fruit have been focused on its characterization, selection, and breeding in Colombia (Restrepo & Kogson, 1998; Espitia *et al*., 2008; Galeano Mendoza *et al*., 2018). However, the sample size, composition, and origin of these studies limit their interpretation and do not allow a broader exploration of the intraspecific variability of this species. Ocampo, Urrea, *et al*. (2013) reported the collection and characterization of 44 yellow passion fruit accessions in different producing areas of Colombia, identifying eight elite materials with high fruit quality (weight >200 g, pulp percentage >50%, and °Brix >14.5) as promising for a breeding program. This same sample of accessions was characterized using microsatellite markers (Ocampo *et al*., 2017), finding relative genetic variability with certain geographic relationships between accessions of the same origin based on fruit-associated characters. While in Colombia variability is currently being explored, in Brazil, several yellow passion fruit cultivars have been released since the year 2000 for fresh consumption and the agroindustry (Meletti *et al*., 2000; Nascimento *et al*., 2003; Cerqueira-Silva *et al*., 2014; Viana *et al*., 2016) with significant impact in the last decade by improving fruit quality in this country (Cerqueira-Silva *et al*., 2016; Faleiro *et al*., 2020).

Characterization of agro-morphological descriptors has been used in several fruit cultivated species, such as *Carica papaya* L. (Ocampo *et al*., 2006), *Theobroma cacao* L. (Santos *et al*., 2012), and *Annona muricata* L. (Moreira-Macías *et al*., 2020), to establish the levels of intra and interspecific variability (Ocampo Pérez & Coppens d'Eeckenbrugge, 2017). These levels are easily accessible, and their information is essential for breeding that cannot be based primarily on DNA sequences. A descriptor is a characteristic or attribute whose expression is easy to measure, register, or evaluate and refers to the shape, structure, or behavior of an accession (Hidalgo, 2003). These descriptors are based on the measurement of quantitative and qualitative characters of different plant organs, such as stem, leaf, flower, and fruit. However, these characters must be measured at the same locality to avoid environmental influence (Hidalgo, 2003). In Brazil, Crochemore *et al.* (2003) established high differentiation in 56 accessions of *Passiflora* species, but with small divergences within yellow passion fruit accessions. Studies carried out by Ocampo Pérez *et al.* (2009) and Ocampo Pérez and Coppens d'Eeckenbrugge (2017) demonstrated that 23 quantitative and 18 qualitative descriptors could detect variability between and within 61 *Passiflora* species, including accessions of *P. edulis* f. *flavicarpa*. Castro *et al.* (2012) defined 16 quantitative and six qualitative descriptors for passion fruit with discriminating capacity by analyzing 24 genotypes grown in Brazil. These studies highlight that the agro-morphological descriptors that contribute most to the total variance are related to flower and fruit traits.

Collection and characterization studies carried out in Colombia by Ocampo, Urrea, *et al.* (2013), Ocampo *et al.* (2017), and Galeano Mendoza *et al.* (2018) are the basis for further research in the search for new planting materials for different ecological niches with greater hardiness and productivity. Therefore, the aim of this study was to establish the genetic relationships between individuals and accessions of different geographical origins through morphological characterization based on desirable agronomic traits, allowing the selection of elite yellow passion fruit accessions.

Materials and methods

Study area

This research was carried out at the experimental station of the Luker farm (5°04'25.95" N; 75°41'4.71" W) located in the municipality of Palestina, department of Caldas (Colombia) at 1023 m a.s.l. The edaphoclimatic conditions registered in this locality were as follows: soil with a loamy texture, pH from 5.5 to 6.0, 9% organic matter, an average environmental temperature of 23°C, heliophany of 190 h per year, average relative humidity of 75.6%, and total annual average precipitation of 2200 mm.

Plant material and field trials

The germplasm included 52 commercial accessions collected in previous studies by Ocampo, Urrea, *et al.* (2013) in different geographical areas of Colombia and other producing countries (Tab. 1). In total, the sample comprised 104 individuals, and each genotype was obtained through open pollination, considering half-siblings within each accession. Seeds of each accession were germinated in 12x17 cm plastic bags (1.9 kg) using substrate composed of soil and rice husk at a 3:1 ratio. Plants were established in the field 40 d after germination. Agronomical practices such as fertilization, weed control, pruning, and pest and disease control were adopted according to the recommendations of Jaramillo Vásquez *et al.* (2009) with emphasis on the use of biological products (*Trichoderma harzianum* and *Pachycrepoideus vindemiae*). Seedlings were sown 3 m between rows and 3 m between plants in a simple trellis system and under a completely randomized block experimental design with 52 treatments (accessions) and five replicates per experimental unit.

TABLE 1. List of yellow passion fruit accessions characterized and evaluated.

No	Country	Department	Code	No. accessions
1	Colombia	Antioquia	AntFla	6
2	Colombia	Amazonas	AmaFla	1
3	Colombia	Caldas	CalFla	4
4	Colombia	Cauca	CauFla	5
5	Colombia	Cundinamarca	Commercial (control)	1
6	Colombia	Cundinamarca	CunFla	1
7	Colombia	Huila	HuiFla	7
8	Colombia	Meta	MetFla	1
9	Colombia	Tolima	TolFla	7
10	Colombia	Valle del Cauca	Unión	1
11	Colombia	Valle del Cauca	ValFla	13
12	Brazil	Sao Paulo	Bra _ 1	1
13	Ecuador	Manabi	Ecu _ 1/2/3	3
14	Costa Rica	Guanacaste	CR _ 1	1

Morphological characterization

In each block, the most vigorous plant per accession was selected, and this plant was characterized with a list of 23 quantitative and 18 qualitative descriptors associated with vegetative, floral and fruit characters selected by Ocampo Pérez *et al.* (2009), Castro *et al.* (2012), and Ocampo Pérez and Coppens d'Eeckenbrugge (2017) (Tab. 2). For the quantitative descriptors, five measurements were made per descriptor in each plant organ using a digital caliper, and weighing was done directly using a digital scale. The traits related to fruit quality were analyzed at the Quality Laboratory at the experimental station of the Luker farm, and total soluble solids (°Brix) per fruit were measured with a manual refractometer on a scale of 0 to 32. All qualitative

TABLE 2. List of morphological descriptors used in 52 yellow passion fruit accessions assessed.

Organ	Quantitative	Code	Qualitative	Code
Stem	Internode length (cm)	INL	Stem anthocyanin	STA
Leaf	Petiole length (cm)	PEL	Stipule color	ESC
	Central lobe length (cm)	CLL	Anthocyanin stipule	AES
Flower	Bract length (cm)	BRL	Pubescence stipule	PUS
	Petal length (cm)	PTL	Petiole anthocyanins	PEA
	Petal width (cm)	PTW	Anthocyanins in abaxial leaf	AAL
	Sepal length (cm)	SPL	Pubescence abaxial bract	PAB
	Nectar chamber length (cm)	NCL	Androgynophore color	ANC
	Nectar chamber diameter (cm)	NCD	Staminal filament color	SFC
	Floral tube length (cm)	FTL	Anthocyanins in styles	ANS
	Floral tube diameter (cm)	FTD	Ovary pubescence	OVP
	Hypanthium length (cm)	HYL	Anthocyanins at the base of the ovary	ABO
	Ovary length (cm)	OVL		
	Staminal filament length (cm)	SFL		
	Operculum length (mm)	OPL		
Fruit	Length (cm)	FRL	Shape	FRS
	Diameter (cm)	FRD	Ripe fruit pubescence	RFP
	Weight (g)	FRW	Ripe fruit color	RFC
	Pulp plus seed weight (g)	PSW	Pulp color (aril)	PUC
	Pulp percentage (%)	PUL%	Seed color	SEC
	Juice weight (g)	JUW		
	Juice percentage (%)	JUI%		
	Shell weight (g)	SHW		
	Seed weight (g)	SEW		
	Seed index (100 seeds)	SEI		
	Total soluble solids (˚Brix)	TSS		

characteristics were recorded as binary numbers, *i.e.*, presence (1) or absence (0), and the colors of each organ were compared with the Royal Horticultural Society color chart (R.H.S., 2001). The shape of the fruit was recorded and divided into three categories as oval (1), spherical (2) and oblate (3) based on the index generated between diameter and length (fruit diameter (FRD)/fruit length (FRL).

Agronomic evaluation

The incidence of pests and diseases (percentage of plants affected) was registered in 10 plants per accession (five replicates) every 15 d for 12 months after sowing the plants of each accession in the field, for a total of 24 phytosanitary evaluations. The number of days after the first flower (DAF; anthesis) was recorded when at least 50% of the plants of each accession bloomed. Fruit yield (kg ha^{-1}, YLD) was determined by the sum of the total of harvests carried out during 8 months after the first harvest. Yield was calculated in kilograms, collecting the fruits of the five plants in each accession per block. The identification of pest insects and diseases was carried out in the Plant health laboratories of Universidad de Caldas and the International Center for Tropical Agriculture (CIAT) - Bioversity International, following the methodologies proposed by Morales *et al.* (2006), Fischer and Rezende (2008), and Hernández *et al.* (2011).

Selection of superior genotypes

A selection index (SI) based on a linear combination of economic weight for the traits yield, precocity (DAF), and fruit quality for market requirements was calculated according to Baker (1986). The values of each variable were standardized (E) using the following equation:

$$E = [(X_{ij} - m)/sd] \quad (1)$$

where X_{ij} is the individual observation, m is the general average of each accession per variable, and sd is the standard deviation.

Once the data were standardized, the SI was established according to the following equation:

$$SI = (FRW \times 0.4) + (PUL\% \times 0.3) + (TSS \text{ x } 0.2) + (DAF \times 0.05) + (FDL \times 0.05) \quad (2)$$

where FRW is the fruit weight estimated in t ha^{-1}, PUL% is the percentage of pulp plus the seeds, TSS is the content of total soluble solids (°Brix), DAF is the days after the first flower or precocity, and FDL is the ratio between fruit diameter and length.

Statistical analysis

The main traits of fruit quality (weight, pulp content, and total soluble solids), and yield were subjected to an analysis of variance (ANOVA; $P \leq 0.05$) and the Tukey's mean difference test. The quantitative descriptors were subjected to univariate and multivariate principal component analyses (PCA) using varimax normalized function. The qualitative descriptors underwent a multiple correspondence factor analysis (MCFA). The analysis of classification by the neighbor-joining method (Saitou & Nei, 1987) was used with the quantitative descriptors utilizing Euclidean distances, and the qualitative descriptors employed the distances of Sokal and Michener (1958). Data were analyzed with the software STATISTICA® v.8.1 and DarWin® v.5.01.

Results

Agronomic evaluation

Precocity

The 52 passion fruit accessions showed an average of 119 DAF with a coefficient of variation of 14.2% (Tab. 3). Likewise, 51% of the accessions reached their first bloom below the average value, and only seven of them between 86 and 105 d (CauFla04, ValFla13, HuiFla04, CalFla04, Ecu_2, Commercial, and ValFla07). In contrast, TolFla01, HuiFla02/3, Ecu_1, and CauFla02/03, with more than 130 DAF, come from regions distant from the area where this study was carried out.

Yield

The ANOVA showed significant differences between the means of each treatment (52 accessions), with a $P \leq 0.05$. The yield during the first eight months after the first fruit harvest registered an average of 19.3 t ha^{-1}, ranging between 14.6 and 25 t ha^{-1} (Tab. 3). Thirty percent of the accessions reached yields of more than 20 t ha^{-1} and were associated with the departments of Valle del Cauca (ValFla05/06/07/08/09/11/12), Tolima (TolFla05/06), Antioquia (AntFla04/05/06), Huila (HuiFla07), Cauca (CauFla03), and Caldas (CalFla04), and the countries Brazil (Bra_1), and Costa Rica (CR_1). In contrast, accessions from Cauca (CauFla04/5), Meta (MetFla01), Ecuador (Ecu_2), and the commercial control showed yields below 16.2 t ha^{-1}. A point to highlight is the discreet yield of the commercial control accession (16.1 t ha^{-1}), with a value lower than 90% of the evaluated accessions. The divergence found in yield between all accessions can be explained by the degree of adaptability of the accessions to the environmental conditions of the study area.

Incidence of pests and diseases

The phytosanitary evaluation carried out for 12 months recorded the incidence of five pest insects during the 24 evaluations carried out (Fig. 1A). Thrips (*Neohydatothrips signifier*) were the most limiting insects in the collection, with a 98% incidence in all accessions, affecting terminal shoots and causing growth retardation. Lance fly (*Dasiops inedulis*) ranked second in importance with 77%, followed by the defoliation worm [*Dione juno* (Cramer)] with 22%, affecting mostly the leaves. Mites (*Tetranychus ludeni* Zacher) and aphids (*Aphis gossypii*) were also detected attacking young and adult leaves with 10% and 21% incidence, respectively.

Accessions with a low pest attack (21% of incidence) were identified. Thrips were the main pest of economic importance affecting yellow passion fruit, particularly the accessions from Valle del Cauca (ValFla01/03/06/08), Huila (HuiFla01), Antioquia (AntFla03), and Tolima (TolFla01). The accession from Ecuador (Ecu_02) showed an incidence of less than 2%, indicating a high potential for the selection of genotypes with pest tolerance.

Four diseases were registered affecting the development of the 52 accessions (Fig. 1B). Scab [*Cladosporium cladosporioides* (Fresen.) G.A.de Vries] was the disease with the highest incidence (100%), affecting branches and fruits at all stages of the crop cycle. Viral symptoms (*Soybean mosaic virus* – SMV) in the leaves were also detected in 96% of the accessions after the first harvest until the end of the evaluation. Bacteriosis [*Xanthomonas axonopodis* pv. *passiflorae* (Pereira) Gonçalves & Rosato] and anthracnose [*Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.] showed severe symptoms in the branches, leaves, and fruits with an incidence of 48% and 83%, respectively. The latter was characterized by partial defoliation and loss of plant vigor, and such symptoms were more affected by increased rainfall and high relative humidity.

TABLE 3. Average of the trait's precocity, yield, and fruit quality for market requirements in the yellow passion fruit germplasm.

Accession	DAF	Yield (t ha^{-1})	Tukey's test	FRW (g)	Tukey's test	PUL%	Tukey's test	TSS	Tukey's test
Bra _ 01	107.0	25.0	f	255.2	d e f	45.0	a b c d e f	12.7	a b c
HuiFla07	121.5	23.5	e f	170.1	a b c d e f	51.1	c d e f	14.2	a b c d e f g h i
CR _ 01	109.0	23.2	e f	155.6	a b c d	37.6	a	12.6	a
ValFla07	104.0	22.9	e f	238.0	b c d e f g	40.7	a b c d	14.8	a b c d e f g h i
ValFla06	106.0	22.2	e f	175.8	a b c d e f	38.9	a b c	15.4	d e f g h i
CalFla04	103.0	21.8	d e f	186.6	a b c d e f g	48.5	a b c d e f	14.3	a b c d e f g h i
ValFla11	120.5	21.4	d e f	220.1	a b c d e f g	47.0	a b c d e f	15.6	e f g h i j
ValFla09	114.5	21.3	d e f	252.0	c d e f g	49.3	a b c d e f	14.7	a b c d e f g h i
TolFla06	108.0	21.1	d e f	201.2	a b c d e f g	50.0	a b c d e f	14.6	a b c d e f g h i
ValFla05	121.5	21.1	d e f	231.6	a b c d e f g	50.6	b c d e f	17.8	j
TolFla05	124.5	20.9	d e f	142.6	a b	46.8	a b c d e f	12.6	a b
CauFla03	117.0	20.8	d e f	233.0	a b c d e f g	50.7	b c d e f	14.1	a b c d e f g h i
AntFla06	124.0	20.4	c d e f	170.8	a b c d e f	56.0	f	15.7	f g h i j
AntFla05	123.0	20.4	c d e f	231.4	a b c d e f g	44.4	a b c d e f	15.4	d e f g h i
ValFla08	107.5	20.3	c d e f	198.1	a b c d e f g	50.2	a b c d e f	14.1	a b c d e f g h i
AntFla04	118.5	20.2	c d e f	247.8	b c d e f g	50.3	a b c d e f	15.1	d e f g h i
ValFla12	149.0	20.1	c d e f	284.3	g	43.9	a b c d e f	15.3	d e f g h i
HuiFla06	106.5	20.0	b c d e f	269.4	e f g	46.4	a b c d e f	14.9	c d e f g h i
TolFla04	108.0	19.9	b c d e f	212.2	a b c d e f g	50.0	a b c d e f	13.7	a b c d e f g
CalFla03	132.5	19.9	b c d e f	255.5	d e f g	40.8	a b c d	16.0	h i j
ValFla02	138.0	19.9	b c d e f	271.6	f g	43.6	a b c d e f	14.7	a b c d e f g h i
AntFla03	117.0	19.7	b c d e f	190.2	a b c d e f g	45.8	a b c d e f	14.8	a b c d e f g h i
TolFla02	131.0	19.5	b c d e f	181.8	a b c d e f g	45.1	a b c d e f	14.5	a b c d e f g h i
CunFla02	119.0	19.5	b c d e f	258.1	d e f g	48.7	a b c d e f	14.8	a b c d e f g h i
Unión	110.5	19.5	b c d e f	178.4	a b c d e f g	45.4	a b c d e f	13.1	a b c d
CalFla02	109.5	19.4	b c d e f	173.1	a b c d e f	50.0	a b c d e f	13.6	a b c d e f
AntFla02	125.5	19.4	b c d e f	177.2	a b c d e f	51.3	c d e f	13.6	a b c d e f g
ValFla10	106.0	19.3	b c d e f	252.8	c d e f g	41.2	a b c d	14.0	a b c d e f g h i
ValFla13	98.0	19.2	b c d e f	176.1	a b c d e f	47.5	a b c d e f	14.1	a b c d e f g h i
CauFla02	158.0	19.0	a b c d e f	204.7	a b c d e f g	45.3	a b c d e f	13.3	a b c d e
HuiFla01	113.5	18.9	a b c d e f	178.0	a b c d e f g	49.9	a b c d e f	15.9	g h i j
CalFla01	107.0	18.7	a b c d e f	182.0	a b c d e f g	46.9	a b c d e f	15.5	e f g h i
ValFla03	124.0	18.6	a b c d e f	211.6	a b c d e f g	39.9	a b c d	15.6	f g h i j
HuiFla04	100.5	18.4	a b c d e f	175.2	a b c d e f	43.9	a b c d e f	13.7	a b c d e f g h
Ecu _ 3	123.0	18.4	a b c d e f	194.2	a b c d e f g	54.3	e f	14.2	a b c d e f g h i
TolFla01	156.0	18.3	a b c d e f	225.4	a b c d e f g	42.2	a b c d e	14.2	a b c d e f g h i
TolFla07	119.0	18.3	a b c d e f	167.2	a b c d e f	38.0	a b	13.8	a b c d e f g h i
HuiFla05	183.5	18.2	a b c d e f	183.3	a b c d e f g	45.4	a b c d e f	15.4	d e f g h i
HuiFla03	136.0	18.2	a b c d e f	202.5	a b c d e f g	42.3	a b c d e	13.7	a b c d e f g
AntFla01	127.0	18.2	a b c d e f	236.4	b c d e f g	49.0	a b c d e f	14.9	b c d e f g h i
TolFla03	127.0	18.2	a b c d e f	171.7	a b c d e f	43.0	a b c d e	14.1	a b c d e f g h i
ValFla01	130.5	17.9	a b c d e f	220.2	a b c d e f g	42.5	a b c d e	14.5	a b c d e f g h i
CauFla05	121.0	17.2	a b c d e f	154.0	a	52.5	e f	13.8	a b c d e f g h i
ValFla04	124.0	17.0	a b c d e f	178.6	a b c d e f g	49.7	a b c d e f	15.3	d e f g h i
AmaFla01	130.5	16.5	a b c d e f	207.4	a b c d e f g	50.7	b c d e f	14.8	a b c d e f g h i
HuiFla02	148.0	16.4	a b c d e	251.5	c d e f g	47.3	a b c d e f	14.0	a b c d e f g h i
Ecu _ 1	131.0	16.1	a b c d	163.4	a b c d e	50.7	b c d e f	14.8	a b c d e f g h i
Commercial	103.0	16.1	a b c d	171.1	a b c d e f	48.7	a b c d e f	14.9	b c d e f g h i
Ecu _ 2	103.0	15.9	a b c d	146.2	a b c	51.5	c d e f	15.3	d e f g h i
CauFla04	86.0	15.6	a b c	200.4	a b c d e f g	49.4	a b c d e f	16.1	i j
MetFla01	137.0	15.3	a b	217.0	a b c d e f g	47.9	a b c d e f	13.6	a b c d e f
CauFla01	128.5	14.6	a	126.9	a b c d	54.6	d e f	13.9	a b c d e f g h i
Mean	121.1	19.3		203.1		47.0		14.5	
S.D.	17.2	2.2		37.5		4.3		1.0	
CV%	14.2	11.3		18.4		9.2		6.7	

DAF - days after the first flower; FRW - fruit fresh weight (g); PUL% - percentage of pulp; TSS - total soluble solids (˚Brix). Lowercase letters indicate the differences between the treatments. S.D. - standard deviation; CV - coefficient of variation.

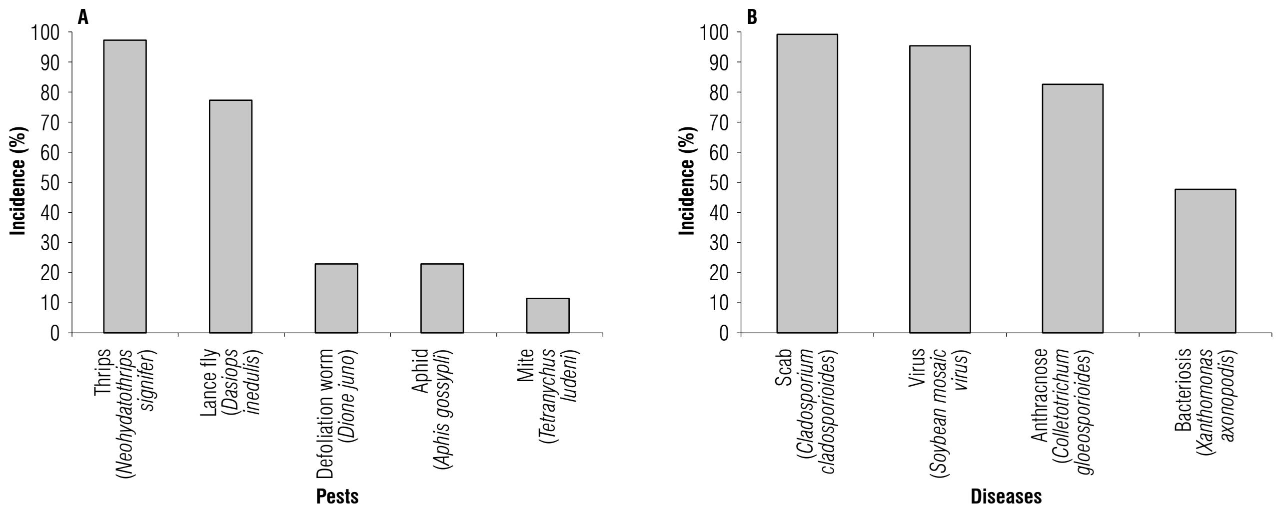

FIGURE 1. Phytosanitary evaluation carried out during 12 months in 52 yellow passion fruit accessions.

The incidence of diseases between the accessions was more variable than the incidence of pests. Seven accessions (TolFla03/06, HuiFla06, CauFla04, CunFla02, Bra_01, and CR_01) showed tolerance to diseases (*C. cladosporioides*) that mainly attack fruit shells lowering their marketing quality as fresh fruit. A positive reaction to the attack of bacteriosis and anthracnose was observed in 57% of the accessions with less than 2% incidence; VaLFla04 and the commercial control showed no visible symptoms. Regarding viral attacks, mainly SMV, accessions AmaFla01, CauFla04, and HuiFla02 did not show symptoms of the disease, and the remaining accessions did not exceed 13% incidence. The results of this phytosanitary evaluation suggest a second agronomic evaluation to confirm these results.

Selection of elite accessions

The SI analysis identified 48% of the accessions with desirable agronomic traits (Fig. 2). These accessions combine the best fruit characteristics, earliness or precocity and yield. Thus, when selection pressure of 10% was applied, six elite accessions were identified: Valle del Cauca (ValFla05/09/12), Antioquia (AntFla04), Cundinamarca (CunFla02), and Huila (HuiFla06) with a SI higher than 0.66 (Tab. 4). Additionally, accession ValFla05 showed the highest values of pulp percentage (50.6%) and total soluble solids (17.8 °Brix), while ValFla09 and HuiFla06 showed high yield (21.3 t ha^{-1}) and a low number of days after the first flower (106.5). The average fruit fresh weight varied between 231.6 to 284.3 g, and the accessions ValFla12 and HuiFla06 were characterized by fruits of more than 269 g. In contrast, 27% of the accessions recorded a SI lower than -0.25, showing lower values of fruit quality, precocity, and yield.

The commercial control showed yield of less than 16.1 t ha^{-1} compared to the general average of 19.2 t ha^{-1} among the 52

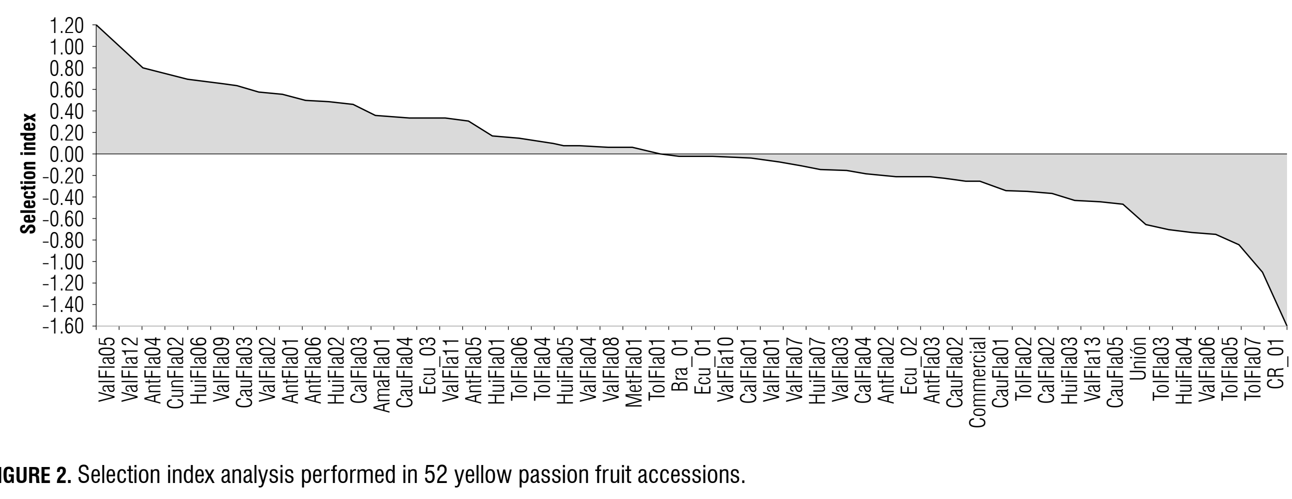

FIGURE 2. Selection index analysis performed in 52 yellow passion fruit accessions.

accessions evaluated. The Brazilian commercial accession Bra_1 showed high qualities regarding average yield (25 t ha^{-1}), precocity (107 DAF), and fruit fresh weight (255.2 g), but pulp percentage (PUL%) of only 45% and low content of total soluble solids (12.7 ˚Brix), which is supported by its low SI (-0.02). The commercial accessions from Ecuador (Ecu_1/2/3) obtained a selection index rate between -0.02 and 0.33, showing yield of less than 18.5 t ha^{-1} and fruit weight <163.5 g, but average PUL% and TSS higher than 50% and 14.5 ˚Brix.

Quantitative morphological variability

Univariate analysis

The estimated genetic variability was calculated for the 26 descriptors analyzed using descriptive statistics (Tab. 5). The average coefficient of variation (CV) was 17.67% and only the descriptors related to petiole length (PEL), and the weights of the fruit (FRW), pulp (PSW), juice (JUW), and shell (SHW) exceeded 25% of the variation. Descriptors associated with the diameter of the nectar chamber (NCD), the length of the stamen filaments (SFL), and the seed index (SEI) showed a CV below 10%, suggesting that these do not contribute to the total variation. The main descriptors related to fruit quality, such as TSS and PUL% showed relative variation (10.66 and 17.21%) that can be considered for the selection of elite individuals. This pomological variation is the consequence of an unsystematic selection of plants with uniform fruit characteristics by the producers in each harvest cycle.

Phenotypic correlations

The average correlation analysis between the morphological and agronomic descriptors was $r = 0.14$ (Tab. 6). The most significant correlations showed values higher than $r \geq 0.70$ and were represented by the most morphologically related descriptors. The most related were floral tube length (FTL) and hypanthium length (HYL) vs. floral tube

TABLE 4. Elite yellow passion fruit accessions selected with desirable agronomic parameters.

Department	**Valle del Cauca**	**Valle del Cauca**	**Antioquia**	**Cundinamarca**	**Valle del Cauca**	**Huila**
Municipally	Ginebra	Palmira	Tamesis	Caparrapi	La Union	Rivera
Accession	ValFla05	ValFla12	AntFla04	CunFla02	ValFla09	HuiFla06
Pest incidence (%)						
Thrips (*Neohydatothrips signifer*)	16.67	20.83	8.33	16.67	16.67	16.67
Lance fly (*Dasiops inedulis*)	4.17	8.33	12.50	4.17	8.33	0.00
Aphid (*Aphis gossypii*)	0.00	0.00	4.17	0.00	0.00	0.00
Defoliation worm (*Dione juno*)	0.00	0.00	4.17	0.00	4.17	4.17
Mite (*Tetranychus ludeni*)	0.00	0.00	0.00	0.00	0.00	0.00
Disease incidence (%)						
Scab (*Cladosporium cladosporioides*)	45.83	75.00	54.17	41.67	62.50	37.50
Anthracnose (*Colletotrichum gloeosporioides*)	0.00	4.17	4.17	4.17	0.00	12.50
Bacteriosis (*Xanthomonas axonopodis*)	4.17	0.00	0.00	4.17	4.17	4.17
Virus (Soybean mosaic virus)	8.33	12.50	12.50	16.67	12.50	8.33
Fruit weight - FRW (g)	231.60	284.30	247.80	258.10	252.00	269.35
Fruit length - FRL (cm)	90.08	103.61	99.22	102.90	101.19	95.40
Fruit diameter - FRD (cm)	81.77	85.29	81.22	77.60	83.69	83.20
Fruit shape (FRD/FRL) - FSH	0.91	0.82	0.82	0.75	0.83	0.87
Pulp percentage - PUL%	50.60	44.00	50.30	48.72	49.30	46.42
Total soluble solids - TSS (˚Brix)	17.80	15.30	15.10	14.82	14.70	14.94
Seed index - SEI (g)	2.40	2.60	2.20	2.40	2.50	2.20
Days after the first flower (DAF)	122.00	149.00	119.00	119.00	115.00	106.50
Yield (t ha^{-1})	21.00	20.10	20.20	19.51	21.30	20.05
Selection index (SI)	1.20	1.00	0.80	0.74	0.67	0.69

TABLE 5. Genetic variability in the yellow passion fruit collection calculated for the 26 descriptors analyzed using descriptive statistics.

Organ	Descriptor	Code	Mean	Minimum	Maximum	Standard deviation	Coefficient of variation (%)
Stem	Internode length (mm)	INL	96.58	60.00	166.10	18.86	19.53
Leaf	Petiole length (mm)	PEL	36.15	16.60	74.00	9.99	27.64
	Central lobe length (mm)	CLL	114.35	72.00	172.00	18.55	16.22
Flower	Bract length (mm)	BRL	24.72	11.60	42.10	5.21	21.07
	Petal length (mm)	PTL	33.38	22.40	42.30	3.35	10.03
	Petal width (mm)	PTW	10.18	6.60	13.90	1.14	11.16
	Sepal length (mm)	SPL	34.69	21.70	49.40	3.94	11.37
	Nectar chamber length (mm)	NCL	5.57	3.00	7.50	0.72	12.92
	Nectar chamber diameter (mm)	NCD	13.01	8.20	16.70	1.13	8.67
	Floral tube length (cm)	FTL	6.01	2.90	8.30	0.91	15.23
	Floral tube diameter (mm)	FTD	16.88	11.20	22.70	1.98	11.73
	Hypanthium length (mm)	HYL	11.58	6.30	15.60	1.43	12.31
	Ovary length (mm)	OVL	7.73	6.00	11.10	0.87	11.25
	Staminal filament length (mm)	SFL	13.56	10.30	16.50	0.86	6.33
	Operculum length (mm)	OPL	2.14	1.10	3.30	0.36	17.07
Fruit	Length (mm)	FRL	91.56	62.50	137.30	11.38	12.43
	Diameter (mm)	FRD	76.86	47.70	109.00	8.83	11.49
	Weight (g)	FRW	203.06	65.00	472.00	67.61	33.30
	Pulp plus seed weight (g)	PSW	94.69	29.00	180.00	32.46	34.29
	Pulp percentage (%)	PUL%	46.96	21.84	71.29	8.08	17.21
	Juice weight (g)	JUW	86.24	19.40	172.10	32.01	37.11
	Juice percentage (%)	JUI%	42.34	17.11	66.84	8.12	19.19
	Shell weight (g)	SHW	108.41	29.00	300.00	42.07	38.81
	Seed weight (g)	SEW	8.45	3.70	16.50	2.00	23.71
	Seed index (100 seeds)	SEI	2.28	1.80	2.80	0.20	10.66
	Total soluble solids (°Brix)	TSS	14.54	10.00	21.00	1.55	8.74

diameter (FTD), petiole length (PEL) vs. central lobe length (CLL), and sepal length (SPL) vs. petal length (PTL) with an $r>0.74$. Regarding the descriptors of fruit quality, only FRW and dimensions (FRL and FRD) showed an average correlation coefficient of $r = 0.77$. Total soluble solids did not show significant correlations, although it is negatively related to the NCD with $r = -0.39$. The yield (t ha^{-1}) exhibited moderate correlations with PEL, CLL, and bract length (BRL), with values between 0.31 and 0.35.

Multivariate analysis

The quantitative descriptors were subjected to a principal component analysis (PCA), and only six were retained with an eigenvalue higher than 1, explaining 71.6% of the total variance (Tab. 7). The first component explains 22.7% of the variance related to flower length descriptors (nectar chamber length (NCL), FTL, FTD, HYL, and operculum length (OPL)). The second component assumed 20.4% of the variance and includes fruit descriptors, such as dimension (FRL and FRD) and weight (FRW, PSW, JUW, and SHW). The third (9.4%) was associated with PUL% and JUW (%). The last three components (4, 5, and 6) explained 19.2% of the variance and were associated with the PTL, SPL, ovary length (OVL), PEL, CLL, as well as seed weight (SEW). The PCA identified 42% of the descriptors proposed in this study and mainly those that were retained in the first two components, with 43.1% of the variance explained.

Plants of each accession were projected in the main plane (Fig. 3). In the first component on the left side, accessions ValFla13ab and AntFla06ab appear characterized by having small flowers, while in the other extreme, MetFla01a,b, CalFla02b, and ValFla09b showed the largest flowers. In the upper part of the second component, the accessions with the largest fruit size and weight such as CunFla02b, TolFla01b, HuiFla02b, HuiFla02a, ValFla09b, and ValFla12b

TABLE 6. Phenotypic correlations between the morphological and agronomic descriptors used in the yellow passion fruit collection assessed.

Descriptor																							
Internode length	INL																						
Petiole length	-0.11	PEL																					
Central lobe length	-0.07	**0.80**	CLL																				
Bract length	-0.02	0.20	0.21	BRL																			
Petal length	0.01	0.06	-0.02	0.42	PTL																		
Petal width	0.05	0.10	0.12	0.13	0.41	PTW																	
Sepal length	-0.09	-0.08	-0.05	0.56	**0.75**	0.21	SPL																
Nectar chamber length	-0.01	-0.12	-0.10	0.06	0.23	0.25	0.38	NCL															
Nectar chamber diameter	0.23	-0.09	-0.01	0.48	0.38	0.14	0.49	0.42	NCD														
Floral tube length	0.09	-0.08	-0.11	0.13	0.37	0.39	0.38	**0.70**	0.32	FTL													
Floral tube diameter	0.13	-0.10	-0.09	0.16	0.33	0.24	0.38	0.62	0.51	**0.80**	FTD												
Hypanthium length	0.06	-0.11	-0.11	0.11	0.34	0.36	0.41	**0.89**	0.39	**0.95**	**0.79**	HYL											
Ovary length (mm)	0.08	-0.02	0.03	0.52	0.49	0.43	0.64	0.35	0.52	0.40	0.38	0.41	OVL										
Staminal filament length	-0.01	0.19	0.14	0.13	0.36	0.31	0.28	0.29	0.38	0.34	0.30	0.35	0.29	SFL									
Operculum length	0.08	0.00	0.08	0.13	0.34	0.25	0.44	0.62	0.44	0.65	0.58	**0.70**	0.53	0.34	OPL								
Fruit length	0.13	0.02	0.07	0.55	0.47	0.37	0.39	0.02	0.27	0.26	0.24	0.18	0.50	0.25	0.22	FRL							
Fruit diameter	0.04	0.10	0.12	0.51	0.45	0.36	0.30	-0.03	0.27	0.20	0.21	0.12	0.35	0.22	0.13	**0.75**	FRD						
Fruit weight	-0.04	0.13	0.09	0.39	0.34	0.26	0.19	-0.13	0.07	0.09	0.08	0.00	0.25	0.15	0.01	**0.75**	**0.81**	FRW					
Pulp percentage	-0.03	0.02	-0.06	-0.32	-0.15	0.00	-0.25	-0.12	-0.26	-0.11	-0.15	-0.12	-0.30	-0.15	-0.17	-0.27	-0.29	-0.24	PUL%				
Seed weight	0.15	0.09	0.10	-0.11	-0.17	0.19	-0.20	-0.04	-0.04	-0.01	-0.04	-0.03	0.02	-0.04	0.10	0.02	0.05	0.10	0.20	SEW			
Seed index	0.18	0.03	0.05	0.20	0.08	0.16	-0.06	0.08	0.17	0.26	0.30	0.20	0.31	0.00	0.23	0.48	0.52	0.47	-0.22	0.34	SEI		
Total soluble solids	-0.02	0.04	0.04	-0.08	-0.18	-0.05	-0.21	-0.19	-0.39	-0.09	-0.18	-0.14	-0.13	0.02	-0.12	0.13	0.16	0.24	0.13	0.24	0.26	TSS	
Days after first flower	-0.02	-0.05	-0.15	0.17	0.15	0.30	0.17	0.24	0.00	0.19	0.09	0.23	0.23	-0.10	0.15	0.25	0.23	0.17	-0.10	-0.15	0.21	0.00	DAF
Yield	-0.22	0.31	0.35	0.35	0.13	-0.15	0.10	-0.14	0.28	-0.28	-0.20	-0.24	0.12	0.08	-0.09	0.12	0.23	0.27	-0.26	-0.10	-0.03	-0.10	-0.22	YLD

TABLE 7. Principal component analysis (PCA) performed on the quantitative descriptors used in the yellow passion fruit collection evaluated.

			Components					
Organ	**Descriptor**	**Code**	**1**	**2**	**3**	**4**	**5**	**6**
Stem	Internode length	INL	-0.032	-0.101	0.022	0.107	-0.016	0.511
Leaf	Petiole length	PEL	-0.086	0.046	-0.083	-0.032	**0.917**	0.034
	Central lobe length	CLL	-0.035	0.042	0.062	0.073	**0.906**	0.002
Flower	Bract length	BRL	0.035	0.324	0.252	0.533	0.160	-0.295
	Petal length	PTL	0.157	0.088	-0.086	**0.828**	-0.030	-0.014
	Petal width	PTW	0.171	0.000	-0.143	0.518	0.142	0.369
	Sepal length	SPL	0.232	0.031	0.027	**0.814**	-0.102	-0.261
	Nectar chamber length	NCL	**0.801**	-0.073	0.019	0.195	-0.100	-0.154
	Nectar chamber diameter	NCD	0.326	-0.038	0.169	0.627	-0.027	-0.100
	Floral tube length	FTL	**0.896**	0.024	0.005	0.150	-0.023	0.086
	Floral tube diameter	FTD	**0.832**	-0.021	0.083	0.216	-0.016	-0.033
	Hypanthium length	HYL	**0.953**	-0.019	0.012	0.188	-0.062	-0.016
	Ovary length	OVL	0.270	0.131	0.109	**0.736**	0.043	0.104
	Staminal filament length	SFL	0.251	0.022	0.075	0.371	0.292	0.138
	Operculum length	OPL	**0.705**	0.027	0.064	0.241	0.085	0.033
Fruit	Length	FRL	0.031	**0.831**	0.142	0.211	-0.004	0.096
	Diameter	FRD	0.007	**0.893**	0.151	0.135	-0.003	0.089
	Weight	FRW	-0.031	**0.978**	0.014	0.013	0.048	0.010
	Pulp plus seed weight	PSW	-0.050	**0.910**	-0.373	-0.001	0.044	-0.004
	Pulp percentage	PUL%	-0.069	-0.119	**-0.947**	-0.079	-0.021	0.036
	Juice weight	JUW	-0.044	**0.906**	-0.384	0.009	0.052	-0.037
	Juice percentage	JUI%	-0.059	0.063	**-0.966**	-0.059	0.017	-0.075
	Shell weight	SHW	-0.016	**0.913**	0.311	0.023	0.044	0.019
	Seed weight	SEW	-0.125	0.332	-0.230	-0.203	0.020	0.439
	Seed index	SEI	-0.020	0.165	0.067	-0.194	0.075	**0.666**
	Total soluble solids	TSS	0.162	0.445	0.307	0.007	-0.028	0.429
Total variance			22.718	20.392	9.357	7.659	6.800	4.703

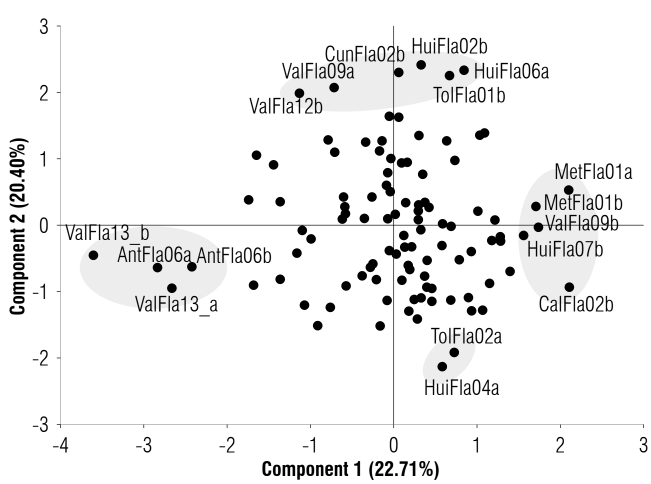

FIGURE 3. Yellow passion fruit accessions projected in the main plane generated by the principal component analysis (PCA) using quantitative descriptors.

are distributed. In the lower part, two plants of the accessions TolFla02a and HuiFla04a are differentiated with small and light fruits. In general, the main plane is dominated by a central cloud, where only 30% of the total accessions move away from the point of origin (0). These results suggest that the descriptors retained in the first two components do not allow grouping or clear differentiating of the accessions studied according to their geographical origin. However, descriptors related to flower and fruit size can contribute to the selection of elite individuals.

Tree classification

Classification analysis of the accessions utilizing the neighbor-joining method was carried out based on the results of the three principal components (PCA) that explained 52.45% of the total variance (Fig. 4). The dendrogram shows five main groups with little differentiation (bootstrap<30) and with high heterogeneity among the individuals of the same accession. The distances among the plants of each accession are higher than among the main groups. This is clearly observed in the accessions from Valle del Cauca (ValFla), Huila (HuiFla), and Tolima (TolFla) that are distributed in all groups. The accessions from Ecuador (Ecu_1/2/3) and Brazil (Bra_1) do not show a considerable differentiation in relation to the Colombian accessions; additionally, plants within the same accession are placed in different groups. In general, grouping of individuals is unsystematic, with some exceptions such as the accessions Union and TolFla03 (group I), ValFla13, AntFla06, and HuiFl06 (group II), and TolFla05 and CauFla04 (group V). The six elite accessions (ValFla05, ValFla09, ValFla12, AntFla04, CunFla02, and HuiFla06)

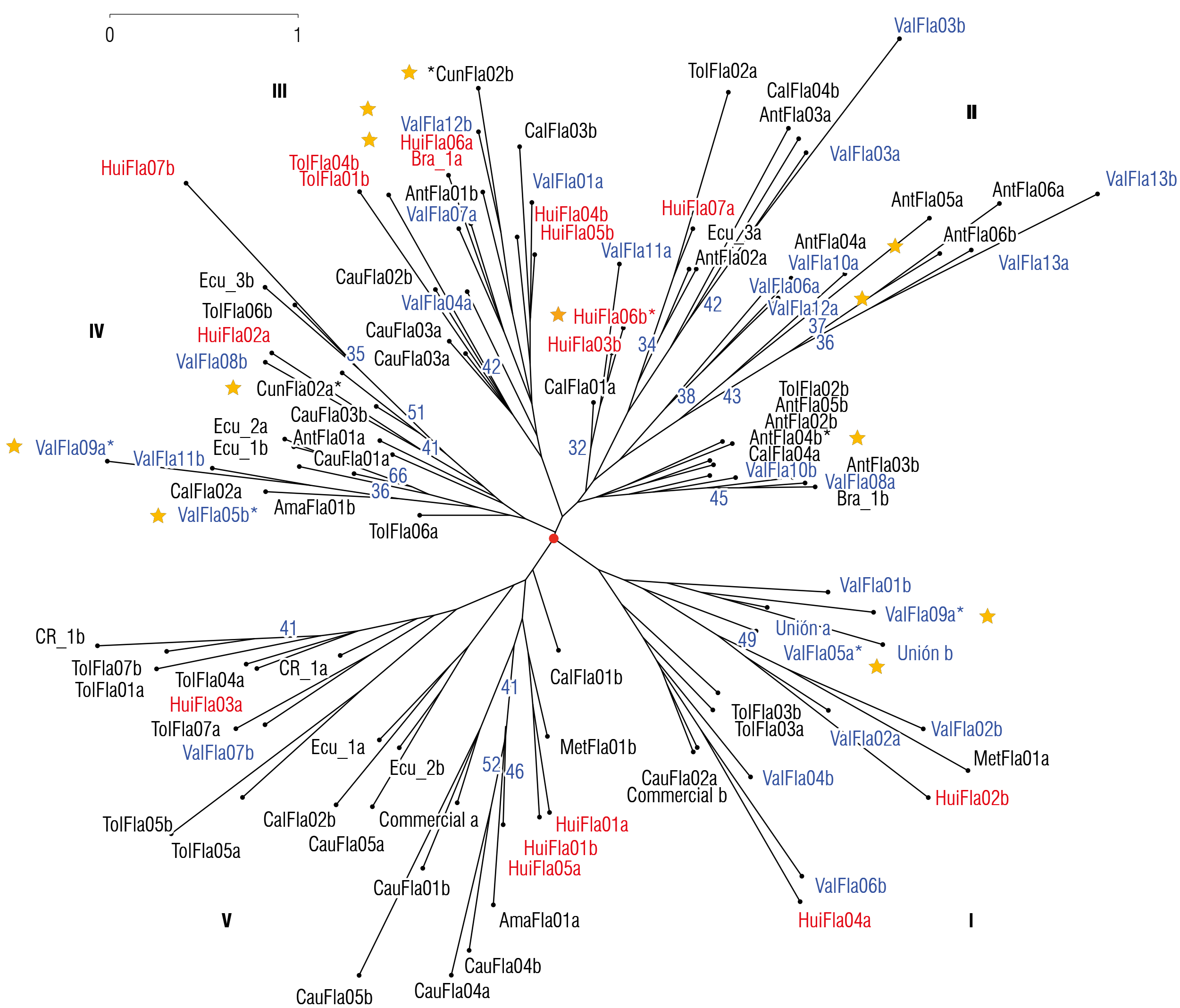

FIGURE 4. Classification analysis of yellow passion fruit accessions using the neighbor-joining (NJ) method and employing quantitative descriptors. Yellow stars indicate elite accessions.

are distributed in four groups and their individuals are well differentiated.

Qualitative morphological variability

Multiple correspondence factor analysis

The characterization showed that descriptors related to the color of the androgynophore (ANC) and staminal filaments (SFC), the pubescence of the ovary (OVP), and the yellow color of ripe fruit (RCF) did not show variability in the evaluated accessions. These descriptors were excluded, and a MCFA was performed with 13 qualitative polymorphic descriptors. The MCFA was represented by four factors that explain 44.4% of the total variability (inertia). The first factor retained 13.4% of the variability and was related to the absence of pubescence in the upper part of the bract (PAB01), anthocyanins in the petiole (PEA01), anthocyanins in the abaxial surface of the leaf (AAL01), and the pale-yellow color in the fruit (RFC03). In the second factor (11.5%), the descriptors related to the presence of anthocyanins at the base of the ovary (ABO02) and in the androgynophore (ANA02), absence of anthocyanins in the stem (STA01), pubescence in the mature fruit (RFP01) and dark green color in the stipule (EST02) stood out. The presence of pubescence (PUS02), absence of anthocyanins (AES01) in the stipule, and fruit shape (FRS) represented 19.5% of the total variability (factors 3 and 4). The FRS showed three categories, ovate, spherical and oblate, across the total accessions with an index of shape (ISH = FRD/FRL) that varied between 0.65 and 1.17. Thus, 96% of the harvested fruits were ovate (ISH<1), 3% spherical (ValFla05, TolFla05, AntFla02, Bra_1, and Union), and some plants of the accessions HuiFla03, CauFla03 and AntFla03 were oblate (ISH>1).

The accessions were projected in the principal plane generated by the MCFA and showed a similar distribution compared to the PCA with the quantitative descriptors. In this main plane, the accessions were not clearly grouped, and in most cases, they did not follow a pattern according to the geographical origin (Fig. 5). In the first factor, the Cauca accessions (CauFla03b/04a) stood out with the absence of pubescence in the bract (PAB01). On the opposite side of factor 1, some accessions from Valle del Cauca (ValFla07a/08a), Antioquia (AntFla01b/06b), Huila (HuiFla04b), Tolima (TolFla04b/07a), and Caldas (CalFla04a) were characterized by the absence of anthocyanin on the petiole (PEA01) and on the abaxial surface of the leaf (AAL01), and by a pale-yellow color of the fruit (RFC03). For the second factor, accessions MetFla01a,b (Meta), AntFla02a,b (Antioquia), ValFla02a/09a,b (Valle del Cauca) and Ecu_2a/b (Ecuador) showed anthocyanin at the base of the ovary (ABO02) and on the androgynophore (ANA02), and its absence in the stem (STA01).

Tree classification

The maximum variability determined by the MCFA with the four factors (44.4% of inertia) was retained to construct the classification analysis of the qualitative descriptors (Fig. 6). Thus, the dendrogram showed five poorly supported main groups (bootstrap<30) with a similar structure to that obtained with the quantitative descriptors and shorter distances between the individuals of each accession. The accessions from Valle del Cauca (ValFla), Huila (HuiFla), and Tolima (TolFla) are distributed in the five branches, with no association among the plants that comprise them. Despite the high heritability of the qualitative traits, there is a high divergence between individuals of the same accession, with some exceptions such as ValFla01, ValFla03,

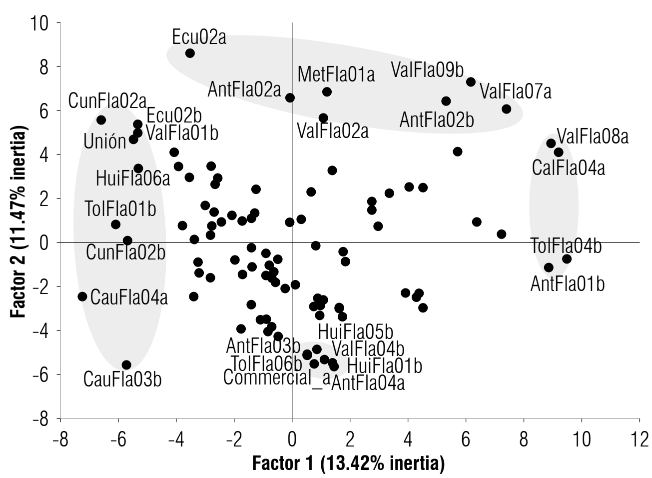

FIGURE 5. Yellow passion fruit accessions projected in the main plane generated by the multiple correspondence factor analysis (MCFA) using qualitative descriptors.

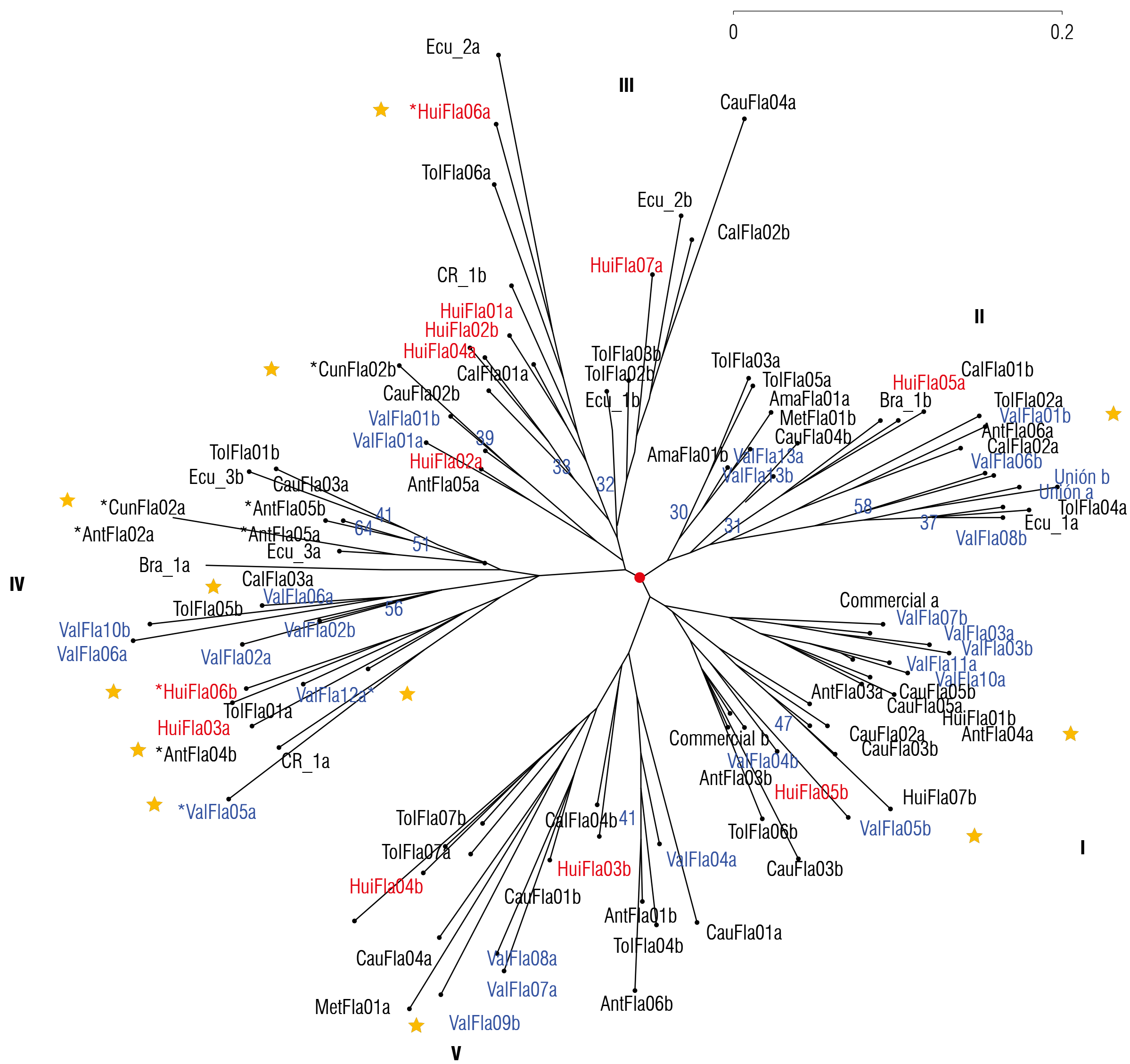

FIGURE 6. Classification analysis of yellow passion fruit accessions using the neighbor-joining (NJ) method employing qualitative descriptors. Yellow stars indicate elite accessions.

TolFla07, ValFla13, and Union. These two accessions are selections from Valle del Cauca and show some uniformity compared to both types of descriptors.

The plants of the six elite accessions are scattered in the five groups and especially in group IV, where the accessions from Brazil (Bra_01), Costa Rica (CR_01), and Ecuador (Ecu_03) are also grouped. Despite the high heritability of qualitative descriptors, the plants of each accession appear separated into different groups (ValFla05 – groups I and IV; ValFla12 – groups II and IV; AntFla04 – groups I and IV; CunFla02 – groups III and IV; ValFla09 – groups IV and V, and HuiFla06 – groups III and IV). Overall, the qualitative descriptors show similar structure variability regarding the quantitative descriptors in the yellow passion fruit collection assessed, reflected by the differences between the accessions and their plants. The low geographical structure of the individuals makes it challenging to identify duplicates within the collection for conservation if the origin criterion of the accessions is used.

Discussion

Pomological variability

In this study, 11 characters related to the fruit register high heterogeneity as reported by Ocampo, Urrea, *et al.* (2013), with a CV higher than 33% in FRW, PSW, JUW, and SHW. However, in this study, TSS showed values between 10 to

21 °Brix with little variation (CV = 11%). The differences between studies can be explained by the origin of the germplasm collected by Ocampo, Urrea *et al.* (2013) which was characterized *in situ* in different producing areas. This germplasm was influenced by edaphoclimatic offer and determined by the genotype x environment interaction.

The high degree of genetic exchange in the species also contributes to the heterogeneity in the quality of the harvested fruits and the progressive genetic degeneration of the cultivated genotypes. However, the variability found in the 52 characterized accessions allows selecting plants with large to small fruits depending on the market's demand for fresh or processed consumption (Tab. 4). The results showed that several accessions with yield higher than 20.0 t ha^{-1} have a PUL% lower than 45%. This suggests that the shell represents more than half the weight of the fruit and is an agronomic parameter not desirable for the yellow passion fruit market (Viera *et al.*, 2020) that should be considered in the selection process.

Precocity and yield

Yield is a function of the production obtained in each harvest and depends largely on the days after the first flower (DAF) and the environmental conditions where the orchards are established (Wallace & Enriquez, 1980; Viera *et al.*, 2020). The precocity of the harvest is beneficial as it brings advantages to geneticists for breeding processes and is excellent in economic terms for producers. In this way, 46% of the accessions have an average of less than 121 DAF; accessions CauFla04 and ValFla13 were particularly more precocious with 86 and 98 d, respectively. This agronomic trait that estimates the precocity in flower production showed that most of the accessions can adapt to the ecological conditions of the area where they were evaluated. However, the yield of most of these precocious accessions was lower than the average (19.3 t ha^{-1}). The correlation between precocity and yield was r = -0.22 (Tab. 6), indicating that these traits are not always related, and in some cases, the effect is inverse. The commercial control accession showed a high precocity with 103 DAF; however, its yield reached only 16.1 t ha^{-1}. Although precocity is a highly heritable trait (Acquaah, 2012), the results shown in this study suggest that it would not be effective in the selection processes in yellow passion fruit. Precocity must complement yield and fruit quality; thus, breeders should pay more attention to these precocious genotypes.

The national annual average yield in Colombia is 17.1 t ha^{-1} (Agronet, 2019), with maximum values between 19.9 and 20.6 t ha^{-1} in the departments of Meta and Antioquia, respectively. In this research, 83% of the evaluated accessions exceed this value and ten exceed 21 t ha^{-1} (ValFla05/06/07/09/11, TolFla06, HuiFla07, CalFla04, Bra_01, and CR_01). The low yield of the commercial control (16.1 t ha^{-1}) questions the crop quality of this material that is currently offered to Colombian growers without any agronomic and scientific validation. The period between the fertilization of the flower and fruit ripening for harvest in yellow passion fruit varies between 50 to 60 d (Arias-Suárez *et al.*, 2014), and if this is considered, the precocious accessions (DAF<121) with good yield (>21 t ha^{-1}) constitute an important promising genetic material since their first harvest could start before six months. The good yield reached by most of the accessions in Valle del Cauca is explained by the selection processes of the best genotypes carried out by growers since the 1950s when passion fruit was introduced to Colombia (Morton, 1967; Ocampo, Urrea, *et al.*, 2013). This department is a reference for the cultivation of yellow passion fruit and many growers from other regions have established new orchards with seed from this area. However, in recent years, phytosanitary problems, climate variability, and the absence of cultivars have affected the crop, preventing its expansion to new areas (Ocampo, Arias, *et al.*, 2013; Galeano Mendoza *et al.*, 2018). The differences in precocity and yield of the accessions were due to the interaction of the genotype x environment that affects the agronomic behavior of the different passion fruit materials that come from different regions in Colombia and other countries (Viera *et al.*, 2020).

Reaction to phytosanitary problems

Plant pests and diseases are a complex problem that generates significant losses in yield and income for farmers. These effects are immediate and impose chemical treatments for the protection of orchards, reducing productivity and the cycle duration and leading to the loss of competitiveness of agricultural systems (Fischer & Rezende, 2008; Faleiro *et al.*, 2019; 2020). Phytosanitary evaluations show that thrips (*Neohydatothrips signifer*), lance fly (*Dasiops inedulis*), scab (*Cladosporium cladosporioides*), viruses (SMV), and anthracnose (*Colletotrichum gloeosporioides*) are the insects and pathogens with the highest incidence (>75%). These phytosanitary problems have been reported throughout the producing areas of the country (Morales *et al.*, 2006; Wyckhuys *et al.*, 2010; Monje *et al.*, 2012), with a higher prevalence in orchards located between 500 and 1,000 m a.s.l. The accessions collected by Ocampo, Urrea *et al.* (2013) and evaluated in this study were also severely affected by these phytosanitary problems. Pest insects are favored (>30%) during summer, while diseases increase (>40) their frequency during rainy periods. Thrips, viruses and collar rot (*Fusarium solani*) are the major limitations for passion fruit production in Colombia, since they cause considerable economic losses (Ocampo, Arias, *et al*., 2013; Osorio Cardona *et al*., 2020). In the evaluated germplasm, several accessions such as ValFla05/12/09, AntFla04 and HuiFla06 responded positively to the pressure of these agents during the 24 phytosanitary evaluations carried out, showing high fruit quality and yield higher than 20 t ha^{-1}. This study shows that accession ValFla09, identified by Ocampo, Urrea *et al.* (2013) as promising, does indeed show good agronomic traits that can respond to the needs of producers.

Selection of elite accessions

The genetic potential in crops determines growth, yield, and quality traits (Acquaah, 2012). However, identifying genotypes with yield potential and good fruit quality traits is a job of extreme precision for geneticists (Moraes *et al*., 2005; Chavarría-Perez *et al*., 2020). Selection indices are a strategy widely used by breeders in various crops for plant selection and the establishment of working collections with the best genotypes (Bos & Caligari, 2008; Lagos *et al*., 2015). The six elite accessions identified in the study with a selection index higher than 0.66 (ValFla05, ValFla09, ValFla12, AntFla04, CunFla02, and HuiFla06) constitute significant progress for future work on the breeding of yellow passion fruit in Colombia. Nevertheless, this germplasm must be evaluated in other regions of the country under a recurrent selection scheme to determine its genetic potential in other agroecological zones. Thus, this strategy will enable the increase in the frequency of favorable alleles that control the quantitative traits, such as total soluble solids (°Brix), pulp weight, and yield. This methodology, combined with SI, has been used successfully in Brazil as an alternative to accumulate intrapopulation genetic gain (Silva *et al*., 2016) and selection of superior progeny through recurrent selection in passion fruit (Silva *et al*., 2009; Rodrigues *et al*., 2020). Recurrent selection has also been used in Brazil to develop the yellow passion fruit cultivar 'UENF Rio Dourado' (Viana *et al*., 2016) with yields of up to 25 t ha^{-1}. In Colombia, using an SI, 30 promising passion fruit accessions were identified by Galeano Mendoza *et al.* (2018) as possible parents for a first cycle of recurrent selection. Another important genetic resource is the elite passion fruit accessions identified by Ocampo, Urrea *et al.* (2013) in Colombia, whose desirable agronomic traits are evidenced in this research. The results confirm the genetic potential of each accession and suggest that productivity does not only depend on agronomic management and environmental factors, as reported in other studies on passion fruit (Araújo *et al*., 2006). This germplasm should also be used to obtain cultivars that respond better to water stress and tolerate saline-sodium soils, which might be key in the near future.

On the other hand, the commercial accessions from Ecuador (Ecu1/2/3) obtained a selection index rate between -0.02 and 0.33, showing yields below 18.5 t ha^{-1} (16.1 to 18.4) and fruit weight <163.5 g, although the average PUL% and TSS were higher than 50% and 14.5 °Brix. These accessions show agronomic characteristics similar to the Colombian commercial control and superior to those reported by Viera *et al.* (2020) in the Ecuadorian Pacific coast, where the orchards do not exceed average yield of 6 t ha^{-1} and 14 °Brix.

Morphological relationships

The use of descriptors in the morphological characterization of germplasm is essential for structuring breeding programs (Bioversity International, 2007). Phenotypic descriptors allow direct access to variability and must be evaluated in the same location due to environmental influence (Bioversity International, 2007). The multivariate analysis grouped the 23 selected quantitative descriptors into six principal components explaining 71.6% of the variance, of which 11 are those that contribute the most to the variability. These descriptors were related to flower length (NCL, FTL, FTD, HYL, and OPL), dimension (FRL and FRD), and fruit fresh weight (FRW, PSW, JUW, and SHW). Ocampo Pérez *et al.* (2009) report similar values, with 77.9% of the variance explained by the same set of descriptors in *P. edulis* f. *flavicarpa*. Castro *et al.* (2012) mention that the descriptors related to the fruit are essential to estimate the genetic variability in yellow passion fruit. At the interspecific level, the descriptors associated with the flower are those that contribute the most to the variability and allow ranking the species of the genus *Passiflora* (Villacis *et al*., 1998; Ocampo Pérez & Coppens d'Eeckenbrugge, 2017). However, Crochemore *et al.* (2003) mention that vegetative descriptors, such as leaf dimensions, contribute to interspecific variability in *Passiflora*. The quantitative descriptors identified in this study are confirmed as a powerful tool to explore variability in yellow passion fruit accessions. The neighbor-joining classification analysis showed five main groups with low geographical structure. This may be a consequence of the inter-cross pollination present in *P. edulis* f. *flavicarpa* since the plants of each accession originate from seeds of half-siblings and constitute different genotypes.

The five groups identified are higher than the groups reported by Ocampo *et al.* (2017) with microsatellite markers, with the same germplasm size and constitution. In the molecular study, these 52 accessions were distributed into three main groups with high heterogeneity among the materials, but with a higher association among the individuals of the same accession. In general, in both morphological and molecular characterizations, the six elite accessions appeared distributed in different groups, as well as the two plants that compose each accession.

Regarding the qualitative descriptors, the presence or absence of pubescence and anthocyanins in the stem, petiole, abaxial surface of the leaf, bract, androgynophore, base of the ovary, and the mature fruit are the most influential descriptors and separate accessions. The accessions in the dendrogram showed a similar distribution to the results of the PCA, indicating that these characteristics are not systematically associated with groups of accessions according to their geographical origin. In general, 53% of the accessions were discriminated by the qualitative descriptors with the highest contribution to variability in the MCFA; some accessions that possess these qualitative characteristics of high heritability showed tolerance to thrips and viruses (VaLFla07/09, TolFla07, CauFla04, AntFla02/06, Ecu_2, and MetFla01). This germplasm should also be considered for future studies of selection of genotypes with tolerance to biotic factors.

The genetic variability estimated in this study is higher than the molecular variability found by Ocampo *et al.* (2017); this high heterogeneity in the fruit variables is the result of cross-pollination between different genotypes in the same orchard. The dispersion of the accessions from Valle del Cauca (ValFla) in all dendrogram groups confirms that this department has been the source of seed dispersal for all the producing areas of the country since the 1950s, when yellow passion fruit was introduced to Colombia (Morton, 1967). The accessions of Ecuador (Ecu_1/2/3) and Brazil (Bra_1) did not show considerable differentiation, which is supported by the recent introductions of Brazilian germplasm in the last decade to Ecuador (Viera *et al.*, 2020; personal observations). However, these new introductions do not show the desirable quality characteristics of the fruit for the concentrated juice export market, which demands fruits with >45% pulp and °Brix>14.0.

Current status and future prospects for breeding

In Colombia, yellow passion fruit was introduced to Valle del Cauca in the early 1950s from Hawaii through Venezuela. For nearly 70 years, producers have contributed to its domestication process with new agronomic practices. Despite this, there are no selected cultivars in Colombia available for the producers that meet the demands of the different fresh and processed consumption markets. Colombian growers, for many generations, have carried out the massal selection of fruits from the best yellow passion fruit plants (Ocampo, Urrea, *et al.*, 2013). However, growers do not distinguish different local varieties based on the morphology of fruit characters due to the high heterogeneity in fruit shape, size, and color within the orchards. This pomological variability is also supported by outcrossing pollination (Arias-Suárez *et al.*, 2014) and by the exchange of seeds between producers in different areas (Ocampo *et al.*, 2017).

Yellow passion fruit breeding programs in Colombia can benefit from the results obtained from the germplasm evaluation and characterization in this study, together with information reported by Ocampo *et al.* (2017) employing molecular markers. These studies establish considerable variability in the cultivated yellow passion fruit materials present in Colombia, structured in four and five clusters by the neighbor-joining analysis. Pre-breeding efforts can improve fruit quality and yield, by emphasizing the elite accessions identified. Elite selections with specific quality characteristics (TSS or PUL%) are of utmost importance for breeders establishing the current genetic structure and variability of cultivated yellow passion fruit for Colombia, in the search for more productive cultivars with resistance to specific phytosanitary problems, such as thrips, collar rot, and viruses that are spread across the country. These accessions must be evaluated in the field in other localities at different selection cycles to establish interesting traits with controlled pollinations to avoid gene flow with unwanted genotypes, through a multivariate analysis. An alternative for a yellow passion fruit breeding program in Colombia is employing the residual or restricted maximum likelihood/best linear unbiased prediction (REML/BLUP) methodology aimed at the selection of progeny or individuals from recurrent selection cycles. This allows accumulating genetic gains in the traits of interest while maximizing heterosis to develop new cultivars (Santos *et al.*, 2015; Viana *et al.*, 2016; Silva *et al.*, 2017).

Advances in phenotype and molecular genetics may overcome some of the limitations of the traditional breeding methods based on sexual recombination by increasing selection efficiency using high-throughput phenotyping (Moreira *et al.*, 2020), molecular markers (genotyping by sequencing – GBS), and transgene technology. In these methods, individual genes, or the recent emergence of genome-editing technologies such as CRISPR/Cas9 (Limera *et al.*, 2017; Fernie & Yan, 2019), have been a significant milestone in crop breeding. Breeding programs could implement all these strategies in Colombia. Grafting with relative species of *P. edulis* f. *flavicarpa* is another technology that must be considered to solve soil-borne diseases such as *Fusarium solani* (Ambrósio *et al*., 2018), one of the leading causes of economic losses in yellow passion fruit crops in Colombia (Osorio Cardona *et al*., 2020).

The selection of genotypes with abiotic stress tolerance used as rootstock could also be an effective strategy to overcome/mitigate constraints for the adequate development of yellow passion fruit orchards (Hurtado-Salazar *et al*., 2017; 2020). Additionally, all elite accessions identified in this study must be included to enrich the primary gene pool of the cultivated germplasm in Colombia as an *ex situ* conservation strategy in gene banks and to improve the genetic resources of this species for future research studies (Ocampo Pérez *et al*., 2021).

Conclusions

The agro-morphological characterization of 52 yellow passion fruit accessions was highly informative, showing important levels of variability in 11 qualitative and 19 quantitative descriptors. The genetic variability was structured in five main groups, with low genetic differentiation among different geographic origins of the accessions due to several factors, such as outcross pollination and seed exchange between growers in different regions. The results of the agronomic evaluation identified six elite accessions (ValFla05, ValFla09, ValFla12, AntFla04, CunFla02, and HuiFla06) that constitute a genetic reservoir for establishing a working collection for future selection and recombination cycles, with well-differentiated and agronomically outstanding parents for passion fruit breeding programs with a commercial focus in the country.

Author's contributions

JO and RU designed the experiments and conducted the research; VM and JO carried out the field experiment; JO performed the data analysis, and JO, RU and VM wrote the manuscript.

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Conflict of interest statement

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Agronomic and physiological characteristics of soybean cultivars and their seeds according to plant population

Características agronómicas y fisiológicas de los cultivares de soya y sus semillas, según población vegetal

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ABSTRACT

Soybean (*Glycine max*) yield can be influenced by the choice of genetic material along with the use of an adequate plant population and selection of high-quality seeds. This study aimed to evaluate agronomic and physiological characteristics of soybean cultivars and their seeds according to different plant populations. For this purpose, an experiment was conducted during the 2017/2018 harvest, under field conditions, in a randomized block design in a 3 x 4 factorial arrangement with four replicates. The treatments consisted of three soybean cultivars, TMG2181 IPRO, M 7739 IPRO, and BMX Power IPRO, at four population densities: 180,000; 220,000; 280,000 and 320,000 plants ha^{-1}. The agronomic characteristics of the plants (plant height, stem diameter, number of branches, number of pods per plant, number of seeds per plant, number of seeds per pod, weight of a 100 seeds and seed yield) and physiological quality (germination, first count of germination, seedling length, dry mass of the shoot and root, and accelerated aging test) of these seeds were evaluated. The experiment showed that the agronomic characteristics of soybean cultivars and their seeds varied according to the genotypes, and that these phenotypic expressions can be altered according to the plant population. The indeterminate cultivar BMX Power IPRO obtained higher seed yield compared to the semi-determinate and determinate cultivars M 7739 IPRO and TMG2181 IPRO, mainly due to their field conformation and size. The indeterminate cultivar BMX Power IPRO showed higher average germination and seed hardiness and produced normal seedlings with good initial growth rate and dry weight. The yield and quality of soybean seeds were not affected by plant population.

Key words: *Glycine max,* plant arrangement, seed yield, germination and vigor.

RESUMEN

El rendimiento de la soya (*Glycine max*) puede verse influenciado por la elección del material genético, junto con el uso de una población de plantas adecuada y la selección de semillas de alta calidad. El objetivo de este estudio fue evaluar las características agronómicas y fisiológicas de cultivares de soya y sus semillas, según diferentes poblaciones de plantas. Para ello, se realizó un experimento durante la cosecha 2017/2018, bajo condiciones de campo, en un diseño de bloques al azar en un arreglo factorial 3 × 4 con cuatro repeticiones. Los tratamientos consistieron en tres cultivares de soya, TMG2181 IPRO, M 7739 IPRO y BMX Power IPRO, en cuatro densidades de población: 180,000; 220,000; 280,000 y 320,000 plantas ha^{-1}. Se evaluaron las características agronómicas de las plantas (altura de la planta, diámetro del tallo, número de ramas, número de vainas por planta, número de semillas por planta, número de semillas por vaina, peso de 100 semillas y rendimiento de semilla) y la calidad fisiológica (germinación, primer conteo de germinación, longitud de la plántula, masa vegetal seca de la parte aérea y raíz y prueba de envejecimiento acelerado) de estas semillas. El experimento mostró que las características agronómicas de los cultivares de soya y sus semillas variaron de acuerdo con los genotipos y que estas expresiones fenotípicas pueden alterarse según la población de plantas. El cultivar indeterminado BMX Power IPRO obtuvo un mayor rendimiento de semilla en comparación con los cultivares semideterminados y determinados M 7739 IPRO y TMG2181 IPRO, principalmente debido a la conformación y tamaño de las plantas en el campo. El cultivar indeterminado BMX Power IPRO mostró mayor promedio de germinación y dureza de la semilla, y produjo plántulas normales con buena tasa de crecimiento inicial y peso seco. El rendimiento y la calidad de las semillas de soya no se vieron afectados por la población de plantas.

Palabras clave: *Glycine max*, arreglo de plantas, rendimiento de semilla, germinación y vigor.

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Introduction

Soybean is the oilseed most cultivated worldwide, and the USA and Brazil are the greatest producers. Soybean currently occupies the largest planted area of legumes in the world, with Brazil producing 135.9 million of grain in 2020/2021, close to 36% of world production (Conab, 2021). Oil and bran are the main products from soy processing. Additionally, soybean oil can be used in the production of biodiesel (Shehata *et al*., 2015; Colombo *et al*., 2019).

The need for superior quality seeds is one of the obstacles to overcome in the search for higher soybean yields, especially in countries with tropical climate such as Brazil, where seeds are produced during the season of cultivation destined to obtain grains. In Brazil, seeds are produced during the spring-summer season, when the harvest usually coincides with the rainfall period (Ávila *et al*., 2011). This is due to the prohibition of soybean cultivation in winter to control the Asian rust (*Phakopsora pachyrhizi*) (Godoy *et al*., 2016). However, the winter season is ideal to produce higher quality seed lots, given the absence of rains during the harvest.

Seed productivity, like other agronomic characteristics such as components and yield, varies as a result of genotype selection, soil management, reduced interspecific competition between plants and the interaction of these factors (Baron *et al*., 2018). However, the soybean plant can adapt to its exposed arrangement, due to the morphophysiological modifications known as plant plasticity (Balbinot Junior *et al*., 2018; Ferreira *et al*., 2020). These modifications include the ability to change the main components (number of pods per plant, number of grains per pod, and seed weight) and grain yield according to the number of plants per area, thus, maintaining constant productivity over a wide range of plant densities (Board & Kahlon, 2013; De Luca & Hungría, 2014; Suhre *et al*., 2014; Petter *et al*., 2016; Souza *et al*., 2016).

In recent years, most of the soybean cultivars released in the market are indeterminate (modern cultivars), with less branching (Werner *et al*., 2016), that allow the use of lower densities without negatively impacting soybean seed yield (Corassa *et al*., 2018; Carciochi *et al*., 2019). Carpenter and Board (1997) indicate that the response of seed yield to plant density is defined by a balance between the reduced yield per plant and increased yield per unit area, due to the effect of plant addition.

Cultivars of soybean with determinate growth (e.g., NS 8590 IPRO, BRS 7780IPRO, and TMG2181 IPRO) are characterized by the completion of around 90% of vegetative growth, starting from the sprouting onset (reproductive phase) (Perini *et al*., 2012). The size of the vegetative growth of plants of indeterminate growth (e.g., NS6906 IPRO, NA 7337 RR, and BMX Power IPRO) increases from two to four times after the sprouting onset (Baigorri & Gassen, 2009). These materials feature a longer period of overlap of vegetative and reproductive phases, growth in height and emission of the first or the beginning of the flora in comparison with determined cultivars (Zanon *et al*., 2016). Plants of semi-determinate growth (e.g., NA 7337 RR, NS 6209 RR, and M 7739 IPRO), reach 70% of their final height when they sprout (Nogueira *et al*., 2014).

Research studies on the trend of soybean cultivars in response to the different sowing densities show contradictory results; some cases confirm the interaction between soybean genetic materials and sowing density, and others do not (Soares *et al*., 2015). These studies demonstrate that a plant population of 300,000 plants ha^{-1} is the most suitable for soybean yield (Balbinot Junior *et al*., 2015; Enciso-Maldonado *et al*., 2021). However, Ribeiro *et al*. (2017) observed that, regardless of the soybean cultivar, a plant density of up to 600,000 plants ha^{-1} did not affect grain yield, plant height, lodging, harvest index or number of grains per pod. Similar results were obtained by Prusiński and Nowicki (2020), who did not observe the influence of the density and the space between lines on soybean yield.

In addition, Rahman *et al*. (2011) found that the yield and accumulation of protein and nutrients in soybean seeds decrease as the density of seedlings increased. Thus, there is a need for research to fill in these gaps and to generate information on the choice of suitable plant populations per area for the genetic materials of soybean currently available to farmers.

This study aimed to evaluate the agronomic characteristics (plant height, stem diameter, number of branches, number of pods per plant, number of seeds per plant, number of seeds per pod, weight of 100 seeds and seed yield) and physiological quality (germination, first count of germination, seedling length, dry mass of the shoot and root, and accelerated aging test) of soybean seeds of cultivars TMG2181 IPRO, M 7739 IPRO, and BMX Power IPRO, considering different plant populations.

Materials and methods

The experiment was carried out during the 2017/2018 agricultural harvest at the experimental facility of Agência

Goiana de Assistência Técnica, Extensão Rural e Pesquisa Agropecuária (EMATER), Anápolis-GO, Brazil, at an average altitude of 1050 m a.s.l. Soil samples, classified as Red-Yellow Latosol (Embrapa, 2013), were collected at the crop location, from the 0-20 cm layer, and sent to the laboratory of the experimental facility for physico-chemical analysis (Tab. 1).

TABLE 1. Results of the physico-chemical analysis of samples (0-20 cm deep) of the soils used in the experiment.

Attributes	Values
pH ($CaCl_2$)	5.3
P (mg dm^{-3})	14.5
K ($cmol_c$ dm^{-3})	0.21
Ca ($cmol_c$ dm^{-3})	2.3
Mg ($cmol_c$ dm^{-3})	0.7
H + Al ($cmol_c$ dm^{-3})	2.0
V (%)	60.0
Organic matter (g dm^{-3})	33.0
Sand (g kg^{-1})	460.0
Silt (g kg^{-1})	110.0
Clay (g kg^{-1})	430.0

The prevailing weather conditions at the time of the experiment are presented in Figure 1.

The experiment was conducted in a completely randomized block design, 3×4 factorial arrangement, with four replicates. The treatments consisted of three soybean cultivars, TMG2181 IPRO, M 7739 IPRO, and BMX Power IPRO, at four plant densities, 9, 11, 14, and 16 plants m^{-1}, corresponding to the following population densities: 180,000, 220,000, 280,000 and 320,000 plants ha^{-1}.

The cultivar TMG2181 IPRO belongs to the maturation group 8.1, has determinate growth and high demand for soil fertility. M 7739 IPRO belongs to the maturation group 7.7, has semi-determinate growth and high demand for soil fertility. BMX Power IPRO belongs to the maturation group 8.0, has indeterminate growth and high demand for soil fertility.

The experiment plots consisted of four rows of 5.0 m length, spaced at 0.50 m. The useful area of the plot included two central rows, with a suppression of 0.50 m from their edges, resulting in 4 m² of total useful area.

The soil was prepared according to the standard practices by plowing (disk plow) and leveling grade, with the application of only regular fertilization for the seeding, using a 05-25-20 compound fertilizer, made up by nitrogen, phosphorus, and potassium (400 kg ha^{-1}). The seeds of the three cultivars were sown manually on 12/01/2017, with the help of laces made of satin previously labeled according to the spacing between seeds. Twenty-five percent more seeds than recommended for each density tested were used. At 10 d after the emergence of seedlings, the plants were pruned to reach the desired population densities.

Manual harvest of the useful area began on 04/01/2018, when the plants reached stage R8, with 95% of the pods in brown color, which indicated ripeness. At first, 10 plants were collected in the useful area of each plot for evaluation of the following characteristics: plant height (PH) - measured in cm between the stalk of the plant and the apical edge of the main stem of the plant, using a measuring tape; stem diameter (SD) - diameter of the stalk, using a digital caliper with an accuracy of 0.01 mm, about 2 cm above the

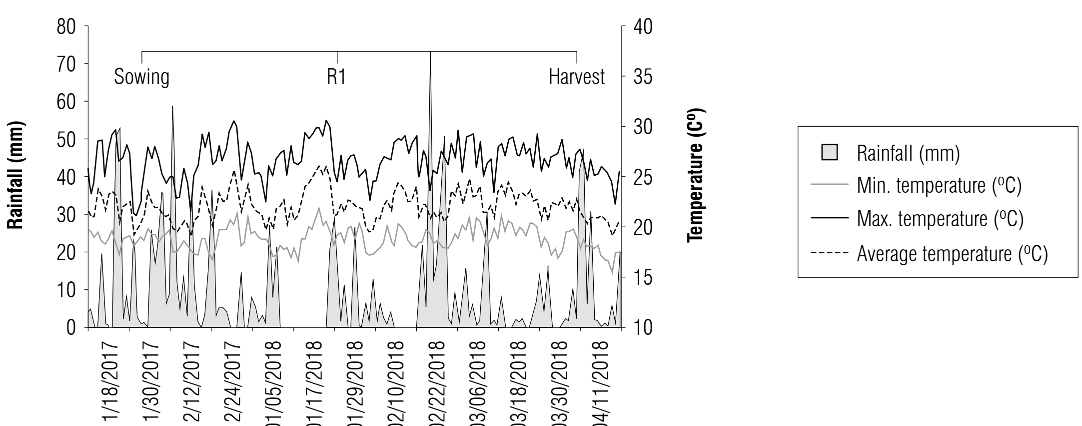

FIGURE 1. Daily climate data: precipitation (mm), maximum temperature (°C), minimum temperature (°C) and average temperature (°C) occurred during the period of the harvest from November 2017 to April 2018, in Anápolis-GO, Brazil. Data provided by the network of stations of SECTEC/SIMEHGO (Secretary of State for Science and Technology - Goiás), Brazil.

soil; number of branches per plant (NBP) - counting the average number of branches in the plant; number of pods per plant (NPP) - calculating the overall mean of pods in the plants; number of seeds per plant (NSP) - counting the seeds formed in the plants; number of seeds per pod (NSPP) - calculating the ratio between the number of seeds per plant and the number of pods per plant; weight of 100 seeds (W100S) - using random samples of 100 seeds. In addition, the seed yield (SY) was computed through an evaluation of the mass of the seeds originated from all the plants collected in the useful area of the plot, weighed using a precision scale of 0.001 g (model TP 200S, OHAUS Corporation, USA), expressed in kg ha^{-1} and corrected to 12% of moisture content (wet base).

With the results of the agronomic characteristics and yield established, the raw batch of seeds obtained in each plot was used to evaluate their physiological quality by performing the following analyses: germination (GER) - carried out with 4 replicates of 50 seeds for each plot and the counting of normal seedlings conducted in the eighth day after the seeding (MAPA - ACS, 2009); first count of germination (FCG) - carried out together with the germination test, by calculating the percentage of normal seedlings in the samples on the fifth day (MAPA - ACS, 2009); seedling length (SL) - using four replicates of 10 seeds for each plot, measuring the total length of the normal seedlings after 8 d (primary root and hypocotyl) (Nakagawa, 1999); dry mass of the shoot and root (DMS and DMR) - in this evaluation, normal seedlings that came from the seedling length test were used, divided into hypocotyl and root system, with the average results expressed in dry mass of the root and dry mass of the hypocotyl in mg per sowing (Nakagawa, 1999); and the accelerated aging test (AA) - with 200 seeds in each plot distributed in translucent plastic boxes (gerbox) (Marcos Filho, 1999).

The obtained data were subjected to analysis of variance by the F test ($P<0.05$), and when significant, the averages of the qualitative treatments (cultivar) were compared by the Tukey's test ($P<0.05$); the regression analysis was applied for the quantitative treatments (densities). The SISVAR software system 5.6 was used for statistical analysis.

Results and discussion

Agronomic characteristics and seed yield

The experiment was carried out from November 2017 to April 2018, the rainy season in the Midwest region of Brazil (Fig. 1), with no water restriction during the entire cycle of the soybean cultivars under study. Regarding plant nutrition, fertilization was carried out in line with the soil analysis (Tab. 1). Therefore, no symptoms of nutrient deficiency or toxicity were observed in the three studied soybean cultivars.

The agronomic characteristics of soybean, such as PH, SD and NBP, were affected by both studied factors, but in an isolated manner. The key components of W100S and NSP were affected by the interaction of the factors soybean cultivars and plant population (C×P), whereas the NPP and the SY were affected only by the factor of the tested cultivars (C). NSPP, on the other hand, was not significant at 5% and 1% of probability in any studied factor.

In agreement with the results of the analysis of variance, the literature reports that the population of the plant factor (seeding density) (P) is a relevant variable in the study of plant arrangement and may be considered the main factor for the morphophysiological characteristics of plants (Suhre *et al*., 2014; Ferreira *et al*., 2016).

For PH, the determinate cultivar TMG2181 IPRO showed greater height average when compared to the other studied cultivars (Tab. 2). Most Brazilian commercial cultivars are around 50 cm to 90 cm in height (Sediyama *et al*., 2016), and determinate plants are higher for soybean cultivation (Sediyama, 2009). For SD, the determinate cultivar TMG 2181 IPRO showed a greater value when compared to the other cultivars, reaching 9.90 mm. SD is an important variable of research that may even characterize the genotypes as favorable or not for bedding.

TABLE 2. Average values of plant height (PH), stem diameter (SD), number of branches per plant (NBP) and number of pods per plant (NPP) for each soybean cultivar.

Cultivars	PH	SD	NBP	NPP
TMG2181 IPRO	77 a	9.90 a	10 a	132 a
M 7739 IPRO	69 b	7.71 b	9 a	90 b
BMX Power IPRO	70 b	7.19 b	5 b	87 b
Averages	72	8.27	8	103

Averages followed by lower-case letters in the same column show no differences among them according to the Tukey's test ($P\leq0.05$ and 0.01).

The average data of NBP differed among the genetic materials studied, given that the determinate cultivar TMG 2181 IPRO produced more branches per plant, but without differences when compared to the semi-determinate M 7739 IPRO. Recent studies (Souza *et al.*, 2016) support the findings of this research that determinate soybean cultivars produce a greater mean of branches per plant since cultivars TMG 2181 IPRO and M 7739 IPRO produced equally a greater number of branches per plant.

The component NPP exhibited a trend behavior similar to that described for PH and SD with the determinate cultivar showing a greater mean among the three genetic materials studied. However, NPP was not significantly affected by the spatial arrangement (at 5% and 1% of probability), although a reduced average of the number of pods produced per plant was observed as the concentration of plants increased. For PH versus different plant populations, regardless of the cultivar, taller plants were observed in arrangements with increased plant population (Fig. 2).

Increased plant population favors the growth of the apical height of the plant, regardless of the managed cultivar (Ferreira *et al.*, 2016). Souza *et al.* (2016) stated that soybean cultivars BRS Valiosa RR (determinate), NA 7337 RR (semi-determinate) and BMX Potência RR (indeterminate), when exposed to populations that ranged from 245,000 to 455,000, showed average height from 91.5 cm to 94.9 cm. They reported that increased density increments the final height of the plant, due to intraspecific competition for light.

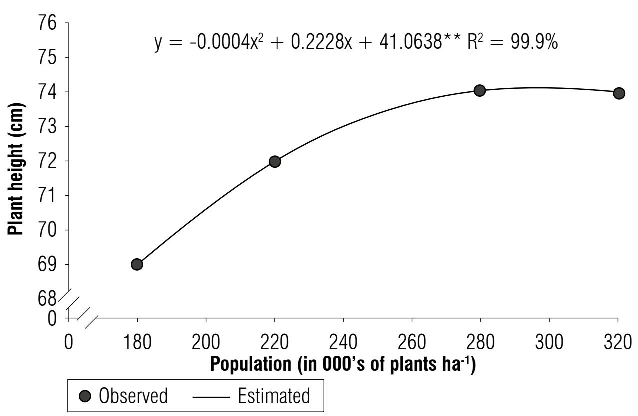

FIGURE 2. Height of soybean plants (cm) according to soybean plant populations (in thousand plants ha^{-1}).

SD was affected by plant population, and a linear decrease in the average values was observed as plant population increased, with values that ranged from 8.82 mm in a population of 180,000 to 7.84 mm in a population of 320,000 plants ha^{-1} (data not shown). A distinct trend was observed for PH, in which the diameter values decreased in a linear manner, while plant height increased.

The average NBP in the three studied cultivars was also affected by the plant population per area, which led to the adjustment of the decreasing linear function of the data in response to the increased plant population (Fig. 3). Heiffig *et al.* (2006) exposed cultivar MG/BR 46 Conquista to populations ranging from 70,000 to 350,000 plants ha^{-1} (1.4 to 7.0 plants m^{-1}) and found a decrease from 3.7 to 1.0 branches per plant. Souza *et al.* (2016) also reports a similar trend of genotypes in their research. This occurs because, in a low-density context, the availability of water, light and nutrients for each subject is higher (De Luca & Hungría, 2014).

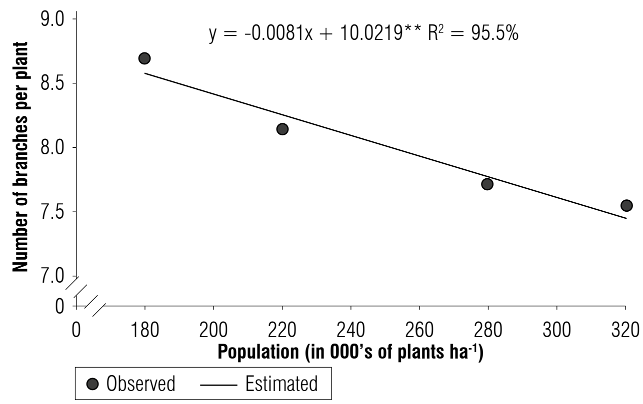

FIGURE 3. Number of branches per plant according to soybean plant populations (in thousand plants ha^{-1}).

The phenotypic trend displayed by the genetic materials in this study was significant and a decreased number of branches per plant was easily observed according to the concentration of plants ha^{-1}, mainly when the cultivar BMX Power IPRO was used (Fig. 4).

FIGURE 4. Plant structure of the indeterminate soybean cultivar BMX Power IPRO according to the populations of plants: A) 180, B) 220, C) 280 and D) 320 thousand plants ha^{-1}.

The effect of the interaction of factors (C×P) on NSP was observed, and the variation of the average of the three cultivars in each studied population was reported. In all populations, cultivar TMG2181 IPRO always stood out from the others, and the overall mean in this category always pointed out to the same result. However, Ribeiro *et al.* (2017) demonstrated that the difference of this factor can be found in soybean cultivars with the same growth habit.

The average data on the NSP of the three genetic materials exposed to the different plant populations revealed the plasticity of each studied genotype, which managed to adapt to the spatial arrangements (Fig. 5). The concentration of the seeding line reduced the spacing between plants, thus decreasing NBP (Fig. 3) and, consequently, the number of seeds per plant.

This trend is observed more frequently in plants of semi-determinate and indeterminate growth, with a linear correlation to the concentration of plants. In other words, when the quantity of plants increases (population of plants ha^{-1}), the quantity of generated seeds decreases. The determinate cultivar TMG2181 IPRO did not show significance in this variable, which demonstrates that the plant population factor did not change its morphophysiological characteristics.

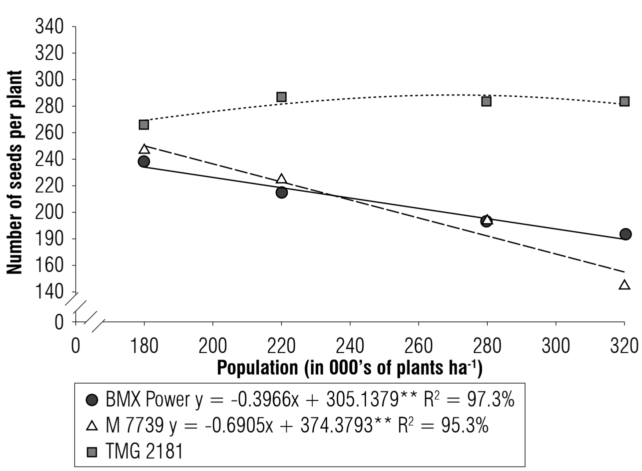

FIGURE 5. Number of seeds per plant of soybean cultivars according to plant populations (in thousand plants ha^{-1}).

Balbinot Junior *et al.* (2018) report that genotypes of indeterminate habits exhibit the highest plasticity rate in response to their exposed plant population. For example, Ribeiro *et al.* (2017), while working with populations of 300,000 to 600,000 plants ha^{-1}, observed a reduction from 114 to 77 grains per plant of indeterminate cultivars. The plant alters its structure in search of a better adjustment to the exposed environment and is able to increase its competition for solar radiation, nutrients, water, and soil biosphere (Ferreira *et al.*, 2016; Petter *et al.*, 2016).

In this study, the NSP showed a trend opposite to that of W100S. As NSP increases, the mass of the product decreases. Among the overall averages of the genetic materials researched, the determinate cultivar TMG2181 IPRO obtained greater average of branches, pods (Tab. 2) and seeds per plant, when compared to the other studied genotypes. However, it obtained the lowest W100S by a large difference. Gulluoglu *et al.* (2017), while working with two soybean cultivars, verified that the plants that produced greater quantities of seeds were the same in which the weights of seeds were lower.

The indeterminate cultivar BMX Power IPRO showed an increase in W100S in plant populations of 280,000 plants ha^{-1} (Fig. 6), followed by the decrease observed in larger studied populations. Seed weight is relevant for its ability to generate more vigorous seedlings during the germination process. This is explained in the physiological quality of seeds section.

These findings are partly consistent with those obtained by Suhre *et al.* (2014), who determined that the increased density of plants in the line promotes a linear increase of W100S, especially in new cultivars, or as characterized by Balbinot Junior *et al.* (2018), in modern cultivars (indeterminate). The component W100S is dependent on the genealogy of the material, which justifies the detection of only the effect of the cultivar factor.

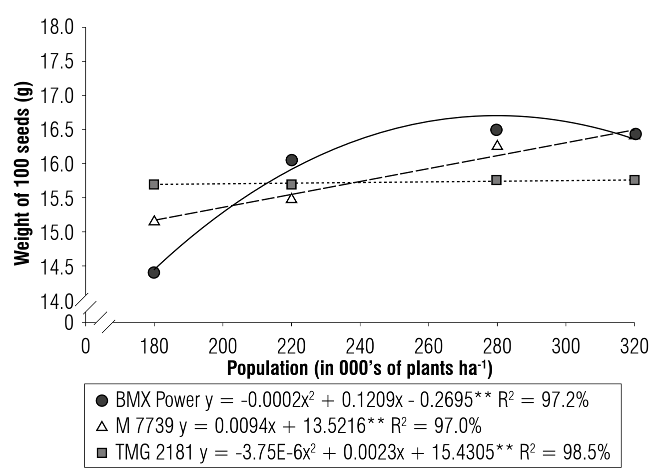

FIGURE 6. Weight of 100 seeds of soybean cultivars according to plant populations (in thousand plants ha^{-1}).

Ferreira *et al.* (2016) and Ferreira *et al.* (2020) verified an increased percentage of grains per plant in lower seeding densities and, consequently, a reduced mass of these grains. These findings are consistent with those of Bellaloui *et al.* (2015), who demonstrated decreased levels of protein, sucrose, glucose, raffinose, boron and phosphorus in grains with decreases in seeding density. Petter *et al.* (2016) report that the changes in the weight of seeds are due to the harnessing of photosynthetic radiation.

Seed yield was greatly affected by just the soybean cultivar factor (C) (Tab. 3). These findings are similar to those from the literature, such as Heiffig *et al.* (2006), who used five populations of plants (70, 140, 210, 280 and 350 thousand plants ha^{-1}) and observed no effect of this factor on the final production of seeds of the cultivar MG/BR 46 Conquista. The lack of an increase response in soybean seed yield with plant population can be usually attributed to the plasticity of plants, thus, corroborating the reports by Balbinot Junior *et al.* (2018) and Ferreira *et al.* (2020).

TABLE 3. Average values of seed yield according to the soybean cultivars.

Cultivars	Seed yield (kg ha^{-1})
TMG2181 IPRO	3316.15 c
M 7739 IPRO	4573.04 b
BMX Power IPRO	5351.03 a
Averages	4413.40

Averages followed by the same lower-case letters in the same column have no difference among them according to the Tukey's test ($P \leq 0.05$ and 0.01).

The three cultivars are characterized as highly producing cultivars based on the final yield, with the indeterminate cultivar BMX Power IPRO standing out from the other two with the highest average (89 bags ha^{-1}), followed by the semi-determinate M 7739 IPRO (76 bags ha^{-1}) and the determinate TMG2181 IPRO (55 bags ha^{-1}).

While working with the three soybean cultivars and three plant populations under the conditions of the Midwest region of Brazil, more specifically in Anápolis-GO, Souza *et al.* (2016) also obtained significance ($P \leq 0.05$) only for the soybean cultivar factor of different growth habits, given that the indeterminate and semi-determinate cultivars demonstrated greater production average than the determinate, with averages of 4,441.95 kg ha^{-1} (74 bags ha^{-1}), 4,529.54 kg ha^{-1} (75 bags ha^{-1}) and 4,111.76 kg ha^{-1} (68 bags ha^{-1}), respectively. These yield values are close to those obtained in this research.

Additionally, the yield levels obtained for the three soybean cultivars studied are considered high worldwide. Moreover, Brazilian soybeans have a lower production cost, compared to North American soybeans, especially in relation to fertilization with nitrogen, which is made via biological N fixation. Also, the adaptation of genetic materials to cultivation under low latitude conditions, especially in the Midwest region of Brazil, associated with the adoption of more efficient management techniques including the definition of an adequate population of plants per area, contributes to lower production costs.

Physiological quality of seeds

The GER and vigor analyses of soybean seeds originated from distinct genetic materials, at different plant populations, were also carried out. The findings demonstrated influence of the soybean cultivars factor (C) only. Only the result of the vigor analysis of the DMR did not show significant differences in the physiological quality of the seed, according to the treatments applied.

Vazquez *et al.* (2008) used two soybean cultivars (BRSMG 68 Vencedora and M-SOY 8001) and found no link between the physiological quality of seeds and the different plant populations (400, 340, 280, 220, and 160 thousand plants ha^{-1}) either. Ferreira *et al.* (2017) also reached the same conclusions. Thus, the results obtained indicate that the increased plant population does not guarantee an improved quality standard of the soybean seeds produced.

The indeterminate cultivar BMX Power IPRO produced seeds with a higher percentage of normal seedlings (93%), followed by the semi-determinate M 7739 IPRO (81%) and the determinate TMG 2181 IPRO (80%) (Tab. 4). These differences between seed lots produced can be attributed to the occurrence of different genotypes among the studied cultivars. These percentages of germination verified by the three studied soybean genetic materials are superior to those required for the trading of soybean seeds in Brazil, above 80-85% (MAPA - ACS, 2009).

The seed vigor data (Tab. 4) showed that the average obtained had a similar trend to that described in the germination test, in which the indeterminate cultivar BMX Power IPRO stood out from the others in all the evaluations. Then, it can be concluded that this cultivar has the potential to produce seeds with a homogenous and faster germination, with more vigorous seedlings.

The vigor of seedlings was inferior to the FCG, due to the initial stressful condition, with high temperature and relative humidity. However, these results are consistent with the methodology applied in the aforementioned analysis.

TABLE 4. Average values of germination (GER), first count of germination (FCG), seedling length (SL), dry mass of the shoot (DMS) and accelerated aging test results (AA) of soybean cultivars.

Cultivars	Germination and vigor				
	GER (%)	FCG (%)	SL (cm)	DMS (mg)	AA (%)
TMG2181 IPRO	80 b	60 b	22.4 b	16 b	50 b
M 7739 IPRO	81 b	62 b	24.4 b	18 b	51 b
BMX Power IPRO	93 a	86 a	27.4 a	25 a	63 a
Averages	84.6	69.3	24.7	19.6	54.3

Averages followed by the same lower-case letters in the same column have no difference among them according to the Tukey's test ($P \leq 0.05$ and 0.01).

The use of seeds of high quality is justified because it helps to adjust the expected population of plants, which is affected by the conditions of the soil and weather, especially in less favorable situations; this did not occur in this study. It is noteworthy that plants originating from seeds with high vigor can produce up to 35% higher grain yield (Cantarelli *et al*., 2015). That is why it is important to use top quality soybean seed lots. Finally, the use of an adequate plant population per area or even smaller populations generally provides better quality soybean seeds, mainly because it provides unfavorable conditions for the emergence of pathogens.

Conclusions

The phenotypic characteristics plant height, number of branches per plant, number of seeds per plant, weight of 100 seeds and grain yield of cultivars TMG2181 IPRO, M 7739 IPRO and BMX Power IPRO were altered according to the population of plants applied. The indeterminate cultivar BMX Power IPRO showed higher yield, vigor, and seed germination percentage. The different populations of plants do not influence seed quality.

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

EEF and IRT formulated the overarching research goals and aims. EEFF and GCS carried out activities to annotate scrub data and to maintain research data for initial and later use. EEF and MEVA applied statistical, mathematical, computational, and other formal techniques to analyze or synthesize study data. IRT obtained the financial support for the project leading to this publication. EEF and GCS conducted the research and investigation process, specifically performing the experiments or data/evidence collection. IRT developed and designed the methodology; created the models. IRT managed and coordinated the research activity planning and execution. IRT, AGS and PCC provided the study materials, reagents, laboratory samples, instrumentation, computing resources, and other analysis tools. EEF, GCS and MEVA implemented the computer code and supporting algorithms/software. GCS, AGS and IRT oversaw and led the research activity planning and execution. IRT and PCC verified the overall replication/reproducibility of results/experiments and other research outputs. EEF and IRT prepared, created, and/or presented the published work and oversaw its visualization/data presentation. EEF, IRT and PCC wrote/translated the initial draft.

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Interaction of triacontanol with other plant growth regulators on morphology and yield of field pea (*Pisum sativum* L.)

Interacción del triacontanol con otros reguladores de crecimiento vegetal en la morfología y el rendimiento de arveja (*Pisum sativum* L.)

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ABSTRACT

The effect of a recent plant growth regulator, triacontanol (TRIA), on plant growth and yield of *Pisum sativum* L. was investigated. The experiment was carried out under field conditions at the Instituto Nacional de Innovación Agraria (INIA), La Molina, Lima, Peru, using a completely randomized block design with eight treatments and three replicates. Treatments consisted in the foliar application of TRIA alone and in all possible combinations with three plant growth regulators based on auxins (AUX), gibberellins (GA), and cytokinins (CK), on pea plants cv. Rondo. The highest green pod yields were obtained with the application of TRIA+AUX+GA+CK, and TRIA+AUX+CK. The TRIA+AUX increased the values of the yield variables while TRIA+GA increased the values of the morphological variables. TRIA+CK showed a stimulating effect on morphological variables and number of grains per pod, while TRIA+AUX+CK acted synergistically on yield variables since their combined effect overweighed the effect of each growth regulator separately. Treatments with TRIA exceeded the control treatment in yield variables, indicating its great potential to be used in sustainable agriculture to guarantee food security in the future.

Key words: auxins, cytokinins, gibberellins, plant physiology, legumes.

RESUMEN

Se investigó el efecto de un regulador del crecimiento vegetal relativamente nuevo, el triacontanol (TRIA), sobre el crecimiento de las plantas y el rendimiento de *Pisum sativum* L. El experimento se llevó a cabo en condiciones de campo en el Instituto Nacional de Innovación Agraria (INIA), Lima, Perú, utilizando un diseño de bloques completamente al azar con ocho tratamientos y tres repeticiones. Los tratamientos consistieron en la aplicación foliar de TRIA solo y en todas las combinaciones posibles con tres reguladores del crecimiento vegetal a base de auxinas (AUX), giberelinas (GA) y citoquininas (CK), en plantas de arveja cv. Rondo. Los mayores rendimientos de vaina verde se obtuvieron con la aplicación de TRIA+AUX+GA+CK y TRIA+AUX+CK. TRIA+AUX incrementó los valores de las variables de rendimiento mientras que TRIA+GA incrementó los valores de las variables morfológicas. TRIA+CK mostró un efecto estimulante sobre las variables morfológicas y el número de granos por vaina, mientras que TRIA+AUX+CK actuó sinérgicamente en las variables de rendimiento, ya que su efecto combinado sobrepesó el efecto de cada regulador de crecimiento por separado. Los tratamientos con TRIA superaron al tratamiento control en las variables de rendimiento, lo que indica su gran potencial de uso en la agricultura sostenible para garantizar la seguridad alimentaria en el futuro.

Palabras clave: auxinas, citoquininas, giberelinas, fisiología vegetal, leguminosas.

Introduction

The field pea (*Pisum sativum* L.) belongs to the Fabaceae family and is one of the oldest domesticated species for human and livestock consumption. It is widely cultivated around the world (Wu *et al*., 2019) and predominant in world trade, representing about 35-40% of the total trade in legumes. It is also among the most consumed vegetables worldwide (Ratnayake *et al*., 2001). The pea is an important nutritional crop (Liu *et al*., 2015) since its grains have high contents of protein (18-30%), vitamins and minerals. Additionally, its shoots and leaves can be used for fresh consumption as leafy vegetables (Santos *et al*., 2014), with a good demand in the national and international markets. Field pea is a profitable agricultural product. However, this profitability is diminished in many regions by various factors such as environmental problems, incorrect agronomic management, and increase in production costs due to the rise in the application of fertilizers and pesticides (Erisman, 2011). Agronomic management is especially critical because long-duration varieties with low yields are still sown in production areas and are susceptible to different diseases and pests (Wu *et al*., 2019). For this crop, correct physiological and nutritional management is imperative,

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with biostimulants and plant hormones playing an important role (Bertolin *et al*., 2010; Cato *et al*., 2013; Martínez González *et al*., 2017).

Plant growth regulators are small molecules that can trigger different physiological processes related to the growth, development, and defense of plants (Pozo *et al*., 2015). They are also known as phytohormones, but this term is not used frequently in agriculture (Davies, 2010). Plant hormones, growth regulators, and inhibitors have been used in practice to increase yield, improve quality, or alleviate the adverse effects induced by biotic or abiotic stresses (Csukasi *et al*., 2009). Different classes of hormones have already been characterized, including abscisic acid, auxins, brassinosteroids, cytokinins, ethylene, gibberellins, jasmonates, strigolactones, etc. (Depuydt & Hardtke, 2011). All of them have been linked in one way or another to growth regulation (Santner *et al*., 2009; Wolters & Jürgens, 2009). Cytokinins, auxins, gibberellins, and brassinosteroids are considered essential for growth of mutant phenotypes in which hormone biosynthesis or perception is disrupted; cytokinins regulate cell proliferation, while gibberellins promote cell elongation and auxins are involved in both processes. Furthermore, brassinosteroids are essential for cell elongation, but may also play a role in cell division (Nakaya *et al*., 2002; Hardtke *et al*., 2007). All these hormones can regulate a high number of processes in a unique and independent way. However, cooperation and interrelationship between signaling metabolic pathways appears to exist, as it follows from the superimposed influence on various cellular processes (Hardtke *et al*., 2007).

New hormones and plant growth regulators are still being discovered, and their interrelationships, mechanisms of action, and relationships at the metabolic level are being exhaustively studied (Gomez-Roldan *et al*., 2008; Kuppusamy *et al*., 2009). Among these substances is triacontanol (TRIA), a growth regulator recently used in commercial applications despite the fact that it was discovered a few decades ago in natural waxes. It can exert stimulating effects, even at considerably low foliar concentrations (Khandaker *et al*., 2013). TRIA is not considered a plant hormone since it is a secondary substance in plant growth (Naeem *et al*., 2012). However, various studies show that its effects on growth and yield in plants are not shown by other plant hormones or growth regulators. The positive role of TRIA in photosynthesis, nitrogen fixation, enzymatic activities, free amino acids, reducing sugars, and soluble proteins in plants has been documented (Borowski *et al*., 2000; Naeem *et al*., 2009; 2010; 2011; Aftab *et al*., 2010; Khandaker *et al*., 2013). The application of TRIA also increases the dry weight, chlorophyll content, protein and net photosynthetic efficiency in rice (*Oryza sativa* L.) (Chen *et al*., 2002). In cotton, it promotes vegetative growth and increases the level of monogalactosyldiacylglycerol (MGDG), a galactolipid that appears to be involved in the synthesis of photosystem I proteins (Naeem *et al*., 2012). The application of TRIA alone or in combination with potassium increases plant height, fresh and dry weight of the plant and leaf area in tomato (Khan *et al*., 2009).

In this exploratory study, we evaluated the effect of the interaction of plant growth regulators with triacontanol on the field pea *Pisum sativum L*. cv. Rondo to determine the roles of growth regulators applied alone and in combination and their impact on yield components and plant growth and development.

Materials and methods

The experiment was carried out in the experimental fields of the Instituto Nacional de Innovación Agraria (INIA), La Molina, Lima, Peru (12°04’48” S, 76°56’44” W at 243 m a.s.l.) between September and December 2015. To determine the effect of the application of triacontanol (TRIA) and the growth regulators on peas, pea seeds cv. Rondo were used. Rondo is a commercial cultivar with a determinate growth habit, semi-late, with straight pod and rough grain suitable for fresh consumption, and with medium maturation appreciated by farmers for its yield and pod size. Climate characteristics of the field site are described in Table 1. Four pea seeds were sown per hole under drip irrigation at a distance between plants of 0.75 x 0.2 m, with 24 experimental plots of 9 m^2 and a total area of 320 m^2 (experimental plots + edge spaces). Average soil characteristics of the field site were: sand, 53%; silt, 28%; clay, 19%; organic matter, 1.12%; P, 17.4 mg kg^{-1}; K, 291 mg kg^{-1}; pH, 7.7, and electrical conductivity, 1.62 dS m^{-1}. The soil analysis was carried out according to the guidelines established by Burt (2014). Subsequently, manual thinning was done, leaving three plants per stroke and a final planting density of 200,000 plants ha^{-1}.

At 43 d after sowing (V4 growth stage), the treatments described in Table 2 were foliar applied. All treatments, except the control, alone or in combination with other plant growth regulators (auxins (AUX), gibberellins (GA) or cytokinins (CK)) contained TRIA, and a foliar-applied potassium fertilizer (K). Other commercial foliar fertilizers from FARMEX S.A. (Peru), such as FX Amino and Powergizer, were also applied to all treatments at the same concentration to replace nitrogen deficiencies.

TABLE 1. Average climate data during the crop cycle.

Month	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)	Mean precipitation (mm/month)
September	21.52	15.34	80.21	0.12
October	22.77	16.16	79.75	0.06
November	22.9	16.64	79.33	0.09
December	25.08	17.97	77.92	0.06

To evaluate the effect of the treatments on growth and yield, the plant height, number of branches per plant, plant fresh weight, pod length, pod width, number of pods per plant, number of grains per pod and green pod yield (t ha^{-1}) were determined at harvest.

A completely randomized block design was used, with eight treatments and three replicates, for a total of 24 experimental units. The results were subjected to a normality test and analysis of variance (ANOVA) by the F test, and the means were compared with the least significant Fisher's test (LSD) at 5% probability of error, using the statistical software R. Pearson's correlation test at $P \leq 0.05$ was applied to assess the significance of correlation coefficients.

TABLE 2. Description of the treatments and doses of the plant growth regulators used in the experiment.

Name	Description
TRIA+AUX	0.5 mg L^{-1} triacontanol + 10 mg L^{-1} NAA
TRIA+GA	0.5 mg L^{-1} triacontanol + 10 mg L^{-1} GA_3
TRIA+CK	0.5 mg L^{-1} triacontanol + 1 mg L^{-1} KIN
TRIA+AUX+CK	0.5 mg L^{-1} triacontanol + 0.5 mg L^{-1} NAA + 1 mg L^{-1} KIN
TRIA+AUX+GA+CK	0.5 mg L^{-1} triacontanol + 0.5 mg L^{-1} NAA + 1 mg L^{-1} GA_3 + 1 mg L^{-1} KIN
TRIA+K	0.5 mg L^{-1} triacontanol + 3000 mg L^{-1} K_2O
TRIA	0.5 mg L^{-1} triacontanol
CONTROL	Treatment without any application of triacontanol or growth regulators

NAA - 1-Naphthaleneacetic acid; GA3 - gibberellic acid; KIN - kinetin; K_2O - potassium oxide.

Results and discussion

Growth parameters

The plant growth regulators applied in this study caused changes in plant height. The application of TRIA + GA obtained the highest plant height (143.5 cm), and the control obtained the lowest value (79.8 cm), which was 15.6% lower than the average obtained in the experiment (Tab. 3). The height of the pea plant is a characteristic determined by its genetics (Weeden, 2007), and the interaction with growth-promoting phytohormones such as GA (Wang *et al*., 2017). The control of this experiment showed a height greater than that reported by Anchivilca Rojas (2018) (68 cm), Santos *et al*. (2018) (60 cm), and Rodríguez Quispe (2015) (52 cm). According to Checa Coral *et al*. (2020), this could be due to environmental conditions and an efficient supply of resources since the resulting phenotype is the interaction of the genotype and the environment. The positive interaction of TRIA and GA agrees with that proposed by Shukla *et al*. (1992), who mention that triacontanol potentiates the effect of GA. The opposite is observed with AUX, since TRIA decreases its influence as a "destruction" effect is produced. The intensity of this effect is specific in each cultivar (Henry & Gordon, 1980); auxins at low concentrations inhibit stem growth in favor of root growth and development (Davies, 2010; Taiz *et al*., 2014). No effects were observed with foliar application of potassium because the soil was rich in this element (291 mg L^{-1}) and the application of potassium would keep the plants under conditions of "luxury consumption" (Marschner, 2011).

Regarding the number of branches per plant, there were no statistical differences in the analysis of variance for the treatments. Most of the treatments obtained similar values to those of the control, except for TRIA+GA, with a value 21.8% less than the average, and TRIA+AUX+GA+CK, with a value 23.5% greater than the control and 26.3% greater than the average (Tab. 3). The trend is repeated for pod length and plant fresh weight regarding the highest and lowest values in the treatments. However, for pod width, the control showed the lowest value of 1.66 cm, TRIA+AUX showed an intermediate value of 1.76 cm, and TRIA+AUX+GA+CK obtained the highest value with 1.83 cm.

The number of branches plant (NBP) and the pod width are also genetically determined characteristics, but the former is more susceptible to variations due to environmental conditions and/or agronomic practices than the latter (presence of the *n* gene, Wehner & Gritton, (1981)). Pod width did not exhibit significant or percentage statistical variation from all variables, showing a less marked incremental effect of the negative interaction of TRIA with AUX and CK applied jointly (Fig. 1). On the other hand, for NBP, the treatments

TABLE 3. Effect of plant growth regulators on growth and yield of pea plants.

Treatment	PH (cm)	NBP	PL (cm)	PW (cm)	NPP	NGP	PFW (g)	Yield (t ha^{-1})
TRIA+AUX	$86.5^{b} \pm 8.3$	$2.7^{ab} \pm 0.4$	$9.67^{ab} \pm 0.7$	$1.77^{ab} \pm 0.03$	$9.5^{abc} \pm 1.0$	$6.1^{c} \pm 0.7$	$130.2^{bc} \pm 21.5$	$7.43^{c} \pm 0.8$
TRIA+GA	$143.5^{a} \pm 4.6$	$2.2^{b} \pm 0.3$	$8.26^{b} \pm 0.4$	$1.83^{ab} \pm 0.09$	$3.7^{c} \pm 0.9$	$4.6^{d} \pm 0.01$	$93.5^{c} \pm 11.5$	$2.15^{d} \pm 0.3$
TRIA+CK	$95.8^{b} \pm 6.4$	$2.8^{ab} \pm 0.2$	$10.67^{a} \pm 0.1$	$1.74^{ab} \pm 0.03$	$12.2^{ab} \pm 3.4$	$7.9^{ab} \pm 0.3$	$143.3^{abc} \pm 15.6$	$8.47^{bc} \pm 0.9$
TRIA+AUX+CK	$85.0^{b} \pm 6.1$	$2.8^{ab} \pm 0.2$	$10.29^{a} \pm 0.4$	$1.68^{ab} \pm 0.02$	$15.0^{a} \pm 1.5$	$7.7^{ab} \pm 0.2$	$154.5^{ab} \pm 7.2$	$10.53^{ab} \pm 0.9$
TRIA+ AUX+GA+CK	$91.5^{b} \pm 4.5$	$3.5^{a} \pm 0.5$	$10.71^{a} \pm 0.7$	$1.83^{a} \pm 0.06$	$14.7^{ab} \pm 3.2$	$8.7^{a} \pm 0.2$	$198^{a} \pm 34.9$	$12.15^{a} \pm 1.4$
TRIA+K	$85.7^{b} \pm 3.3$	$2.7^{ab} \pm 0.2$	$10.46^{a} \pm 0.4$	$1.81^{ab} \pm 0.03$	$9.0^{bc} \pm 1.3$	$7.7^{ab} \pm 0.6$	$120.8^{bc} \pm 15.4$	$7.30^{c} \pm 1.0$
TRIA	$88.7^{b} \pm 3.3$	$2.7^{ab} \pm 0.2$	$10.21^{a} \pm 0.3$	$1.73^{ab} \pm 0.05$	$12.8^{ab} \pm 0.9$	$7.2^{bc} \pm 0.4$	$135.8^{bc} \pm 9.3$	$8.88^{bc} \pm 0.4$
CONTROL	$79.8^{b} \pm 7.2$	$2.8^{ab} \pm 0.2$	$9.33^{ab} \pm 0.5$	$1.66^{b} \pm 0.04$	$13.3^{ab} \pm 1.5$	$5.9^{cd} \pm 0.3$	$122.2^{bc} \pm 14.6$	$7.98^{bc} \pm 1.0$
F-Test	**	ns	ns	ns	*	**	ns	**
Mean	94.56	2.77	9.95	1.75	11.27	6.98	137.29	8.11

TRIA - triacontanol, AUX - auxins, GA - gibberellins, CK - cytokinins, K - foliar-applied potassium, PH - plant height, NBP - number of branches per plant, PL - pod length, PW - pod width, NPP - number of pods per plant, NGP - number of grains per pod, PFW - plant fresh weight. ns - not significant at $P>0.05$; *: $P\leq0.05$; **: $P\leq0.01$.

with CK increased the branching of the shoots (Müller & Leyser, 2011) and the number of branches in the plants (Taiz *et al.*, 2014).

Pearson's correlation coefficients showed interesting data regarding the relationships between variables. Within the vegetative parameters, plant height was negatively correlated with pod length (-0.271) and the number of branches per plant was positively correlated with pod length (0.442*) and yield (0.731**). The variable pod length was positively correlated with plant fresh weight (0.626**), number of grains per pod (0.823**), and green pod yield (0.654**). A positive correlation was also observed between these last three variables. However, these variables were not positively correlated with pod width (Tab. 4). In contrast with the values obtained in the variable plant height, the average pod length in this experiment (9.9 cm) was lower than that obtained by Anchivilca Rojas (2018) (10 cm), Rondinel Ruíz (2014) (10.2 cm), and Rodríguez Quispe (2015) (11.4 cm). Triacontanol application resulted in an increase of 11.3% in yield over the control (Fig. 2). A positive interaction was also observed with treatments containing CK and, to a lesser extent, with foliar potassium. A slight negative interaction was observed with AUX and a higher negative interaction was registered with gibberellins.

The effect of CK is significant since they promote greater pod setting in legumes (Carlson *et al.*, 1987) and increase the width, length, and weight of pods (Mosjidis *et al.*, 1993). Potassium also has a slight incremental effect on fruiting and a higher pod setting in legumes (Marschner, 2011). However, this is not reflected by a luxury consumption of K. The trend of the effect of the hormonal interaction is repeated on pod length, as well as on plant fresh weight, except that a negative interaction with foliar potassium was also recorded. Although gibberellins favor the vegetative growth of the plant when applied in excess (Taiz *et al.*, 2014), this growth is very fast, forming tall but weak stems with lower dry matter content and fresh weight of the plant. The mixture of the three hormones considerably increases the plant fresh weight due to a better regulation of physiological processes and the internal hormonal balance in the plants (Figs. 1-3).

Yield parameters

The lowest values of all yield variables (number of pods per plant, number of grains per pod, and green pod yield) were obtained with TRIA+GA. The highest values were recorded with TRIA+AUX+GA+CK, in which all the hormones were used, except in the number of pods per plant, where the best treatment was TRIA+AUX+CK (Tab. 3). The number of pods per plant showed a different behavior between treatments, although it was highly positively correlated with the number of grains per pod (0.485*) and yield (0.883**) as shown in Table 3. An increase of 308.7% over the lowest value of this variable and 12.52% over the control was recorded. Statistically significant differences between the treatments were observed, since a very strong detrimental effect of GA was seen in this variable. Figures 1A and 1B show the positive interaction of TRIA with the mixture of AUX, CK and GA, but a negative interaction with AUX and CK applied separately.

Regarding the number of grains per pod, significant statistical differences were observed between the treatments. The TRIA+AUX+GA+CK showed an increase of 48.8% over the control and 14.3% over the TRIA applied alone, which, in turn, represents a 22.6% increase over the control.

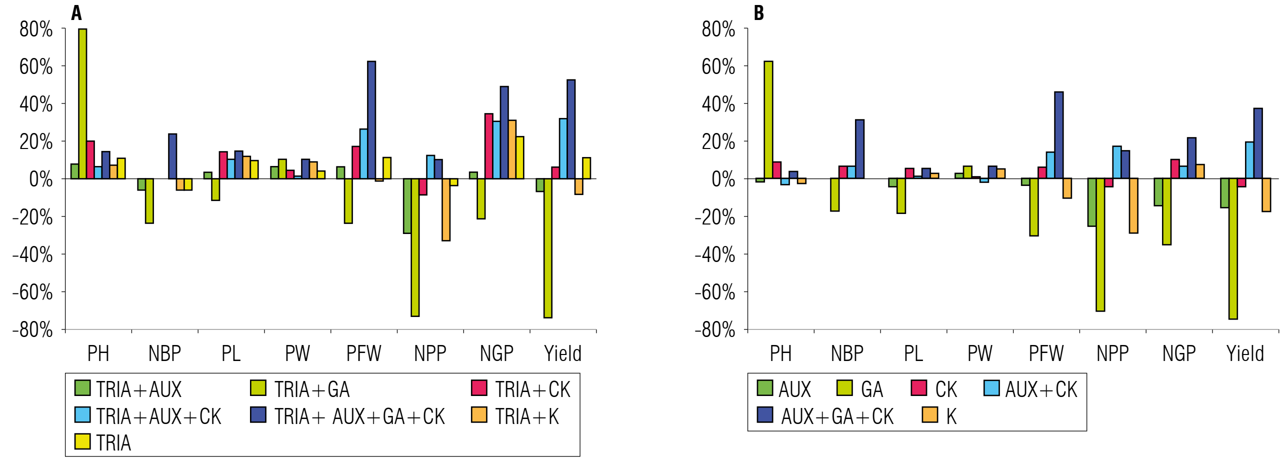

FIGURE 1. Differential effects of the application of A) triacontanol (TRIA) compared to the control and B) the plant growth regulators (AUX - Auxins, GA - Gibberellins, and CK - Cytokinins) alone or mixed compared to triacontanol. PH - plant height; NBP - number of branches per plant; PL - pod length; PW - pod width; NPP - number of pods per plant; NGP - number of grains per pod; PFW - plant fresh weight.

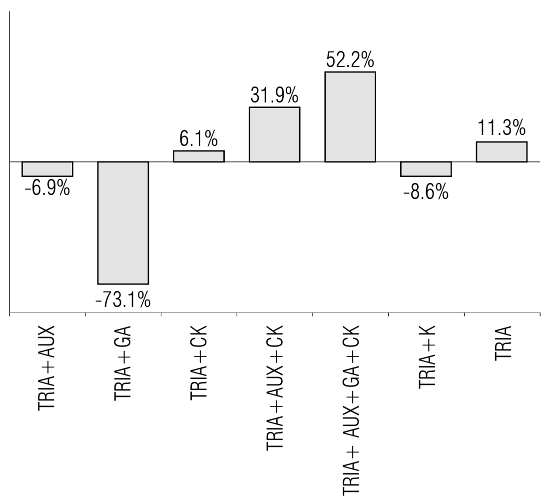

FIGURE 2. Differential effect of the treatments on the yield (t ha^{-1}) compared to the control. TRIA - triacontanol, AUX - auxins, GA - gibberellins, CK - cytokinins, K - foliar-applied potassium.

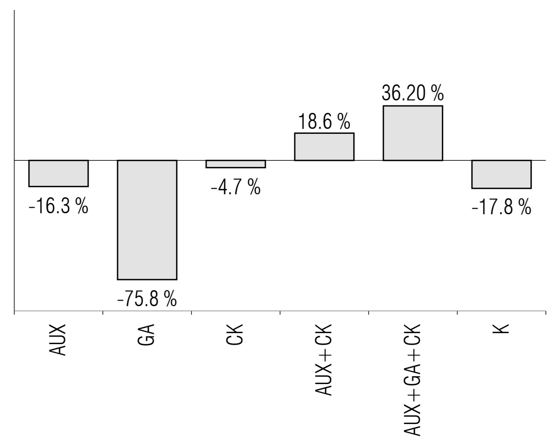

FIGURE 3. Differential effects of growth regulators on the yield (t ha^{-1}) compared to triacontanol. AUX - auxins, GA - gibberellins, CK - cytokinins, K - foliar-applied potassium.

TRIA+AUX, TRIA+GA and the control did not exceed the average value obtained in the experiment of seven grains per pod (Tab. 3).

Within the yield components, the number of pods per plant with the application of TRIA decreased by 3.8% compared to the control (Fig. 1A). No positive interactions were observed with the mixture of AUX and CK or with the mixture of the three hormones. However, a negative interaction was recorded with AUX and CK applied separately since the AUX-CK relationship is imbalanced in physiological processes. A large decrease was observed with GA since they favor vegetative growth (Davies, 2010), and foliar potassium application obtained values below the control (32.5% less).

The number of pods per plant is one of the most important yield components in legumes. GA decreased this yield variable because it promotes growth to the detriment of fruiting and formation of plant flowers (Davies, 2010). CK promote pod formation and show synergism with auxins (Cato *et al*., 2013). The positive impact of CK has been observed in plants, such as *Artemisia* grown *in vitro* (Rasool *et al*., 2013), showing a greater effect with the mixture of the three growth regulators (Cato *et al*., 2013).

Foliar-applied potassium has a positive effect on the control and an interaction with TRIA, similar to that occurring with CK. Regarding the variable green pod yield, the values obtained ranged between 2.15 and 12.15 t ha^{-1} with highly significant statistical differences between treatments (P<0.01). TRIA+AUX+CK and TRIA+AUX+GA+CK

TABLE 4. Pearson's correlation coefficients of the variables tested.

	PH	NBP	NPP	PL	PW	PFW	NGP
NBP	-0.271	----	----	----	----	----	----
NPP	-0.573**	0.655**	----	----	----	----	----
PL	-0.443*	0.442*	0.396	----	----	----	----
PW	0.225	0.243	-0.232	0.131	----	----	----
PFW	-0.255	0.891**	0.670**	0.626**	0.267	----	----
NGP	-0.447*	0.456*	0.485*	0.823**	0.058	0.587**	----
Yield	-0.613**	0.731**	0.883**	0.654**	-0.009	0.845**	0.723**

PH - plant height; NBP - number of branches per plant; NPP - number of pods per plant; PL - pod length; PW - pod width; PFW - plant fresh weight, and NGP - number of grains per pod.

exceeded 10 t ha^{-1}, and TRIA+GA showed a decrease of 73.1% relative to the control. TRIA+AUX and TRIA+K did not exceed the control. The application of TRIA alone showed an increase of 11.3% relative to the control (Fig. 2).

Pearson's correlation coefficients of the yield variables showed that the number of pods per plant was positively correlated with number of grains per pod (0.485*), number of branches per plant (0.655**), plant fresh weight (0.670**), and yield (0.883**), and negatively correlated with plant height (-0.573**). The number of grains per pod was also positively correlated with the other variables ($P<0.05$). The same was observed for the variable green pod yield, except for pod width, which is not correlated with any variable (Tab. 4).

Green pod yield is the agronomic trait that helps to determine the profitability of the crop. It also determines if the crop is a good alternative for the farmer, who looks for high yields with increasing emphasis on quality (Espinosa & Ligarreto, 2005; Checa Coral *et al*., 2017). The high yield values obtained are due to the fact that the values of number of branches per plant, number of pods per plant, and number of grains per pod were also higher. These last two variables are important components of the yield and determine the productivity of plants. The lowest values were obtained in the interaction of TRIA with AUX and GA applied separately. This shows the negative effect of GA on crop yield in general since they decrease pod formation favoring vegetative growth and increasing plant height, an unfavorable issue leading to lodging (Davies, 2010; Hedden, 2016; Wang *et al*., 2017). It also shows the negative effect of AUX applied alone, since they alter the AUX-CK relationship that is key to the growth and development of plant species (Schaller *et al*., 2015).

TRIA is a natural growth regulator widely studied in various species, with notable effects on the growth and development of rice (Jadhav *et al*., 2017), *Capsicum annuum* L. (Sahu *et al*., 2017), willow (Digruber *et al*., 2018), sweet potato (Rajak *et al*., 2018), strawberry (Baba *et al*., 2017), and guava (Singh *et al*., 2017). Pearson's correlation coefficients show a highly positive correlation between yield and the number of pods per plant (0.883**), number of branches per plant (0.731**), number of grains per plant (0.723**), plant fresh weight (0.845**), and pod length (0.654**) (Tab. 4).

The differential effect for TRIA in this experiment was greater, especially in the yield components, with an increase in plant height of 11%, a decrease in number of branches per plant and number of pods per plant of 5.9 and 3.8%, respectively, and increases of 9.5% in pod length, 4.2% in pod width, 11.19% in plant fresh weight and 22.6% in number of grains per pod.

Conclusions

The results of this study showed that TRIA by itself has no remarkable effect on the growth and yield of *Pisum sativum*; only when TRIA is combined with other plant growth regulators such as AUX, GA and CK are noteworthy effects seen. AUX, GA and CK increased morphological variables with the exception of the number of branches per plant, and pod length in the case of GA. Within the yield components, AUX and CK increased the number of grains per pod and GA decreased all these variables, showing a negative interaction with TRIA. AUX and CK acted synergistically in almost all the variables evaluated, except for the number of branches for which similar values were obtained. In all these variables, the synergistic action of AUX and CK exceeds the effect of each growth regulator separately. The joint application of foliar-applied potassium fertilizer with TRIA showed an unfavorable effect on the yield and plant fresh weight. The highest green pod yields were obtained with the application of TRIA plus AUX, GA and CK (12.15 t ha^{-1}), followed by the application of TRIA plus AUX and CK (10.53 t ha^{-1}). The trihormonal application with TRIA obtained the best results in the variables because the hormonal relationship is maintained and is not altered by the imbalance of the others.

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

AHJ obtained the financial support and the experiment site for the project. AHJ and HCS designed the methodology. HCS conducted the research process, performed the experiment, and analyzed the data. HCS wrote the draft and AHJ carried out the revision of the manuscript.

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Causes of fruit cracking in the era of climate change. A review

Causas del rajado de frutos en la era del cambio climático. Una revisión

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ABSTRACT

The objective of this review was to report on advances in environmental, cultural, and physiological aspects of fleshy fruit cracking to reduce or avoid this disorder, which affects many fruit species. Cracking is a physiological disorder that limits the production and quality of fleshy fruits because it affects the exocarp and mesocarp, especially with climate change and variability. Fruit cracking is generated by external factors (agronomic and environmental) and internal factors, several of which require exhaustive study. The incidence of cracking varies widely according to climatic characteristics during fruit development, different fruit species and varieties, growth sites, and crop management. This physiological disorder is aggravated by increases in rain intensity, especially after a dry season or in areas with increased temperatures. Knowledge on causes of cracking has generated management strategies that involve genetic improvement, ecophysiological conditions, agronomic practices such as pruning, irrigation, and fertilization (mainly with Ca, Mg, B, and K), applications of plant growth regulators, and use of plastic covers, etc. For several fruit trees, these strategies are effective, but in species such as the cape gooseberry, cracking remains without a full explanation or effective management.

Key words: mineral nutrition, climate, heavy rains, fleshy fruits, fruit growing, physiological disorders.

RESUMEN

El objetivo de esta revisión es informar sobre los avances en los aspectos ambientales, culturales y fisiológicos del rajado en los frutos carnosos más importantes para disminuir o evitar este desorden el cual afecta numerosas especies frutales. El rajado es una fisiopatía que limita la producción y la calidad de frutos carnosos porque afecta el exocarpio y el mesocarpio, especialmente en escenarios de cambio y variabilidad climática. El rajado del fruto es generado por factores externos (agronómicos y ambientales), e internos, varios de los cuales todavía son motivos de exhaustivo estudio. La incidencia del rajado varía ampliamente según las características climáticas durante el desarrollo del fruto, las diferentes especies frutales y variedades, además de los sitios de crecimiento y el manejo del cultivo. Esta fisiopatía se agrava por el aumento de la intensidad en las lluvias y especialmente cuando estas ocurren después de una época seca, igualmente en zonas donde ha aumentado la temperatura. El conocimiento de las causas del rajado ha permitido generar estrategias de manejo que involucran: mejoramiento genético y de condiciones ecofisiológicas, practicas agronómicas como podas, riego y fertilización (principalmente Ca, Mg, B y K), aplicación de fitorreguladores y uso de cubiertas plásticas, entre otros. En varios frutales estas estrategias son efectivas, pero en especies como la uchuva aún el problema del rajado permanece sin total explicación y manejo efectivo.

Palabras clave: nutrición mineral, clima, lluvias intensas, frutos carnosos, fruticultura, fisiopatías.

Introduction

Food security is strongly influenced by the threat of climate change (CC), not only through global warming but also by altered rain patterns, which generate more extreme weather events (IPCC, 2019). The effects on fruit trees will probably intensify for countries in the tropics and subtropics with higher initial temperatures, which will affect more marginal or degraded lands and underdeveloped regions with a low adaptation potential (Yohannes, 2016). Thus, global warming will affect small farmers more, who, for the most part, depend solely on rain (Sthapit *et al*., 2012). In addition, the Andean tropics will be greatly affected by CC, not only because it would increase precipitation by 20% to 25% but also because warming would increase more at altitude than in valleys (Marengo *et al*., 2011).

Zandalinas *et al*. (2021) highlighted that multifactorial stress, generated by CC, severely affects the growth and survival of crops, for which Dubey *et al*. (2021) pointed out that the intensity and frequency of abiotic stress are constantly increasing. Chmielewski *et al*. (2008) stated that adaptations of fruit plantations to CC require time and long-term research.

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One of the most important limitations for fruit growth is the presence of physiological disorders, especially fruit cracking (Fig. 1), which limits production and quality (Ramteke *et al*., 2017) both at harvest and post-harvest, mainly in berries and drupes. This disorder affects the appearance of fruits, increases water loss and susceptibility to infection by pathogens, and decreases storage and shelf-life, resulting in substantial commercial losses for growers (Ginzberg & Stern, 2016; Yu *et al*., 2020). The peel plays a crucial role in resistance to cracking, portability, storability, and quality during storage (shelf-life quality) (Wang *et al*., 2021).

Cracked fruits lose their commercial value for the fresh market and can only be sold locally or for the processing and transformation, as reported by Rojas Alfonso *et al.* (2012) for cape gooseberry producers in Cienaga (Boyaca, Colombia), where the exporting company returns at least 20% of the fruits because of cracking. Likewise, in times of heavy and prolonged rainfall, exporters can reject up to 50% of cape gooseberry fruits because of cracking (Fischer, 2005). Interestingly, cracked pomegranate fruits developed a higher oil content than intact fruits (Zaouay *et al*., 2020).

For the main causes of this physiological disorder, Yilmaz and Özgüven (2019) mentioned genetic factors, very ripe fruits, and fluctuating soil moisture, apart from nutritional deficiencies, sunburn, injuries, and pathogens on the skin of the fruits. Generally, fruit cracking occurs because of a physical failure of the cuticle or skin as the result of tensions (stresses) and heavy rains (Ramteke *et al*., 2017). Because of fruit cracking, water management and nutrition in crops have become a concern for many countries (Fischer & Orduz-Rodríguez, 2012).

In nature, fruit cracking manifests at the end of fruit development, that is, just after ripening and before seed dispersal (Lichter *et al*., 2002). It is a physiological disorder that affects the exocarp and the mesocarp (Yu *et al*., 2020) and can be distinguished from epidermis cracking, which is more superficial, including the cuticle and epidermal tissue. Deeper cracking can be characterized by an opening to the interior of the pulp, known as splitting (Opara *et al*., 1997; Fischer, 2005). In the cape gooseberry, Gordillo *et al*. (2004) found two types of cracking: superficial (cracking) and deep (splitting) (Fig. 1A). However, in most cases, cracking and splitting are used synonymously (Lopez-Zaplana *et al*., 2020).

In the case of cherries, microcracking results in deterioration of the barrier, involving only the cuticle, whereas macrocracking involves both the barrier and structure,

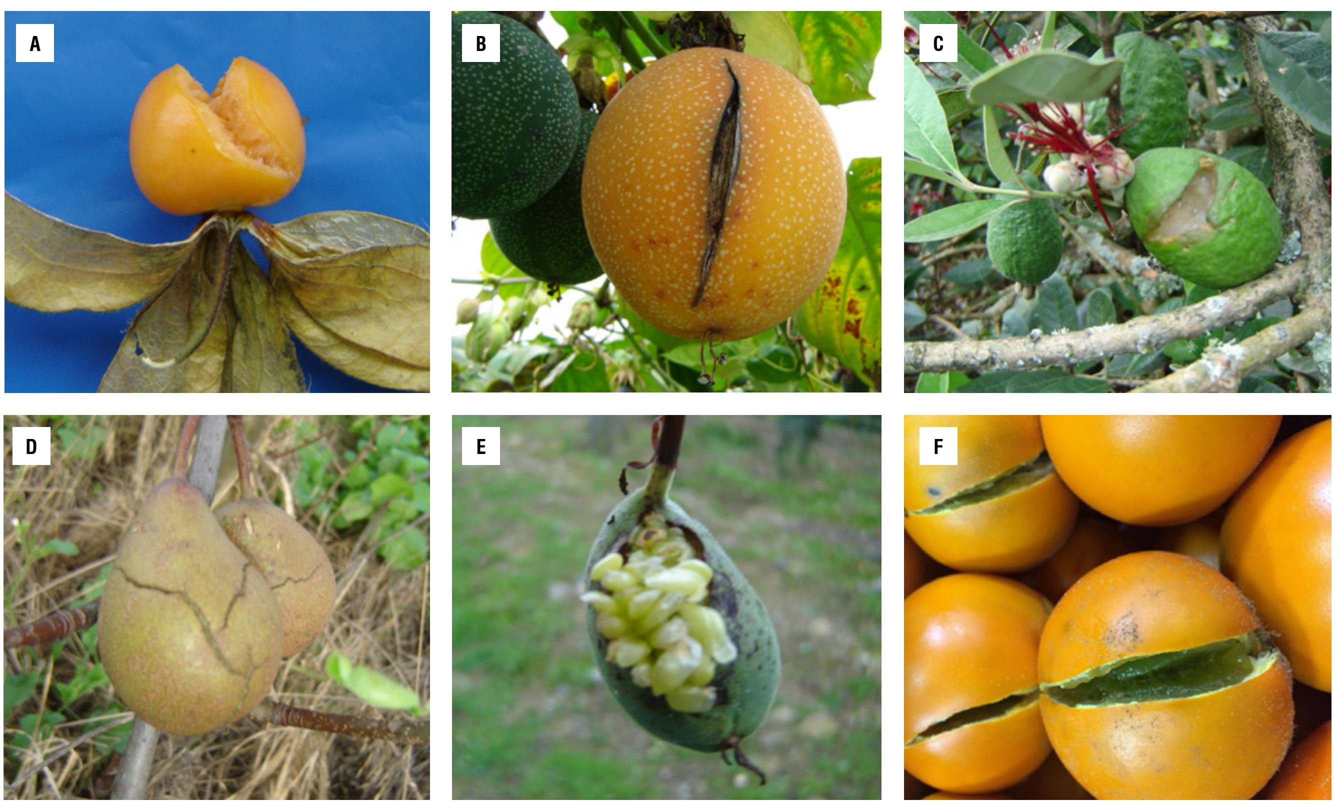

FIGURE 1. Fruit cracking in different fruit species. A) cape gooseberry; B) sweet granadilla; C) feijoa; D) pear; E) banana passion fruit; F) lulo.

deteriorating the cuticle and the internal cell layers (Knoche & Lang, 2017).

The objective of this literature review was to report on advances in environmental, cultural, and physiological aspects of fruit cracking, which are important for making management decisions, and plant breeding programs to reduce and avoid this disorder in many fruit crops, especially in the context of climate change and variability.

Factors causing fruit cracking

Figure 2 shows these factors for the cape gooseberry (*Physalis peruviana* L.), a fruit that shows high percentages of cracking and various causes. Fruit cracking is generated by external factors, including agronomic and environmental, and internal factors (Yu *et al.*, 2020). It is a physiological disorder that involves the exocarp and mesocarp of the fruit and affects its appearance, intensifies the loss of water and predisposition to infection by pathogens, and decreases the postharvest shelf-life, generating considerable losses for fruit growers (Ginzberg & Stern, 2016; Ramteke *et al.*, 2017). Wang *et al.* (2021) characterized fruit cracking as a common physiological disorder, in which the surface cracks because of uncoordinated internal growth and an external environment with high climatic variability.

The incidence of cracking varies widely according to the climatic characteristics during fruit development, the different fruit species and varieties, growth sites, and crop management (Fischer & Orduz-Rodríguez, 2012). As these authors report, fruit cracking can occur occasionally in any season, garden plant, branch, or cultivar.

Environmental factors

Water

Cracking because of rain, especially in fleshy fruit, (Fig. 1) imposes a severe problem in production and is related to huge commercial losses worldwide (Grimm *et al.*, 2019). This situation greatly affects the cultivation of fruit trees in the Andes, where rainfall will increase as a result of climate change (Marengo *et al.*, 2011).

High amounts of rain for several days or short heavy rains after a dry season can cause fruit cracking, such as in the cape gooseberry (Fischer & Melgarejo, 2020; Fischer *et al.*, 2021). There is a disproportion between the amount of water that enters a fruit in a given time and the extensibility of its epidermis (Fischer & Orduz-Rodríguez, 2012). However, this turgor model (the critical turgor hypothesis), initially developed for grapes (*Vitis vinifera* L.) by Considine and Kriedemann (1972), was questioned by Winkler *et al.* (2016) in sweet cherries (*Prunus avium* L.) because its results indicated that rain cracking is a localized phenomenon, a local exposure of a fruit's skin to water that is not related to the net water balance. According to Grimm *et al.* (2019), rain and fruit cracking have a causal relationship, and cracking is related to a change in the water relations of fruits, associated with the humidity of its surface. Also, abundant rains can cause very watery fruits, as in guava (*Psidium guajava* L.), with a reduced content of sugars, ascorbic acid, and titratable acidity, making them more susceptible to cracking (Fischer & Melgarejo, 2021).

The water balance of fruits is associated with several factors that can generate cracks (Saei *et al.*, 2014), where the

FIGURE 2. Diagram of possible factors of cracking in the cape gooseberry fruit.

water potential is the force that produces cracking, while the cell wall and other structures must withstand this pressure (Lichter *et al*., 2002). Thus, the biomechanical characteristics of the epidermis are crucial in maintaining internal pressure and resistance to fruit cracking (Saei *et al*., 2014). In the case of cherries, Knoche (2015) pointed out that an important cause of cracking is the rapid absorption of water by a fruit, which can be generated by direct absorption of the skin of the fruit or absorption by the vascular system of the plant.

In berries, such as the tomato and cape gooseberry, the high-water content and the high concentration of solutes exert high pressure on the epidermis of fruits, which cannot resist and cracks (Peet, 2009; Fischer & Melgarejo, 2020). This situation is accentuated if there are few fruits on the plant, such as the first fruits when the cape gooseberry production cycle begins (Gordillo *et al*., 2004). In postharvest, this problem is also accentuated when cape gooseberry fruits are subjected to immersion treatments in water, which limits the use of technologies such as pre-cooling with water and disinfection with products in liquid form.

In the case of high-water availability in the soil, cracking is very frequent in combination with two other factors, high relative humidity (RH) and low air temperature, conditions that reduce the transpiration of fruits (Fischer, 2005). For the pomegranate, Ikram *et al*. (2020) mentioned environmental factors, such as the imbalance of soil and air humidity, that contribute to intense cracking, that is, factors that have a high influence on fruit growth and development.

Relative humidity and temperature

In several studies in apple, Opara *et al*. (1997) reported greater cracking as the result of a lower formation potential or change in the composition of the cuticle, losing protective capacity, and a high RH that decreases the loss of water through the fruit. Deep splitting occurred when there was noticeable and depressed transpiration for 6 hours or more (Opara *et al*., 1997). On the other hand, Fischer (2005) reported a propitious effect of fruit cracking from low night temperatures in combination with a high RH.

A high RH prevents transpiration, which generates a high pressure inside the fruit, so that the epidermis can crack (Fischer & Orduz-Rodríguez, 2012), especially during prolonged periods of a RH between 99% and 100%, alone or in combination with rain (Fischer, 2005).

In tropical species such as passion fruit (*Passiflora ligularis* Juss, Fig. 1B), temperatures below 12-15°C are conducive not only to floral abortion and lower fertilization but also to fruit cracking (Fischer & Miranda, 2021). In addition, this species cracks more when sudden changes occur between day and night temperatures (Miranda Lasprilla, 2020). Cape gooseberries are susceptible to cracking from high amounts of water (Figs. 1A and 2) when the fruit grows completely inside the closed calyx, which dampens extreme temperatures by up to 5°C, especially with variations in day and night temperatures (Fischer *et al*., 2021).

In the cherry, apple, and peach, Bohlmann (1962) observed a tendency to crack when the temperature of the water on the epidermis increased. Reddy *et al*. (2017) also pointed out that physiological disorders are accentuated by global warming, such as the cracking of lychee fruits. Likewise, in hotter and more humid weather that follows a cold and dry period, there is danger of cracking, especially when young fruits begin to fill (Opara *et al*., 1997).

For the pomegranate, an excessive increase in temperature, hot dry winds, a downpour after a dry season, and large differences between day-night temperatures with temperatures greater than 38°C combined with a RH of 60%, favor fruit cracking in this species (Ikram *et al*., 2020).

Light

Regarding light, the side of the fruit exposed to direct sun can crack more because the epidermal cells on this exposed side become comparatively thicker and inelastic and do not adapt to the rapid increase of the tissues below (Opara *et al*., 1997). Ikram *et al*. (2020) reported that direct sun increases the temperature and evapotranspiration of the fruit surface, which results in a high moisture loss and a greater susceptibility to cracking.

Opara *et al*. (1997) pointed out that, in some apple varieties, cracking occurs preferably on the shady side of the apples, *i.e.,* the phenomenon is exclusively varietal. Fischer (2000) observed greater crack at 2,700 m a.s.l. in the department of Cundinamarca (Colombia) in 'Jonagold' apples exposed directly to the sun and affected by *Venturia inaequalis*.

Ulinnuha *et al*. (2020) found a lower incidence of cracking in tomatoes under shade and proposed further investigation of this phenomenon with associated crops that produce shade. Suzuki *et al*. (2007) harvested more fruits without cracking in this species when the foliage and fruits intercepted a lower amount of light than with other types of conduction (training).

Cultural factors

Mineral nutrition

The mineral nutrition of fruit is highly related to cracking (Yu *et al*., 2020). Ca deficiency has been reported as one of the main causes of cracking, such as in mandarin (Chabbal *et al*., 2020), lemon (Devi *et al*., 2018), grape (Yu *et al*., 2020), fig (Aydin & Kaptan, 2015), sweet cherry (Erogul, 2014), sour cherry (Simon *et al*., 2007), apricot (Nie *et al*., 2017), pomegranate (Davarpanah *et al*., 2018), litchi (Martínez Bolaños *et al*., 2017), loquat (Yilmaz, 2020), sweet granadilla (Fischer & Miranda, 2021; Fig. 1B), and cape gooseberry (Cooman *et al*., 2005; Fig. 1A).

As a macroelement and the most important element for fruit quality (Marschner, 2012), Ca is essential for multiple important functions in plant physiology, mainly because of its role in the structural integrity and stability of cell walls and the middle lamella, not only serving as a bridge between pectin molecules but also improving the integrity of cell membranes through the bonds with phospholipids. Additionally, it participates in stress signaling as a secondary messenger (Ranty *et al*., 2016; Yu *et al*., 2020).

Gordillo *et al*. (2004) observed that a deficient dose of potassium (K), as well as a lack of boron (B) in fertilization, induced greater cracking in cape gooseberry fruits. Cooman *et al*. (2005) also found an increase between 5.5% and 13.0% in the number of cracked fruits in this species when Ca or B was lacking in fertilization (Fig. 2). Fischer *et al*. (2020) reported cracking in banana passion fruit, known in the high tropics of Colombia as "the witch's laugh" (*risa de bruja* in Spanish) (Fig. 1E) because of the visibility of all seeds, attributed to Ca and/or B deficiency. Marschner (2012) underlined the role of B in the structure of cell walls.

On the other hand, Garzón-Acosta *et al*. (2014) recorded the highest cracking in mature and harvested cape gooseberry fruits in a greenhouse in plants that suffered from a magnesium (Mg) deficiency (Fig. 2), compared to plants deficient in Ca (11% and 1% cracked fruits, respectively). Problems with fruit cracking in feijoa (*Acca sellowiana;* Fig. 1C) have also been reported in Colombia with a Ca/Mg ratio ≥10 in the soil, where fruits crack and develop brown pigmentation (Fischer *et al*., 2021). Marschner (2012) stated that part of Mg is firmly bound to pectin in cell walls; therefore, deficiency of this element would contribute to cracking.

On the contrary, high concentrations of N in the soil from chemical or organic overfertilization, especially when the content of organic matter (OM) in the soil exceeded 20%, increased cracking in the cape gooseberry (Fig. 2) (Gordillo *et al*., 2004). Fischer and Orduz-Rodríguez (2012) suggested that the increase in the epidermis is not enough to keep up with the expansion of the fruit. Similarly, Yilmaz (2020) found an overconcentration of N in cracked loquat fruits.

Phytosanitary problems

Superficially or deeply cracked fruits not only exhibit open lesions that facilitate a loss of moisture and wrinkling but also are susceptible to infection by pathogens (Opara *et al*., 1997), which Fischer (2005) described in cracked cape gooseberries affected by Botrytis.

In apples cultivated in the high tropics, the epidermis of the fruit can be burned and cracked by high insolation (sun burning), especially by high amounts of UV light, which facilitates infection by *Venturia inaequalis* (Fischer, 2000). Casierra-Posada (2012) pointed out that B deficiencies can weaken the epidermis of young apples and pears, facilitating infection by *V. inaequalis* and *V. pyrina*, respectively, that later show lesions similar to cracking.

Ikram *et al.* (2020) and Singh *et al.* (2020) reported that attacks by insects, pathogens, and birds especially affect ripe, cracked fruits, which greatly reduces their commercial quality.

Another cause of cracking or aggravation in existing lesions may be the application of agrochemicals, especially mixtures with surfactants since they increase the penetration of water through the cuticle of the fruit (Opara *et al*., 1997).

Fruit factors

The relationships between fruit characteristics and susceptibility to cracking have also been evidenced (Fig. 2). Khadivi-Khub (2015) have reported a series of correlations between cracking and different aspects of the fruit, such as size, shape, and firmness, and the epidermis (resistance, stomata, and cuticular properties), in addition to the osmotic concentration, water capacity of the pulp, and the state of development of the fruit.

In the 'Terigas' mandarin, Hardiyanto and Nirmala (2019) found that fruit cracking increased with decreases in the thickness of the epidermis and, surprisingly, thinning of the peel of the mandarins was not influenced by nutrients (K, Ca, Mg).

In most species, the fruit suffers constant stress during development, because the volume is constantly increasing and, therefore, the surface of the fruit increases as well

(Knoche & Lang, 2017). These authors characterized the skin of fruits as a primary structure, consisting of a polymeric cuticle whose function is similar to that of a barrier that covers the epidermis and hypodermis, cell layers that are responsible for the skin's load-bearing functions.

Mishra *et al.* (2016) in pomegranate (*Punica granatum* L.) and Fischer (2005) in cape gooseberry reported that a prolonged drought hardens the skin (epidermis) prematurely, and the next heavy rain causes the pulp to expand. The same occurs during phases of rapid fruit growth, in which the skin cracks, especially in late harvests of mature fruits (Fig. 2) (Mishra *et al.*, 2016). The parts of the fruit that are most susceptible to cracking are its weak parts, as is the case with lenticels (Fischer, 2005).

In the pomegranate, during water stress, the growth of the peel is limited, as in the aril; however, when the water enters the fruit again, the aril expands more than the peel, which results in fruit cracking (Ikram *et al.*, 2020). This desynchronization when the pulp turgor exceeds a critical threshold causes the fruit skin to stretch beyond its extensibility limit and crack (Considine & Kriedemann, 1972).

In most studies, an increase in the incidence of cracking was found as fruit maturation advanced (Fig. 2). Jiang *et al.* (2019) and Wang *et al.* (2021) stated that, when the fruit is in an advanced state of maturity and a downpour occurs, the risk of cracking is much higher. In addition, fruits in a state of overmaturity are more susceptible to cracking because of the senescence of the epidermis (Fischer & Orduz-Rodríguez, 2012).

Gordillo *et al.* (2004) found in the cape gooseberry that this physiological disorder occurs especially in fruits with a large volume and weight and at the beginning of the harvest season (Figs. 1A and 2), considering that this species has indeterminate growth (Fischer *et al.*, 2021), in which vegetative and reproductive development occur at the same time. This situation was described by Torres Azuero *et al.* (2016) as an imbalance between the source-demand relationship because of an excess of assimilates in the first stage of the harvest, which supposedly generates pressure from assimilates, propitiating an average of 15% of cracked fruits in the cultivation of the cape gooseberry in the Cundiboyacense highlands. Also, Alvarez-Herrera *et al.* (2012) found the highest cracking (81%) in large cape gooseberry fruits (Fig. 2) but only 33% in small fruits in the same greenhouse study; the highest percentage of cracked fruits was observed in the upper stratum (38%) (Fig. 2), as compared to only 25% in the lower stratum of the plant.

In different citrus cultivars, most of the cracking occurs during cell elongation or in the period of fruit maturation. However, in some cultivars, cracking can occur throughout the development of the fruit (Li & Chen, 2017).

In tomato studies, Jiang *et al.* (2019) concluded that ripe fruit is more resistant to cracking as it contains more intact pectins as a result of a good degree of binding between the pectins by Ca^{2+} and other cross-linkages. Additionally, the correlation analysis by these authors verified that tomato cracking is significantly more related to the level of protopectin and cellulose than to that of Ca^{2+}.

On the other hand, a transcriptome analysis of atemoya (*Annona cherimola* × *A. squamosa*) by Chen *et al.* (2019) found that the decomposition of starch into soluble sugars and the metabolism of polysaccharides of the cell wall are closely related to fruit cracking. The hydrolytic enzyme of the cell wall, polygalacturonase (PG), breaks the α-(1-4)-galacturonan bond in the pectin chain, so an increase in PG activity generates fruit softening (Lu & Lin, 2011). On the other hand, research on the non-enzymatic protein expansin (EXP), which is involved in the extension of cell walls, is essential for knowledge on causes of cracking (Balbontín *et al.*, 2013) since varieties that are more resistant to cherry and lychee splitting have a level of EXP expression that was noticeably higher than in those most susceptible to this physiological disorder (Balbontín *et al.*, 2014).

Brüggenwirth and Knoche (2017) and Jiang *et al.* (2019) noted that factors such as disassembly, modification, and composition of the cell wall can affect the mechanical characteristics of the pericarp; therefore, they become determining elements of susceptibility to fruit cracking.

Varietal and genetic factors

As described above, the causes of cracking can be diverse, and the reasons vary between different species (Lu & Lin, 2011) and, in many cases, also between varieties of the same species (Opara *et al.*, 1997). Khadivi-Khub (2015) pointed out that fruit cracking is a quantitative trait that is controlled by several genes. Additionally, this author indicated that the cultivars most resistant to this problem and that show adequate fruit quality can be chosen for subsequent cultivation. Therefore, cultivars that exert greater skin break force and elasticity are more resistant to fruit cracking (Brüggenwirth & Knoche, 2016).

When evaluating 54 cape gooseberry materials from northeastern Colombia, Herrera *et al.* (2011) found large differences in the occurrence of cracking, which was higher in feral cultivars with an average of 23.9%. However, within this group, there was an accession (06Uch0001) without broken fruits, as compared to accession 06Uch0073 with 50% cracked fruits. This result confirmed that cracking depends to a high degree on the genetics of the material (Fischer, 2005), as in other nightshade berries, such as the tomato (Peet, 2009). Criollo *et al.* (2014) compared cracking in three genotypes of cape gooseberry and found that the highest percentages of this disorder occurred in the materials 'Silvania' and 'Kenya' (8.9% and 8.1%, respectively), compared to 'Regional Nariño' with only 3.1%. These results confirmed that, under the same climatic conditions, fruits of different cultivars of the same species show differences in susceptibility to cracking (Khadivi-Khub, 2015). On the other hand, Lagos-Burbano *et al.* (2021) characterized the fruit of 36 hybrids obtained from double haploid lines, with cracking being one of the three parameters that explained the total variability through principal component analysis. They also reported that two of the six clusters registered a high seed content, a low maturity index, and a high percentage of cracking.

Berries are particularly susceptible to splitting (Fischer *et al.*, 2021). However, Medina *et al.* (2009) reported that improved material from 'Lulo de la Selva' was used to find materials more resistant to cracking in the cultivation of lulo (*Solanum quitoense* Lam.) (Fig. 1F), and, after a generation of open pollination, the Jalisco clone showed reduced cracking and large fruits. Fischer and Melgarejo (2020) pointed out that plant breeding programs should also include resistance to cracking, and not only the production of large fruits, because size is highly correlated with the number of seeds (Trevisani *et al.*, 2017; Balaguera-López *et al.*, 2020). This should always be considered in these programs because cracking is an attribute whose gene expression may also depend on environmental conditions.

It is worth mentioning that the development of high-quality varieties resistant to cracking and the identification of genes involved in this resistance should be an important objective in plant breeding programs in fruit trees (Balbontín *et al.*, 2013).

Examples for cracking handling

The best way to reduce fruit cracking today would be proper orchard management that tries to minimize water stress and considers nutrition and physiological factors that contribute to fruit cracking (Khadivi-Khub, 2015).

Table 1 shows examples of Ca applications in cultivation, alone or in combination with B or K, which resulted in a decrease in the incidence of cracking in grapes, citrus, and

TABLE 1. Examples of crop management to reduce the incidence of fruit cracking.

Management type	Species	Effect	Authors
Fertilization			
Cluster immersion with 5 g L^{-1} $CaCl_2$	Grape 'Xiangfei'	Ca inhibited the production of water-soluble proteins, delaying the degradation of protopectin in the cell wall	Yu *et al.* (2020)
Foliar applications of 1% $CaCl_2$, boric acid or a combination	Mandarin 'Shogun'	Significant reduction in the number of cracked fruits	Sdoodee and Chiarawipa (2005)
Application of K_2SO_4 (10%) and $CaCl_2$ (1%)	Lemon 'Eureka'	Reduction of cracking and improvement of fruit quality	Devi *et al.* (2018)
Application of Ca (0, 50, or 100 kg ha^{-1})	Cape gooseberry 'Colombia'	Cracking of 38% in fruits without Ca, decreased cracking to 27% when 100 kg ha^{-1} of Ca was applied	Alvarez-Herrera *et al.* (2012)
Application of B (0, 1, or 3 mg L^{-1}), K (3, 5, or 7 meq L^{-1})	Cape gooseberry 'Colombia'	The two levels of B decreased the number of cracked fruits, as did the application of 3.5 meq L^{-1} of K	Sabino-López *et al.* (2018)
Irrigation			
Irrigation levels with net coefficients of 0.7, 0.9, 1.1, and 1.3 evaporation from tank class A	Cape gooseberry 'Colombia'	The 1.3 and 1.1 irrigation levels produced the fruits with the lowest percentage of cracking	Álvarez-Herrera *et al.* (2021)
Plant hormones			
Application of 50 mg L^{-1} naphthaleneacetic acid (NAA)	Lemon 'Pant Lemon-1'	Lower number of cracked fruits	Bhatt *et al.* (2016)
Double application of 40 mg L^{-1} NAA	Lemon 'Eureka'	More effective treatment in minimizing cracking	Devi *et al.* (2018)
Application of 0, 5, 10, or 15 mg L^{-1} GA_3	Cape gooseberry 'Colombia'	Plants with 10 mg L^{-1} of GA_3 resulted in the lowest cracking percentage, and the highest was with 5 mg L^{-1}	Amézquita *et al.* (2008)
Covers			
Plastic film cover (Oroplus®), 5 m above the plants	Cherry 'Lapins'	The plastic cover reduced cracking from 20% to 2% in both seasons, without affecting fruit yield	Mika *et al.* (2019)

cape gooseberries. Cronjé *et al.* (2013) suggested an interaction of Ca with B, forming a stabilizing complex in the middle lamella of cells. For these effects, Yu *et al.* (2020) carried out applications of Ca during the maturation phase to prevent a localized deficiency of this element since it is necessary for fruit quality. Also, in the cape gooseberry, B or K alone (Tab. 1) reduced the percentage of fruit cracking (Sabino-López *et al.*, 2018). In the pomegranate fruits highly susceptible to cracking with losses of up to 40-60% (Ikram *et al.*, 2020), 0.2% B reduced the presence of this physiological disorder (Sharma & Belsare, 2011).

Applications of naphthaleneacetic acid have shown good results for controlling cracking in lemons (Tab. 1), which Devi *et al.* (2018) attributed to the significant role of this plant hormone in increasing resistance and plasticity of the peel, which determine the intensity of cracking. Furthermore, Amiri *et al.* (2012) specified that synthetic auxins decrease cracking in citrus fruits because of the increased thickness of the rind. In the pomegranate, an application of 5 mg L^{-1} of forchlorfenuron (CPPU), a cytokinin, significantly reduced cracking (Sahu & Sharma, 2019), as did applications with paclobutrazol (an anti-gibberellic compound) (Khalil & Aly, 2013). The results of the application of gibberellins in cultivation are not consistent (Amézquita *et al.*, 2008), possibly because this regulator can increase the size of fruits. However, Amézquita *et al.* (2008) found that applications of 10 mg L^{-1} of GA_3 generated the lowest percentage of cracking of cape gooseberry fruits (Tab. 1; Fig. 2).

Besides proper management with nutrition, irrigation, and growth regulators, Ikram *et al.* (2020) reported other practices to reduce cracking for the pomegranate, such as protecting the fruits from direct solar radiation by bagging or putting a shade net, as well as the application of antitranspirants based on kaolin.

Additionally, in different crops, specific management methods should be sought, such as the elimination of the first flowers at the beginning of the productive cycle in the cape gooseberry (Fischer & Miranda, 2012). Also, in the cape gooseberry 'Silvania', cracking was reduced by pruning (training) with an increase in primary branches, from three to four (Criollo *et al.*, 2014). By increasing the number of branches, the plants had a greater transpiratory volume, an increase in cell mass, and a better water distribution (Marsal *et al.*, 2006). Criollo *et al.* (2014) assumed that they reduced the risk of breaking the fruit surface because of a decrease in pressure exerted on the cell walls at the pericarp level.

Conclusions

Fruit cracking is a physiological disorder that seriously affects the quality and marketability of many fruit species.

Because of climate change, this physiological disorder is aggravated in areas where the intensity of rains is higher, but also in regions where there is an increase in temperatures and solar radiation and in the dry seasons that will be followed by rains, which is the main cause of this disorder. In general, fruit cracking is due to the stated climatic conditions, but also due to prolonged rains in combination with a high RH that suppress the transpiration of fruits.

High temperatures and/or sunburn age the epidermis of fruits, which cannot withstand the pressure when the fruit fills up with more turgor again. Likewise, sudden changes between daytime and nighttime temperatures or hot seasons after cold ones promote cracking in several species.

The deficiency of nutrients that are important for the stability of the cell wall, such as Ca, B, and Mg, is another important cause of this physiological disorder. Additionally, the lack of K that regulates the water rate in plants, influences cracking. Likewise, excess N can increase the pressure of the pulp on the epidermis of the fruit, causing it to crack.

Measures are proposed to control this disorder, such as the selection of more resistant varieties, adequate nutrition to strengthen the resistance of the cell wall, the avoidance of water stress in plantations, and the application of growth regulators such as NAA or GA_3, depending on the species. Additionally, plastic covers should be placed on species, such as cherries, that easily crack as a result of water absorption on the skin of the fruit. Specific tasks are important according to the species, such as eliminating the first flowers or increasing the number of primary branches in the cape gooseberry to reduce the pressure of water and solutes on a small number of fruits when the plant is in full growth and just beginning production.

Finally, since many fruit species lack successful research on fruit cracking, this literature review provides initiatives to lessen this problem with proper crop management and breeding programs for the affected species and varieties that consider the aforementioned traits.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contribution

GF wrote the initial draft and carried out the final revision of the manuscript. HEB wrote, carried out the revision of the manuscript and translated the initial draft. JAH carried out the revision and complemented the manuscript.

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Sampling methods of symphylids in pineapple (*Ananas comosus* L.) crops in Santander, Colombia

Metodologías de muestreo de sinfílidos en cultivos de piña (*Ananas comosus* L.) en Santander, Colombia

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ABSTRACT

In the pineapple growing region of Lebrija, Santander, one of the largest such regions in Colombia, five farms were selected, and a sampling of symphylids associated with this crop was carried out in the first months of cultivation in the variety Perolera and hybrid MD2. Two collecting methods were compared: i) the destructive method, with soil inspection around the roots, and ii) the bait-trap method with pieces of potato mixed with soil. These two methods were implemented in the same plots during five bimonthly samplings. Additionally, the percentage of root damage was estimated, defined as the percentage of roots that show bifurcation due to the damage caused by symphylids. All symphylids were identified as *Hanseniella* sp. Sampling of symphylids based on underground potato bait traps requires fewer samples to estimate population density and is a predictor of root damage while destructive sampling is not. Consequently, trap sampling could be a useful tool for monitoring and managing symphylids on pineapple. The abundance was related to soil moisture, and not to soil pH.

Key words: arthropods, monitoring, Myriapoda, Symphyla, pests, traps.

RESUMEN

En la zona productora de piña de Lebrija, Santander, una de las más grandes de Colombia, se seleccionaron cinco fincas y se realizó un muestreo de sinfílidos asociados al cultivo en los primeros meses en la variedad Perolera y el híbrido MD2. Se compararon dos métodos de recolección: i) método destructivo, con revisión de suelo alrededor de las raíces, y ii) método con trampas con cebo de trozos de papa mezclado con suelo. Estos dos métodos se implementaron en las mismas parcelas durante cinco muestreos bimensuales. Adicionalmente, se estimó el porcentaje de daño en raíces, definido como el porcentaje de raíces que muestran bifurcación por el daño de los sinfílidos. Todos los sinfílidos se identificaron como *Hanseniella* sp. El muestreo de sinfílidos basado en trampas subterráneas con cebo de papa requiere menos muestras para estimar la densidad poblacional, y es un predictor del daño en las raíces, mientras que el método destructivo no lo es. De esta manera, el muestreo con trampas podría ser una herramienta útil para el monitoreo y manejo de sinfílidos en piña. La abundancia se correlacionó con la humedad del suelo y no con el pH del suelo.

Palabras clave: artrópodos, monitoreo, Myriapoda, Symphyla, plagas, trampas.

Introduction

The department of Santander has the highest pineapple production in Colombia, with 11,444 ha planted and a yield per area below the national average (Ministerio de Agricultura y Desarrollo Rural, 2017). Two pineapple materials predominate, the traditional variety Perolera and the hybrid MD2, also called “Oro miel”. The latter has increased in planted area in recent years and has a higher market price. However, the variety Perolera is widely accepted by farmers, has a market for agribusiness, and a higher tolerance to pests and diseases, according to growers. Although, there is information about arthropods associated with the variety Perolera (Morales Granados & López González, 2002), little is known about the susceptibility of the hybrid MD2 to pest arthropods due to its relatively short introduction.

During the pineapple vegetative growth phase and especially the first five months after planting, symphylids (Class Symphyla) are of special importance (Rohrbach & Johnson, 2003). They are thin, whitish arthropods between 1 - 8 mm long, and the adults have 12 pairs of legs and long antennae (Domínguez Camacho, 2015). They are generally associated with detritivore and omnivorous habits and there are few records of agricultural importance (Gerdeman & Diehl, 2021). In pineapple plants, symphylids consume tender

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roots and induce the formation of very short and not very functional roots that impede adequate nutrition, delay growth, and affect the anchoring of the plant (Saavedra, 1990; León, 1997). Due to damage by symphylids, a reduction of up to 67% of the roots fresh weight is reported under pot conditions (Agredo *et al*., 1988) and up to 70% in pineapple crops (Morales Granados & López González, 2002). They can also favor the proliferation of root diseases (Saavedra, 1990; Castañeda, 1998). Despite their importance, many aspects of the biology and ecology of neotropical symphylids are unknown, and this information could be the key to improving management and control practices in favor of less polluting and more sustainable procedures.

Symphylids have an aggregate distribution (Soler *et al.,* 2011). Therefore, locating their colonies within the crop would be the first step for their management. This activity is difficult because the symptoms caused by their damage, such as growth retardation and the reddish coloration of leaves, can also be confused with the damage of mealybugs (Agredo *et al.,* 1988; García Reyes, 1994). Symphylid monitoring has been mainly based on uprooting pineapple plants and observing their presence in the soil and roots (Morales Granados & López González, 2002). This destructive method can be inaccurate and difficult to standardize, making it hard to use as a decision tool. A new sampling methodology based on traps with a bait of potato pieces and soil has shown to be efficient and easy to use (Soler *et al*., 2011). However, its benefits have been neither evaluated nor compared with the traditional sampling methodology, nor has this method been related to the damage of symphylids in pineapple roots.

The objectives for this study were i) sampling and monitoring symphylids associated with pineapple plantations on the vegetative growth stage, ii) comparing the efficiency of two sampling methods and their relationship to root damage caused by symphylids, and iii) evaluating the influence of soil pH and moisture on the presence of symphylids.

Materials and methods

Selection of commercial pineapple plots

Five farms were selected in the pineapple growing area of the municipality of Lebrija, Santander with plots close to being planted, recently planted, or two weeks after planting, with the aim of monitoring symphylids in the vegetative growth phase (Tab. 1). Depending on the availability, they were searched so that the traps in both varieties were well distributed in the region.

Identifying and monitoring symphylids with bait traps

Bimonthly monitoring was carried out from the planting of the crop or a few weeks later to 8 months after planting, for a total of five samplings. In each farm, 10 to 20 symphylid traps were set within the pineapple crop with a separation between traps of at least 20 m. According to Soler *et al.* (2011), symphylid colonies have a width of between 4 - 6 m, and they are stable over time with little lateral displacement (Gerdeman & Diehl, 2021). Under these circumstances, a distance of 20 m was established to ensure that the traps were independent of each other. At each sampling time, 60 traps were installed, 30 for the variety Perolera and 30 for the hybrid MD2. The traps were installed at the same sampling points at each sampling time.

The symphylid traps consisted of plastic containers with perforations (Fig. 1A), to which potato pieces mixed with soil were introduced as bait; they were then buried and checked after 3 d (Fig. 1B). The traps had the same capacity (250 ml) as those originally proposed by Soler *et al.* (2011) and with enough perforations to expose the bait to the symphylids.

TABLE 1. Sampled pineapple farms of the municipality of Lebrija, Santander.

Rural district - farm	Georeferenced position	Altitude (m a.s.l.)	Variety/hybrid (n)	Planted area
La Aguada - El Remolino (REM)	7°11’42.1”N 73°10’22.8”W	834	Perolera (10)	2 ha
La Aguada - La Esperanza (ESP)	7°12’03.8”N 73°10’24.2”W	905	MD2 (10)	2 ha
La Aguada - Hoya Larga (HLA)	7°11’52.9”N 73°10’03.6”W	970	Perolera (5) and MD2 (5)	3 ha
La Aguada - El Diviso (DIV)	7°11’48.16”N 73°10’17.2”W	973	MD2 (10)	6 ha
La Puente - La Trinidad (TRI)	7°04’44.3”N 73°12’12”W	1317	Perolera (15) and MD2 (5)	4 ha

The values of (n) indicate the number of sampling points.

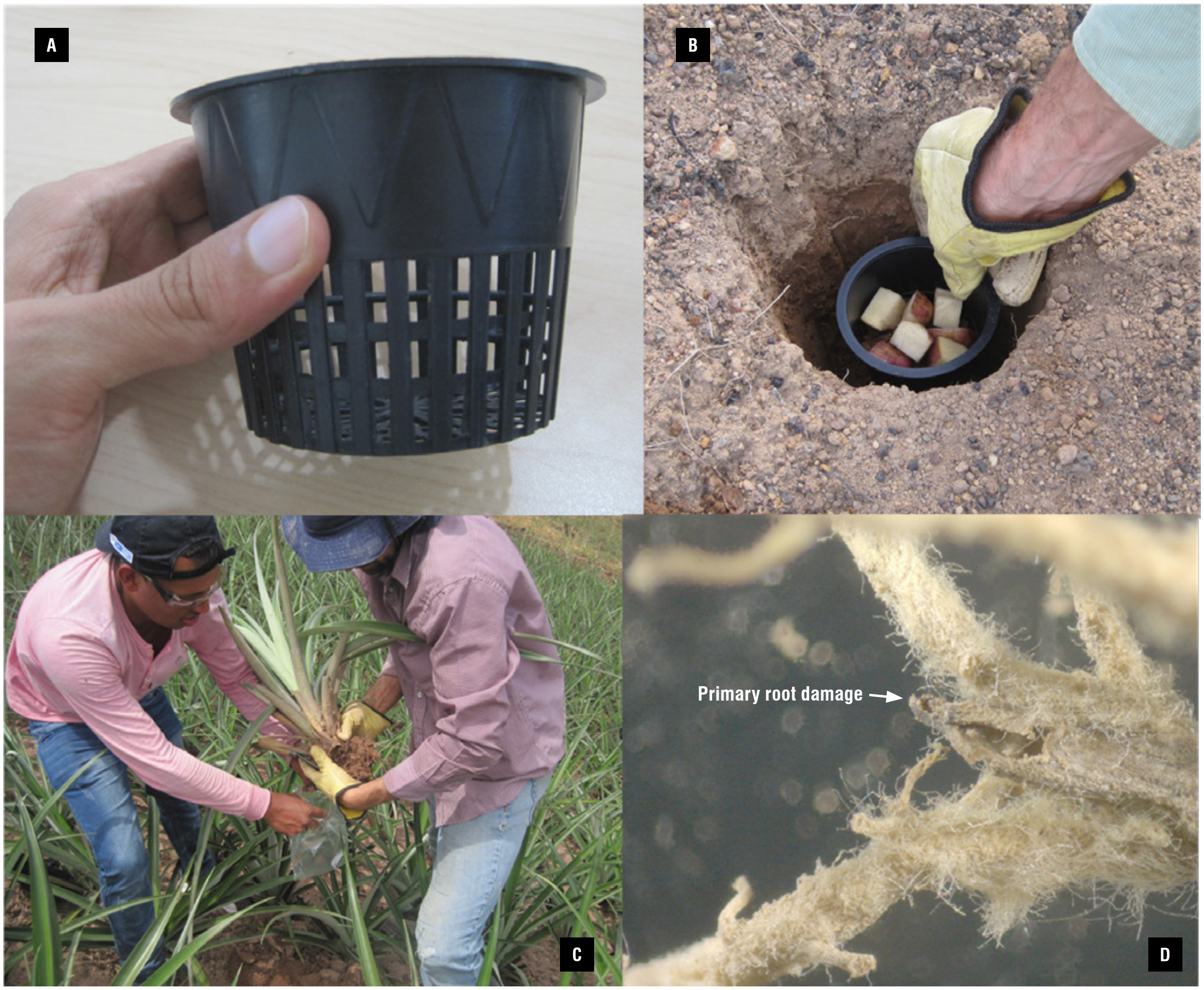

FIGURE 1. Symphylid sampling methodology. A) PVC plastic container used for sampling. B) Container with 50 grams of potato used as bait. C) Collection of plants. D) Bifurcation in the roots caused by symphylid damage.

The traps were transferred to the laboratory of La Suiza Research Center of the Colombian Agricultural Research Corporation - AGROSAVIA. There, they were thoroughly checked, spreading the soil and the bait in a dark-colored plastic tray where the captured specimens were collected with brushes and deposited in 75% ethyl alcohol.

All symphylids collected were placed into labeled alcohol vials. They were identified to genus with the taxonomic key of Scheller and Adis (1996) for the neotropical region. To compare the number of symphylids between sampling times for each farm, the non-parametric Kruskall-Wallis test and Mann-Withney pairwise comparison, Bonferroni corrected, were used in the PAST program version 1.86b (Hammer *et al.*, 2001). The collection of arthropods was carried out under the collecting permit 1466 of 2014 granted by the National Authority of Environmental Licenses (ANLA).

Comparison of sampling methods and their relationship with symphylid damage

The sampling with the destructive method consisted of taking the closest pineapple plant to each bait trap for each sampling time, trying to collect the root with approximately 1 kg of the surrounding soil (Fig. 1C). These roots and the soil were thoroughly inspected. Symphylids were identified as previously explained for the bait trap method. The soil was weighed to obtain the number of symphylids kg^{-1} of soil.

The damage caused by symphylids was quantified for each plant and measured as a percentage of affected roots. The affected root was estimated by the apex damage and subsequent bifurcation (Fig. 1D). This damage has been reported to be exclusively caused by symphylids (Agredo *et al.*, 1988; Soler *et al.*, 2011). Although it would have been desirable to evaluate a higher number of roots for this study, the number of roots per plant was low and only 10 were measured. The percentage of damage per sampling point was estimated using Equation 1:

$$\text{Percentage of root damage (\%)} = \frac{\text{affected roots}}{\text{total roots}} \times 100 \qquad (1)$$

To evaluate which of the two symphylid sampling methods was more efficient to estimate population density with fewer samples, the following equations proposed by Soler *et al.* (2011) were used:

$$n = (t_{0.005})^2 \times \frac{\left(\frac{1}{x} + \frac{1}{k}\right)}{d^2} \times 100 \qquad (2)$$

$$k = \frac{\bar{x}^2}{s^2 - \bar{x}} \qquad (3)$$

where n represents the necessary number of samples to estimate the symphylid density population in each farm and at each sampling time; k is the aggregation coefficient for a population that has a binomial distribution; $t_{0.05}$ is the t-student distribution value, and d the acceptable deviation from the population mean which in this case is 0.25, that is 25%. This analysis was performed only on farms with 10 or more samples of the same variety/hybrid.

Spearman's rank correlations coefficient (ρ) matrix was also carried out between the percentage of damage and the current and previous number of symphylids collected with the two sampling methods four months after planting. This was not done before because the plants had not rooted. This analysis sought to evaluate which of the two methods provided a better predictor of the percentage of root damage and to evaluate if the damage found was old or caused several weeks before. In that case, it would be more related to the symphylid registries of the two previous months than with the recent ones. The time after planting and plant weight were also added to the analysis. Additionally, symphylids in traps and symphylids captured by the destructive method were also correlated for each sampling time: 4, 6, and 8 months after planting, using the PAST program version 1.86b (Hammer *et al.*, 2001).

Relationship between soil pH and moisture and symphylid abundance

In three of the sampling times, one at planting, four months after planting, and at the end of the trial eight months after planting, the pH was measured using a potentiometer, and soil moisture was estimated by heating a sample of 20 g of soil taken from the same trap hole, 30 cm deep, in a microwave oven for 12 min, according to the methodology of Kramarenko *et al.* (2016).

To evaluate the relationship between pH and the percentage of soil moisture and symphylids, a correlation analysis was performed as previously described.

Results and discussion

Identification and monitoring of the incidence of symphylids

In general terms, symphylid abundance increased from planting to the end of the establishment of the crop eight months later (Fig. 2). The differences between farms in terms of the variations in the populations of symphylids were notable (Fig. 2). The context: slope level, resources, and farmer experience of each farm determined differences in the management practices that most affected the symphylids, such as plowing and pest management. A better understanding of the variations in their populations could be achieved if each farm were analyzed independently.

In all farms, land preparation for planting included plowing and burning, two practices that decrease the populations of symphylids (Gerdeman & Diehl, 2021). After that, populations of symphylids can increase up to five times in only two months (Fig. 2). Symphylids migrate vertically and, although they are superficially concentrated in the first 15 cm, they can go deeper up to 90 cm depending on soil conditions such as structure and water storage capacity, thus avoiding the effect of burning and plowing (Umble *et al.*, 2006; Gerdeman & Diehl, 2021). This way, surviving symphylids can recolonize the soil surface, coming from below after soil preparation (Sarah, 1990). Despite the benefits of plowing as a method of symphylid control, its practice is debatable because pineapple crops are located on land with slopes between 10% and 60% (García Reyes, 1994), where soil conservation practices such as minimal tillage would be recommended to decrease erosion.

Also, the use of pesticides at planting is a widely used and effective strategy for managing symphylids (Agredo *et al.*, 1988; Gerdeman & Diehl, 2021). Soler *et al.* (2011) record the absence of symphylids for up to four months after edaphic applications of insecticides with the active ingredient ethoprophos at the time of planting. Although ethoprophos has also been recommended in the growing area of Santander (Morales Granados & López González, 2002), farmers prefer to apply insecticides, mainly chlorpyrifos, in drench application at the time of planting or a few weeks later. However, other active ingredients are also used such as carbofuran, acephate, and thiamethoxan. These applications are more efficient at controlling the pineapple mealybug *Dysmicoccus brevipes* than symphylids, matching the farmer perception that symphylids are not a major pest in their crops. As a result, symphylid populations are maintained or increased a few weeks after applications, demonstrating the low efficacy of these products in controlling symphylids (Fig. 2).

The results of the taxonomic identification confirmed that the collected symphylids belong to the genus *Hanseniella*. Although *Scutigerella immaculata* (Newport) has been repeatedly recorded in the growing region of Santander (Morales Granados & López González, 2002) and other regions such as the eastern plains and Valle del Cauca (Agredo *et al.*, 1988; León, 1997), this species was not found in the samplings.

Comparison of sampling methods and their relationship to symphylid damage

Fewer specimens were collected with the destructive symphylid sampling method, while symphylids were captured in 70% of the samples with the traps. Using destructive sampling, they were only found between 12% and 19%. Checking the soil around plants is a destructive and inaccurate method since symphylids are quick and removing the soil alerts them to easily escape from the sample. Other factors such as daytime and the observer's skill can be crucial in generating bias. All these factors decrease the sensitivity of the destructive method and underestimate symphylid populations.

Both sampling methods performed a sample variance higher than their average, except in cases where the records are null or very few, showing that the distribution of symphylids is aggregated (Soler *et al.*, 2011). In most cases, sampling with bait traps requires fewer samples than the destructive method (Tab. 2). In the two methods, a similar time was spent per sampling point, so it was expected that sampling with traps would have values closer to the population mean when using the two methods (destructive and traps) with the same sample size or sampling effort.

The correlation matrix shows that root damage is related to sampling with bait traps and not to the destructive method (Tab. 3). The previous reading of symphylids with traps two months before was decisive in the percentage of damage. This may indicate that part of the damage registered was old damage that occurred several weeks before. Roots that are no longer functional because of symphylid attacks may take several weeks to decompose. Therefore, the registry of symphylids that is carried out at a certain moment will serve more to predict future damage to the roots than to infer current damage.

Damage decreases significantly with the time after planting (Tab. 3). Symphylid damage and impact depend on the age of the plant and is greatest in the first months after planting (Rohrbach & Johnson, 2003). According to Saavedra (1990), symphylid damage has the greatest impact in the period of root emission that, in our case, is the first four months after planting. Roots increase their growth gradually until anthesis, when they reach their greatest number (Malézieux *et al.*, 2003). This way, damage is compensated with an increase in radical growth six months after planting. Plants at intermediate or advanced stages of development and

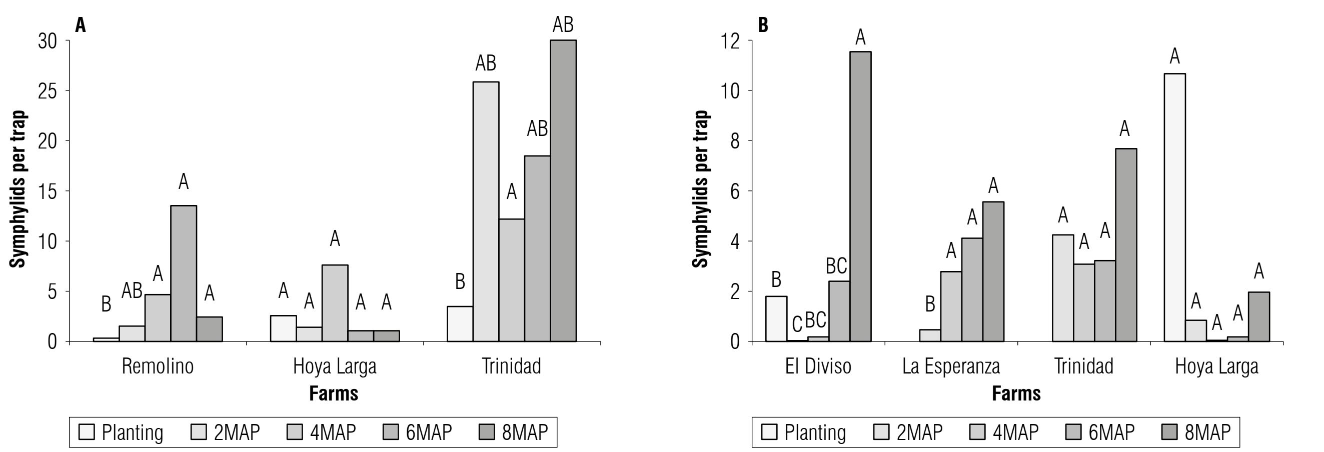

FIGURE 2. Symphylids in the establishment stage of the pineapple crop collected with traps with potato bait + soil. A) Variety Perolera; B) hybrid MD2. MAP - months after planting. Same letters indicate not statistically significant differences according to the Kruskall Wallis test and Mann-Withney pairwise comparison, Bonferroni corrected. For the hybrid MD2, there was no information on the captures of symphylids at the time of planting in the farms La Esperanza and Trinidad.

TABLE 2. Values of means, variance, dispersion coefficients (*k*), and number of samples necessary to sample symphylids (*n*) at four farms in the municipality of Lebrija, Santander.

Sampling	Farm - variety / hybrid	Method	Mean	s^2	*K*	*n* rounded
4 MAP	TRI - Perolera	Traps	12	90.4	1.8	49
		Destructive	0.5	3	0.1	788
	REM - Perolera	Traps	4.4	18.2	1.4	74
		Destructive	0.5	1.2	0.3	354
	DIV - MD2	Traps	0.2	0.1	-1.8	350
		Destructive	0	0	NA	NA
	ESP - MD2	Traps	2.8	6.8	1.9	69
		Destructive	1.7	8.5	0.4	228
6 MAP	TRI - Perolera	Traps	18.1	328.7	1.05	79
		Destructive	0.7	3.2	0.2	532
	REM - Perolera	Traps	13.1	989	0.2	455
		Destructive	0.8	1.8	0.6	230
	DIV - MD2	Traps	2.4	6.2	1.4	86
		Destructive	0.07	0.05	- 0.3	789
	ESP - MD2	Traps	4.1	27.2	0.7	127
		Destructive	1.2	6.6	0.2	356
8 MAP	TRI - Perolera	Traps	30.3	1952.6	0.5	168
		Destructive	0.9	1.3	2.6	112
	REM - Perolera	Traps	2.2	3.07	5.5	50
		Destructive	0.9	1.07	6.3	97
	DIV - MD2	Traps	11.5	70.9	2.2	42
		Destructive	0.3	0.8	0.3	439
	ESP - MD2	Traps	5.5	26.7	1.4	70
		Destructive	0.7	1.6	0.4	289

MAP - months after planting. Farm names: TRI - La Trinidad, REM - El Remolino, DIV - El Diviso, and ESP - La Esperanza.

TABLE 3. Spearman rank coefficient correlation (ρ) matrix between root damage percentage, plant weight, months after planting and the two symphylid sampling methods used.

	Plant weight	Root damage	Bait traps	Previous two months bait traps	Destructive method	Previous two months destructive method
Month after planting	0.29 **	-0.26**	0.16*	0.2*	0.19*	0.19*
Plant weight	------				0.18*	
Root damage		------		0.23**		
Bait traps			------	0.29**	0.27**	
Previous bait traps				------		
Destructive method					------	

Only statistically significant values are shown. Statistically significant correlations: *$P<0.05$; **$P<0.01$.

under good fertility conditions tolerate high populations of symphylids without affecting their growth and production (Castañeda, 1998).

The size of the plant has an influence on symphylid numbers in the destructive method (Tab. 3). Large plants may allow symphylids to shelter on the leaves that are in contact with the soil. This makes the base of the plant a more favorable environment for symphylids.

Although the correlation is significant between the two symphylid sampling methods (Tab. 3), when estimating the correlation for each sampling time, a significant correlation was found only in the months of September (ρ = 0.5; P = 0.009) and November (ρ = 0.41; P = 0.017) for the variety Perolera, while for this same variety for July (ρ = 0.39 and P = 0.06) it was not, nor for the three samplings in the hybrid MD2 (July ρ = 0.21, P = 0.27; September ρ = -0.01, P = 0.95; November ρ = 0.2, P = 0.28). Sampling in the variety Perolera for September and November showed the highest population density of symphylids from 10.8 to 11.7 symphylids per trap. When the density of symphylids was lower between 1.5 and 7.9 symphylids per trap, no correlation was found between the two sampling methods. This would indicate that the destructive method only achieves similar results as the traps when the density of symphylids is high. This result confirms the advantage of traps to detect low populations of symphylids so as to apply appropriate management strategies when populations and damage are at low levels.

Due to the greater susceptibility of the roots of seedlings at early stages of development, symphylid management practices should begin before land preparation. Sampling with baited traps would be useful to detect colonies of symphylids that can remain constant from year to year with little lateral displacement (Gerdeman & Diehl, 2021). To estimate the population density of symphylids, Gerdeman and Diehl (2021) recommend a sampling of at least 50 baited traps depending on the size of the lot and the time of year, but they do not recommend a specific number of traps ha^{-1}. In our case, in most of the evaluations, an adequate estimate would be reached (except for some farms with more variability) with 75 well distributed samples. This could be an adequate minimum of samples in pineapple crops of 1 ha. Once the colonies are detected, control strategies can be targeted, reducing costs and pesticide applications.

To carry out sampling with baited traps, an estimated time of 8 h for installation and 4 h for the collection of traps is required. The inspection and extraction of symphylids required around 15 to 20 min per trap, which would indicate that a total of 19 - 25 h is needed for the 75 traps. This way, all the sampling would be carried out in 31 - 37 h (4 - 5 d) by a worker trained in recognizing symphylids. This investment in time might seem too high if the farmer does not see a tangible benefit from this effort. Although the damage of symphylids is notable and on average affects 40% of the roots, the effects on the reduction of production, delay in the life cycle, and costs of foliar fertilizers and pesticides have not been quantified, and a large percentage of farmers have not recognized symphylids as a severe problem. A research study considering these factors will be necessary to demonstrate the convenience and profitability of integrated symphylid management based on sampling with traps and appropriate and environmentally friendly management practices.

Relationship between soil pH and moisture and the presence of symphylids

The correlation between symphylids in traps and soil moisture is statistically significant (P = 0.282; P = 0.00012), confirming that percentage of soil moisture is a crucial factor in the distribution of symphylids. Although, the correlation coefficient was positive, it was expected that high soil moisture values could also decrease symphylid mobility and development (Sarah, 1990). When evaluating the preferences of soil arthropods, Ghiglieno *et al.* (2020) found that Symphyla is correlated with low moisture soils (<35%). In pineapple crops, the soils were in the range between 6.7% and 25%. This correlation is limited to this range of moisture. Similarly, Edwards (1961) found a significant correlation between the abundance of symphylids and soil moisture in the range between 7.5% and 15.5%.

Regarding soil pH, values were in the range from 3.1 to 5.6 that indicates strongly to extremely acid soils. No significant correlation was reported between pH and symphylids in traps (P = -0.17; P = 0.572). Umble *et al.* (2006) mention that the presence of the symphylid *Scutigerella immaculata* is not related to pH and can be found in very acidic to alkaline soils. Salazar-Moncada *et al.* (2015) and Ghiglieno *et al.* (2020) also find no relationship between the Symphyla and soil pH.

Conclusions

Symphylid sampling based on potato bait traps is superior to the practice of checking pineapple roots to sample symphylids. This methodology is easier to standardize, requires fewer samples, and is a predictor of root damage. Its incorporation into this production system can be a very useful tool for monitoring and managing symphylids.

Evaluation of symphylid monitoring based on baited traps is recommended as a decision tool in pineapple crops before planting. A number of at least 75 traps ha^{-1} is recommended to have an estimate of the population of symphylids.

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

JMM designed the methodology. JFO and JMM carried out field sampling, laboratory processing, data analysis, writing and revision of the final document.

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Abundance and flight activity of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in a female chrysanthemum crop for seeding, Colombia

Abundancia y actividad de vuelo de *Frankliniella occidentalis* (Thysanoptera: Thripidae) en un cultivo hembra de crisantemo para obtención de semillas, Colombia

Andrés Felipe Silva-Castaño[1] and Helena Luisa Brochero[2*]

ABSTRACT

Commercial flower crops in Colombia are largely grown within plastic greenhouses in the Sabana de Bogotá (Bogota Plateau). This study examined the abundance and flight activity of thrips in a chrysanthemum crop, estimated from plant samples, commercial yellow sticky traps, and non-commercial (self-crafted) yellow sticky tape traps installed in the crop. *Frankliniella occidentalis* was the dominant species present associated with all plant phenological stages. Abundance of larvae and adults was not evenly distributed throughout the greenhouse. An absolute method of plant sampling found 2.5 times more thrips larvae than the relative method of sampling plants by beating. For non-commercial traps, there was no difference in the number of thrips at different trap heights; however, traps facing the south-west caught significantly more thrips than traps facing north-west, north-east, and south-east. A moderate positive correlation was found between the number of thrips sampled on plants and the spatial location of the commercial sticky traps. The results found here provide a basis to design and standardize direct sampling methods for thrips on plants and indirect sampling using yellow sticky traps for monitoring and managing thrips in ornamental crops under plastic covers in Colombia.

Key words: sticky traps, western flower thrips, pest monitoring, floriculture.

RESUMEN

Los cultivos comerciales de flores en Colombia se realizan principalmente en invernaderos de cubierta plástica en la Sabana de Bogotá. Este estudio examinó la abundancia y la actividad de vuelo de los thrips en un cultivo de crisantemo, a partir de los individuos capturados directamente en las plantas y los obtenidos de trampas amarillas con pegante, tanto comerciales como no comerciales (artesanales), instaladas en el cultivo. *Frankliniella occidentalis* fue la especie dominante y presentó asociación con los diferentes estadios fenológicos de las plantas muestreadas. La abundancia de larvas y adultos no se distribuyó uniformemente en el invernadero. El método absoluto de muestreo de plantas encontró 2.5 veces más larvas de thrips que el método relativo por golpeo en plantas. Para las trampas no comerciales, no hubo diferencia en el número de thrips a diferentes alturas de las trampas; sin embargo, las trampas orientadas hacia el suroeste capturaron significativamente más thrips que las trampas orientadas al noroeste, noreste y sureste. Se encontró una moderada correlación positiva entre el número de thrips muestreados en las plantas y la ubicación espacial de las trampas comerciales. Los resultados que aquí se presentan proporcionan una base para diseñar y estandarizar métodos de muestreo directo para thrips en plantas y muestreo indirecto utilizando trampas amarillas con pegante para el monitoreo y manejo de thrips en cultivos ornamentales bajo cubiertas plásticas en Colombia.

Palabras clave: trampas adhesivas, thrips occidental de las flores, monitoreo de plagas, floricultura.

Introduction

Colombia is the second country for fresh cut flower exports in the world (Vanegas López *et al*., 2017). Floriculture for exports is concentrated in six municipalities of the Sabana de Bogotá (Bogota Plateau), with production of roses, carnations, alstroemerias, gerberas, and mixed floral bouquets with sales of around 1.5 billion US dollars/year (González *et al*., 2014). The flower sector constitutes a non-traditional agricultural export that is based on cheap labor, fertile soil, an equatorial climate that provides around 12 h of sunshine per day throughout the year, and more than fifty years of experience (Iizuka & Gebreeyesus, 2018). This industry provides the financial and social support for workers in rural areas with nearly 200,000 employees and an important participation of women who are mainly single heads of households (Lee, 2008; Patel-Campillo, 2010).

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Frankliniella occidentalis (Pergande, 1895) (Thysanoptera: Thripidae) is considered a significant pest for flower production and exports in Colombia (Loyola *et al*., 2019). This thrips species feeds and lays eggs directly in plant tissues causing malformation of plant structures during production, detrimental cosmetic damage for exports, transmission of virus to plants in crops that cause reduction of yield (Reitz *et al*., 2020) and quarantine issues for international trade (Nicholas & Follett, 2018). In Colombia, control of western flower thrips (WFT) is based on weekly insecticide applications during crop production and phosphine treatments for postharvest management (Kim *et al*., 2016) despite the initial development of some biological control strategies (Rueda-Ramírez *et al*., 2018).

For monitoring thrips populations in commercial greenhouses for exports in the Sabana de Bogotá, a direct sampling of thrips from the buds and flowers by a gentle beating of plants is carried out to determine the density of thrips per plot (relative sampling) (Pizzol *et al*., 2010). Additionally, plastic or acrylic yellow, white, and blue cards with insect glue are installed in the crop to determine the relative abundance and spreading of flying thrips (Corredor, 1999). In general, for standard direct plant sampling protocols in Colombia, there is no discrimination between the different species present or of population structure (larvae versus adults). This is given by the fact that, due to quarantine export regulations, no thrips should be allowed in any container (threshold = 0). In contrast, sticky cards require technical personnel for selecting colors and materials for application of glue for their use, periodic installation, counting of insects, and the incorporation of these data into entomological surveillance for thrips management strategies. These traps do not have standardized colors determined by spectral measurements or studies to define the cardinal orientation optimizing trap capture for monitoring or trapping of thrips, as is recommended by several authors (Pearsall & Myers, 2001; Ben-Yakir & Chen, 2008; Aliakbarpour & Rawi, 2011). In Colombia, information obtained from colored sticky traps is difficult to interpret by crop managers and management consultants. There are no studies showing the relationship between thrips caught on self-crafted or commercial, colored sticky traps and thrips infestation on plants and thrips damage. In general, since there are no clear criteria to incorporate data from colored sticky cards for surveillance or mass capture of thrips, they are usually abandoned by the phytosanitary programs of each floriculture company. The aim of this study was to establish the abundance and flight activity of *F. occidentalis* in a female chrysanthemum crop for seed production by using two different methods of direct sampling and one indirect sampling (yellow sticky traps) for thrips on plants at different phenological stages.

Materials and methods

Chrysanthemum crop conditions

This study was carried out in a female chrysanthemum crop for seed production in a plastic greenhouse located in Madrid, Cundinamarca, in the Sabana de Bogotá, Colombia (4°44' N, 74°17' W). The floor area of the greenhouse was of 1,088 m^2 divided into five compartments, each separated by 0.45 m. The first area had five flower beds 0.6 m wide by 30 m length on soil substrate, and the other four areas had six flower beds of the same size. Average (18.4°C), maximum (40.3°C), and minimum (5.6°C) temperatures and relative air humidity (67.9%) were recorded every minute per day using three dataloggers distributed inside the greenhouse.

During this study, several varieties of chrysanthemum at different plant phenological stages (*e.g.*, vegetative, flowering, senescence, and seed harvest) were present in the greenhouse at the same time, in different beds due to the production cycle. Thrips control was carried out with regular use of chemical insecticides directed at all plant phenological stages, except for those associated with the collection of pollen. The greenhouse had a main entrance with trap doors to prevent thrips from entering and moving within the greenhouse and a disinfection area for workers to get dressed and clean their shoes before entering the production area. No thrips control was done in external greenhouse areas. During the day, greenhouse temperature and relative humidity were regulated using vertical screens that could be raised and lowered between areas, but during the night, the temperature was regulated by three heaters located on the headers of areas one to three, while six fans distributed the heat. Ventilation was enabled through two longitudinal openings on the ceiling of areas one and five.

The plant material sampled for control of WFT contained plants at different stages of development: vegetative stage (VS) (1 to 8 weeks after planting), flowering stage (FS) (9 to 13 weeks after planting), senescence stage (SS) (14 to 18 weeks after planting), and seed harvest (SH) (19 weeks after planting). A grid of 454 cells (each one of 0.3 m x 0.24 m) was superimposed over the five beds to assist the selection of plants to be sampled.

Thrips plant infestation

Two direct sampling methods were used to estimate the abundance of WFT on chrysanthemum plants within the greenhouse. The relative method consisted of the gentle beating of buds and flowers (~20 sec for each plant) from a total of 240 plants (FS = 123, SS = 101, SH = 16) over a white surface (~0.15 m of diameter) where the adults and immature thrips were collected with a soft brush and preserved in 70% ethanol in plastic 1.5 ml vials labelled with the phenological stage of plants, date, and hour of collecting. The sample unit corresponded to one plant per cell (*i.e.*, 240 plants). At the time that the relative samples were undertaken, there were no plants in the vegetative stage. The absolute method included the removal of 93 whole plants (VS = 12, FS = 51, SS = 30) from the planting beds, which were immediately immersed into 70% ethanol in plastic bags and preserved at -20°C. To protect the seeds obtained in the harvest sample (SH), plants at this phenological stage were not sampled. In the laboratory, adult thrips and immatures were counted from the ethanol solution in the sample and from the plant after careful dissection of plant material under a Nikon SMZ800® stereomicroscope (80X). Sampling was carried out in the greenhouse in March (relative method) and April (absolute method) 2018 between 9:00 and 11:00 h. Thrips were identified; 1% of the total females from direct sampling and 0.5% of females from the sticky traps were macerated and mounted on microscope slides (Mirab-Balou & Chen, 2010). Taxonomic identification was based on morphological characters of the females (Mound & Kibby, 1998; Cavalleri & Mound, 2012). The entomological material was processed at the Laboratorio de Entomología, Área Genética de Insectos de Interés Económico, Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogotá.

The mean number of adult and immature thrips per plant was obtained for each plant development stage. Kruskal-Wallis test (Conover & Iman, 1981) followed by Dunn's multiple comparison test (Dunn, 1964) were conducted to determine if the density of thrips changed with the plant developmental stage for each sampling method. Also, a Spearman's correlation (Puth *et al*., 2015) was made for the two sampling methods to assess the relationships between number of thrips and phenological stages.

Thrips flight activity

Non-commercial (self-crafted) yellow sticky tape traps (1.35 m x 0.2 m) with manually applied adhesive glue (Biotrapper®, Agrotrampas Colombiana Ltda. Bogotá, Colombia) on both faces were used to examine thrips flight activity. To examine the effect of trap height and cardinal orientation for monitoring thrips, 28 yellow sticky tape traps were placed in the greenhouse for three consecutive days in February. The bottom of each trap was placed at 0.50 m above of ground and the total height of each one was 1.85 m. Each of the yellow sticky tape traps were divided in nine squares of 0.15 m x 0.15 m, where each square (S) was represented by a different height of capture in meters from the ground (S1 = 1.85 to 1.70, S2 = 1.70 to 1.55, S3 = 1.55 to 1.40, S4 = 1.40 to 1.25, S5 = 1.25 to 1.10, S6 = 1.10 to 0.95, S7 = 0.95 to 0.80, S8 = 0.80 to 0.65, and S9 = 0.65 to 0.50). Of these 28 traps, ten yellow sticky tape traps were positioned with trapping surfaces facing the north-west/south-east and 18 yellow sticky tapes with trapping surfaces facing the south-west/north-east. The mean number of adult thrips per trap was estimated by visual observation of the thrips on the traps and the number determined for each combination of trap height and orientation. Statistical differences were estimated using Kruskal-Wallis test (Conover & Iman, 1981) and multiple comparison of Dunn's test (Dunn, 1964). The relationships between the number of thrips vs. height and orientation of the non-commercial yellow sticky tape traps were estimated using Spearman's correlation (Puth *et al*., 2015).

Fifteen commercial yellow sticky traps (40 cm x 25 cm, Impact Sticky Board Yellow - Russell IPM®) were placed 7.5 m apart in the crop throughout the greenhouse. The commercial traps were inspected over a period of 10 d in March. The cumulative number of thrips per trap at different locations in the greenhouse was compared using a one-way ANOVA and a multiple comparison Duncan test (Zar, 2010). The relationships between number of thrips and the spatial location of the commercial traps were estimated using a Pearson's correlation (Obilor & Amadi, 2018).

For each commercial yellow sticky trap and non-commercial yellow sticky tape trap, the total number of thrips was counted under a Nikon SMZ800® stereomicroscope (80X) and 1% of the randomly selected samples of each trap were taxonomically identified based on morphological characters (Mound & Kibby, 1998; Cavalleri & Mound, 2012). All statistical analyses were done using the software R v 3.5.0 using $\alpha = 0.05$.

Results and discussion

Thrips plant infestation

A total of 3,688 larvae, 8,513 adult females, and 325 adult males were collected. *Frankliniella occidentalis* (N = 7,856; 62.7%) was represented by three morphotypes based on the exoskeleton color. WFT morphotypes have been previously reported (Bryan & Smith, 1956; Elimem *et al*., 2011) and thought to have developed in response to changes in seasonal and environmental factors, such as temperature.

Additionally, two more species of thrips were recorded: *Frankliniella* sp. 1 (female N = 627; 5%) and *Frankliniella* sp. 2 (female N = 30; 0.2%). Since *F. occidentalis* was the dominant species in this study with a ratio of 40 females:1 male, it is recognized as the main pest in chrysanthemum crops for seeding (Wu *et al*., 2021). As there are no taxonomic keys based on morphological characters for larvae and males of thrips, these developmental insect stages were assumed to belong to WFT. For statistical analyses, only females of WFT were included.

The relative beating sampling method allowed the recovery of 1,033 larvae, 146 adult males, and 5,301 adult females of *F. occidentalis,* 519 females of *Frankliniella* sp. 1, and 22 females of *Frankliniellla* sp. 2 from 240 plants (29 thrips/plant). There were significant differences in thrips abundance (*P*<0.01) associated with the plant phenological stages with higher larvae infestation on the flowering stage (Tab. 1).

The spatial distribution of larvae was not correlated with the adult distribution inside the greenhouse (Fig. 1). The highest abundance of total adult thrips was found at the senescence stage sample followed by the flowering stage (Tab. 1) with the greatest abundance of thrips located at the north end of the greenhouse (Fig. 1, large, dotted circle). Unlike the adults, the larvae were found in greater abundance in the south of the study area and in the plants at flowering stage (Fig. 1, small, dotted circles). The abundance of several thrips species is influenced by the phenological stage of plants (Cárdenas & Corredor, 1989; Mejía *et al*., 2018), because larvae and adults have different feeding habits, nutritional requirements, and physiological pathways to detoxify plant defenses (Kirk, 1997; Chau & Heinz, 2006; Mouden *et al*., 2017). Additionally, the distribution of immatures and adults can be different since once females have laid eggs on a plant, they migrate to other neighboring plants (Rhainds & Shipp, 2004).

The absolute sampling method resulted in a total of 2,655 larvae, 179 adult males, and 2,555 adult females of *F. occidentalis,* 108 adult females of *Frankliniella* sp. 1, and 8 adult females of *Frankliniella* sp. 2 from 93 whole plants that represented an average infestation of 59 thrips per plant (Tab. 2). Larvae, female, and male infestation were found from flowering to senescence stages (*P*<0.01) (Tab. 2).

No correlation (*P*>0.09) was found between number of thrips and those phenological stages sampled with the relative sampling method; however, a moderate negative correlation (rho = -0.4, *P*<0.01) was found between the number of thrips and those phenological stages sampled using the absolute method. The larvae were detected twice as much with the absolute whole plant samples compared to the relative samples (beating of flowers). The larvae may prefer to take refuge and remain in the leaves of the plant (Hansen *et al*., 2003), while the natural thigmotactic behavior of adult thrips allows them to escape (fly) during the removal of plants. Since the relative sampling for monitoring thrips in ornamental production in Colombia is focused on the flowering stages, the real infestation in the crop is probably underestimated (Sutherland & Parrella, 2011). Discriminating the structure of thrips populations in the greenhouse is important, not only as an indicator of resident populations that complete their full life cycle in the greenhouse (like those in this study) but also for the sensitivity of larvae to acquire and transmit orthophotospovirus to plants, where chrysanthemums are particularly susceptible to being infected (He *et al*., 2020; Zhao & Rosa, 2020). Because males are more efficient at transmitting viruses to plants (Ogada & Poehling, 2015), the absolute sampling that allows detection of more larvae and males is an important tool for preventing virus outbreaks in chrysanthemums.

TABLE 1. Mean number (± standard error) of larvae and adult thrips collected using the relative beating sampling method at different plant phenological stages.

Thysanoptera (Thripidae)	Plant phenological stages			
	Vegetative N = 0	Flowering N = 123	Senescence N = 101	Seed harvest N = 16
Frankliniella occidentalis (females)	-	17.2 (± 0.3)[a]	29.3 (± 0.5)[b]	13.9 (± 0.6)[ab]
Frankliniella sp. 1 (females)	-	2.3 (± 0.2)[b]	2.4 (± 0.3)[b]	0.1 (± 0.2)[a]
Frankliniella sp. 2 (females)	-	0.1 (± 0.1)	0.1 (± 0.1)	0
Males	-	0.8 (± 0.1)[b]	0.5 (± 0.1)[ab]	0.1 (± 0.3)[a]
Larvae	-	7.5 (± 0.4)[b]	1.1 (± 0.2)[a]	0.1 (± 0.2)[a]

The letters represent the groups formed by the Dunn's test between stages.

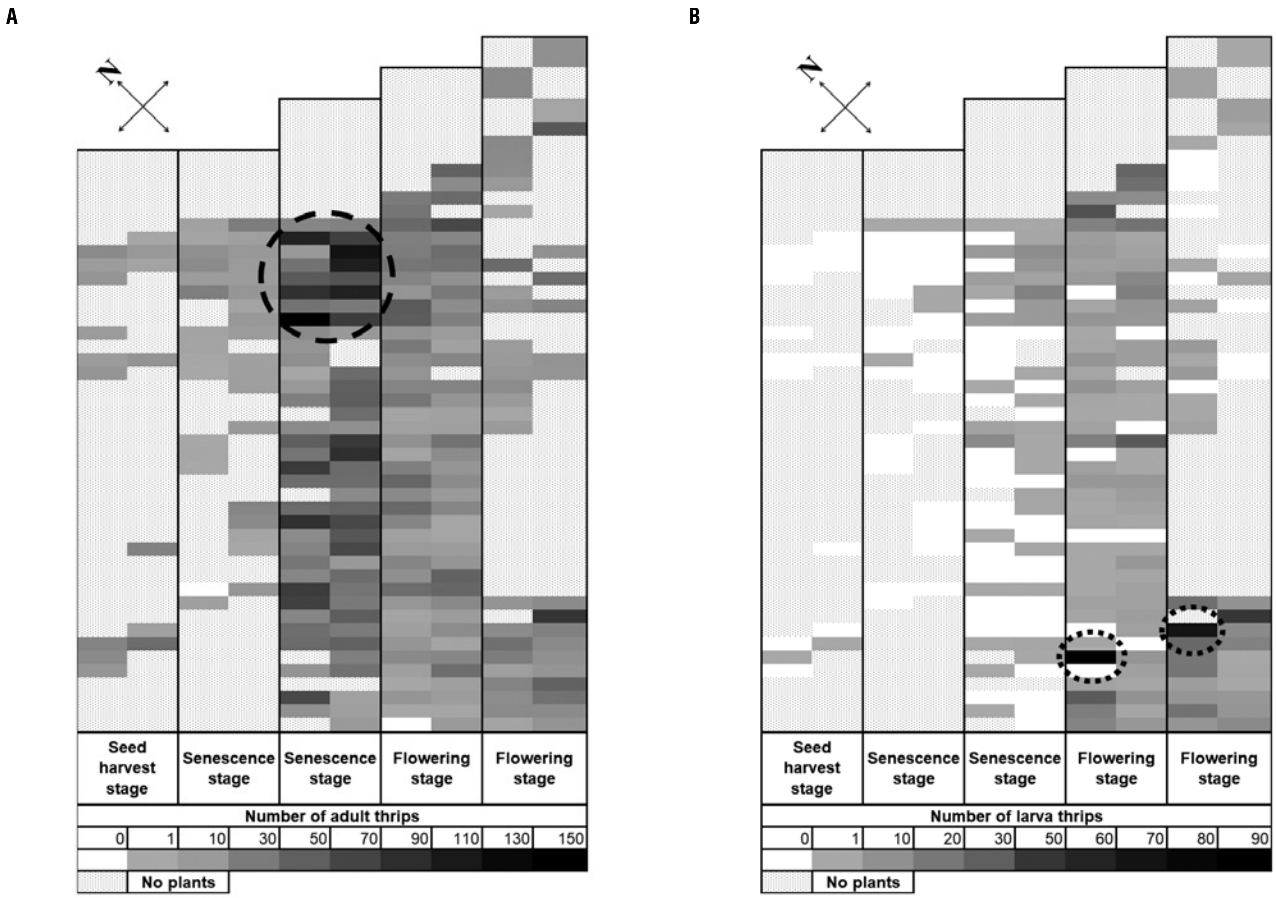

FIGURE 1. Heat map illustrating the spatial distribution of A) adults and B) larvae of thrips sampled from a range of phenological stages on a female chrysanthemum crop for seeding in a greenhouse at the Sabana de Bogotá, Colombia.

TABLE 2. Mean number (± standard error) of larvae and adult thrips collected using the absolute sampling method at different plant phenological stages.

Thysanoptera (Thripidae)	Plant phenological stages			
	Vegetative N = 12	Flowering N = 51	Senescence N = 30	Seed harvest N = 0
Frankliniella occidentalis (females)	0.25 (± 0.26)[c]	38.6 (± 0.8)[a]	19.5 (± 0.5)[b]	-
Frankliniella sp. 1 (females)	0	0.6 (± 0.4)[a]	2.6 (± 0.4)[b]	-
Frankliniella sp. 2 (females)	0	0.03 (± 0.1)	0.2 (± 0.1)	-
Males	0	1.9 (± 0.2)[a]	2.7 (± 0.3)[a]	-
Larvae	0	24.1 (± 1.4)[a]	47.6 (± 1.7)[a]	-

The letters represent the groups formed by the Dunn's test between stages.

In ornamental crops in the Sabana de Bogotá, thrips populations can reside permanently in the greenhouse because of relatively stable biotic conditions, weak plant defenses since breeders select the cosmetic attributes of the crop, and good food sources as plant fertilization is regularly applied. The genetic background of these resident thrips is improved by genes from dispersal and migrant thrips that enter through ventilation openings and main doors of the greenhouse. In this scenario, historical data on thrips plant infestation through sampling by relative and absolute methods is important to determine the structure, variation and patterns of spatial and temporal distribution of the thrips species in the greenhouse. This may help to reduce sampling costs by early detection of peak populations and sampling efforts focusing on hotspots of thrips and receptive zones for new colonization (Natwick *et al.*, 2007). This can be done without compromising robust information for the monitoring and control of thrips, not only to reduce losses in production but also to reduce quarantine interceptions during exports.

Thrips flight activity

Non-commercial yellow sticky tape traps (self-crafted) that faced south-west caught significantly (*P*<0.01) more thrips (n = 9.937[c]) than traps facing north-west (n = 1.979[a]), north-east (n = 960[bc]), and south-east (n = 1.267[ab]). No statistical differences were found (*P*>0.42) between the number of thrips versus the height for thrips caught on the vertical oriented non-commercial traps: S1 (1.85 to 1.70 m) = 1,046 thrips; S2 (1.70 to 1.55 m) = 1,224 thrips; S3 (1.55 to 1.40 m) = 1,472 thrips; S4 (1.40 to 1.25 m) = 1,427 thrips; S5 (1.25 to 1.10 m) = 1,555 thrips; S6 (1.10 to 0.95 m) = 1,715 thrips; S7 (0.95 to 0.80 m) = 1,830 thrips; S8 (0.80 to 0.65 m) = 1,988 thrips, and S9 (0.65 to 0.50 m) = 1,886 thrips. Sticky traps are generally installed at the height of the plants or a few inches above them (Brødsgaard, 1989); however, this study showed that at different heights, a high number of thrips were captured. The positive attraction response of each thrips species or thrips populations at different wavelengths using passive colors such as those on colored sticky cards and a high number of thrips infesting a crop (as in our study) can influence the effectiveness of traps to capture thrips for monitoring and mass trapping (Prema *et al*., 2018; Stukenberg *et al*., 2020). In rose crops, the blue traps at canopy level capture significantly more thrips than the yellow traps; however, the yellow traps show no significant differences for capturing thrips relating to the height from the ground (Khavand *et al*., 2019). A low positive correlation was found between the number of thrips vs. the height and orientation of the non-commercial traps (*rho* = 0.11, *P* = 0.01; *rho* = 0.24, *P*<0.01, respectively). This may be due to the limited sampling time used in this study. Nevertheless, the height and cardinal orientation of the traps increase the attraction of thrips species to the traps (Mao *et al*., 2018; Shin *et al*., 2020).

Commercial traps caught significantly more thrips (*P*<0.01) in the northern zone (N = 5,895[a]) of the greenhouse in comparison with the central zone (N = 4,305[ab]), the edge of the northern zone (N = 3,789[ab]), the southern zone (N = 2,681[bc]) and the edge of the southern zone (N = 1,116[c]) of the study area. A moderate positive correlation was found between number of thrips in plant samples and the spatial location of the commercial traps (rho>0.6, *P*<0.01).

This study showed that the trap's cardinal orientation and thrips spatial distribution within a crop and the standardized color and glue in sticky traps play an important role for the monitoring and mass trapping of thrips as an essential compound of insect pest management. Sticky colored traps allow a relative estimation of the density of adult flying thrips (Rhodes *et al*., 2011; Liansheng *et al*., 2013). This information is fundamental for developing models of spatial thrips distribution patterns using geostatistical techniques to predict dispersion of thrips inside the greenhouse; along with the abiotic conditions, these could contribute to define an early warning system for thrips management. However, colored sticky traps do not provide real information about the species and the population structure of thrips in greenhouses (Lewis, 1997) because larvae, as the biological stage for acquisition of orthotospovirus, are not represented in these (Rotenberg *et al*., 2015).

Frankliniella occidentalis has shown different levels of attraction to different shapes of sticky traps (Mainali & Lim, 2010), and the species is positively attracted to blue (Otieno *et al*., 2018; Khavand *et al*., 2019) and yellow colors (Rőth *et al*., 2016). Despite this, in chrysanthemum crops in the Antioquia region of Colombia, growers use yellow sticky traps for thrips monitoring (Mejía *et al*., 2018). In the Sabana de Bogotá, western flower thrips also show attraction for white and purple sticky cards (Cárdenas & Corredor, 1989). The positive phototaxis of thrips to colored cards is mediated by the specific photoreceptors of the species (Matteson *et al*., 1992; Röth *et al*., 2016) and the surface of the material and the glue (van Tol *et al.*, 2021) that define specular or diffuse sunlight reflection (Davidson *et al*., 2015). The cardinal orientation of traps facing the sunlight directly or indirectly affects the reflectance of the light as an important factor for thrips response to the passive light. The architecture of the crop in its different phenological stages and external interferences, such as UV filters in plastics or nets in the greenhouse, affect thrips flight and their response to attraction cues. For example, *F. occidentalis* shows reduced dispersal and lower rates of attraction to yellow sticky traps under UV-deficient environments (Kigathi & Poehling, 2012). Optical response of western flower thrips populations in Colombia as an essential component of thrips monitoring and control in ornamental crops in the Sabana de Bogotá requires more studies to assess the response to different wavelengths, alone and in combination with semiochemicals (Sampson & Kirk, 2013; Abdullah *et al*., 2015; Cao *et al*., 2018; Kirk *et al*., 2021) and to evaluate their impact on beneficial insects for biological control of thrips and pollinators (Ogino *et al.,* 2016; van Tol *et al.,* 2020).

Floriculture systems in the Sabana de Bogotá are based on crops under plastic cover with different varieties and phenological stages for one or several plant species. Critical fluctuations in abiotic factors are managed through heaters, fans, and UV filters on plastic covers to protect the physiology of plants and to synchronize crop management (De Gelder *et al*., 2012; Shamshiri & Ismail, 2013). Density of plants and the aeration systems based on openings located in the ceiling of the greenhouse generate micro and macro environments that modulate the interaction between thrips and their hosts (Rhainds *et al*., 2007). All these conditions produce an ideal matrix for the establishment and maintenance of thrips populations according to their nutritional requirements, strategies to find shelters, and escape from control strategies, particularly during the spraying of chemical pesticides. Thrips management in flower crops for export from the Sabana de Bogotá is based on weekly chemical insecticide spraying. However, as the results in this research have shown, studies on thrips population dynamics, population structure, and insect-plant relationships provide a base line for implementing control strategies for thrips (Hillocks, 2002). It is necessary to design and standardize relative and absolute sampling methods to evaluate thrips management and complement the information obtained from sticky color traps. Only through these methods, it will be possible to implement control strategies for thrips that are environmentally friendly and that allow a pest and residue free production of export flowers to maintain the market in accordance with the regulations of quarantine and chemical traceability required by each country.

Conclusions

Taxonomic identification of thrips species collected by direct and indirect sampling provide the basis to develop effective integrated pest management strategies. This study has shown a resident population of *F. occidentalis* with high numbers of females in flowering plants and a differential spatial distribution of the larvae and adults in the greenhouse. Direct sampling of plants by relative and absolute methods provides information to determine the species and stages of development of thrips infesting plants at different phenological stages and to infer patterns of the distribution of thrips using geostatistical models. Yellow sticky traps faced south-west captured more thrips, but the height of these in the crop was not significant to increase their capture. Standardized sticky traps must be assessed in different crops and greenhouse conditions to enhance this strategy for monitoring and mass trapping thrips within an integrated pest management framework. Despite the limitations in the sampling time because the crop was eradicated by high infestation of external and resident populations of thrips, this study contributed to the knowledge of the western flower thrips populations in Colombian greenhouses and thrips monitoring in chrysanthemum crops.

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Conflict of interest statement
The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions
AFSC and HLB planned and supervised the study; AFSC performed the experiments and the analysis of data; both authors discussed the results and wrote the manuscript.

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Acaricidal activity and repellency of commercial essential oils on *Tetranychus urticae in vitro* and protected cultivation

Actividad acaricida y repelencia de aceites esenciales comerciales sobre *Tetranychus urticae in vitro* y cultivo protegido

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ABSTRACT

This study analyzed the toxicity by residual contact and the repellency effect of the essential oils of *Rosmarinus officinalis*, *Mentha piperita*, *Melaleuca alternifolia,* and *Commiphora myrrha* and their mixtures on adult females of *Tetranychus urticae* in laboratory and protected cultivation. The essential oil of *C. myrrha* exhibited a LC_{50} of 0.55 ml L^{-1}, and the mixtures *R. officinalis + M. piperita + M. alternifolia + C. myrrha, C. myrrha + M. piperita,* and *C. myrrha + M. alternifolia* showed 68%, 60%, and 36% mortality, respectively. The essential oils of *C. myrrha* and *M. alternifolia* showed 95 and 70% of repellency, respectively. Mixtures of *C. myrrha + M. alternifolia*, *C. myrrha + M. piperita,* and *C. myrrha + R. officinalis* provided repellency of 85, 74 and 73%, respectively. Toxicity by residual contact of the essential oil of *C. myrrha* in protected cultivation exhibited 93% mortality, while the acaricide fenpyroximate showed 80%. The constituents of essential oils were eucalyptol (49.66%), *M. piperita* menthol (48.53%), *M. alternifolia* terpinen-4-ol (48.93%), and *C. myrrha* benzyl benzoate (97.71%). The essential oil of *C. myrrha* and the mixtures *R. officinalis + M. piperita + M. alternifolia + C. myrrha* and *C. myrrha + M. piperita* showed significant mortality. However, further studies are needed to assess the cost/benefit ratio and the effects on non-target organisms.

Key words: spider mite, mixtures, toxicity.

RESUMEN

El presente estudio analizó la toxicidad por contacto residual y el efecto repelente de los aceites esenciales de *Rosmarinus officinalis*, *Mentha piperita*, *Melaleuca alternifolia* y *Commiphora myrrha* y sus mezclas en hembras adultas de *Tetranychus urticae* en laboratorio y cultivo protegido. El aceite esencial de *C. myrrha* exhibió una Cl_{50} de 0.55 ml L^{-1} y las mezclas *R. officinalis + M. piperita + M. alternifolia + C. myrrha*, *C. myrrha + M. piperita,* y *C. myrrha + M. alternifolia* presentaron 68%, 60%, y 36% de mortalidad, respectivamente. Los aceites esenciales de *C. myrrha* y *M. alternifolia* mostraron 95 y 70% de repelencia, respectivamente. Las mezclas de *C. myrrha + M. alternifolia*, *C. mirrha + M. piperita* y *C. myrrha + R. officinalis* proporcionaron repelencia de 85, 74 y 73%, respectivamente. La toxicidad por contacto residual del aceite esencial de *C. myrrha* en cultivo protegido presentó una mortalidad del 93%, mientras que el acaricida fenpiroximato presentó un 80%. Los componentes de los aceites esenciales fueron: eucaliptol (49.66%), *M. piperita* mentol (48.53%), *M. alternifolia* terpinen-4-ol (48.93%), y *C. myrrha* bencil benzoato (97.71%). El aceite esencial de *C. myrrha* y las mezclas *R. officinalis + M. piperita + M. alternifolia + C. myrrha* y *C. myrrha + M. piperita* mostraron una mortalidad significativa. Sin embargo, se necesitan más estudios para evaluar la relación costo/beneficio y los efectos en organismos no objetivo.

Palabras clave: ácaro araña, mezclas, toxicidad.

Introduction

The two-spotted spider mite*, Tetranychus urticae* Koch *(*Acari: Tetranychidae*)*, is a polyphagous cosmopolitan pest responsible for serious damage to crops of economic interest in irrigated and protected cultivation systems worldwide (Migeon *et al*., 2019). The considerable negative effect of this pest on agriculture is related to its short biological cycle, high fertility rate and ability to cause injuries (Araújo *et al*., 2020). The main form of control is through the application of synthetic acaricides, widely used throughout Brazil (Araújo *et al*., 2020). The indiscriminate use of these synthetic products has favored the selection of resistant populations (Monteiro *et al*., 2015). *T. urticae* is currently resistant to 95 active ingredients with different modes of action, with records of more than 500 cases of resistant populations in various regions of the world and Brazil (Mota-Sanchez & Wise, 2021). Additionally, these acaricides can cause other undesirable effects, such as biological imbalance due to the elimination of beneficial organisms (Efrom *et al*., 2012). Thus, there is a need to

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look for control alternatives that can minimize such problems. To this end, researchers have been investigating new acaricides based on essential oils extracted from plants. The literature reports the effects of essential oils and their constituents on several arthropods, including *T. urticae* (Mar *et al.*, 2018). However, few studies have addressed the acaricidal properties of essential oils and their mixtures for the management of *T. urticae* in protected cultivation (Born *et al.*, 2018).

This study evaluated the residual contact toxicity and the repelling effect of the essential oils of *Rosmarinus officinalis* (Lamiaceae), *Mentha piperita* (Lamiaceae), *Melaleuca alternifolia* (Myrtaceae) and *Commiphora myrrha* (Burseraceae) and their mixtures on adult females of *T. urticae* under laboratory conditions and protected cultivation.

Materials and methods

Biological material

The adult females of *T. urticae* were bred on jack bean (*Canavalia ensiformis* L. (Fabaceae) plants in air-conditioned rooms with 25±1ºC, 65±3% relative humidity and 12 h photoperiod in the Núcleo de Desenvolvimento Científico e Tecnológico em Manejo de Pragas e Doenças (NUDEMAFI) at the Centro de Ciências Agrárias e Engenharia at Universidade Federal do Espírito Santo - Campus de Alegre, Espírito Santo, Brazil (CCAE-UFES).

Obtaining the essential oils

Essential oils with 100% purity were purchased from the company Casa do Saboeiro® Ltda, located at Rio Grande do Sul, São Paulo, Brazil.

Chemical characterization of essential oils

The essential oils were analyzed by gas chromatography with flame ionization detector (GC/FID) (GC-2010 Plus, Shimadzu, Tokyo, Japan) and gas chromatography coupled to mass spectrometry (GC/MS) (QP2010 Plus, Shimadzu, Tokyo, Japan) adapting the methodology of Souza *et al.* (2017). The following chromatographic conditions were used in both analyses: capillary column of fused silica (30 m x 0.25 mm) with stationary phase Rtx®-5MS (0.25 µm of film thickness); N_2 (in GC/FID analysis) and He (in GC/MS analysis) as carrier gas with a flow rate of 3.0 ml/min; the oven temperature followed a schedule in which it remained at an initial temperature of 40°C for 3 min and then gradually increased by 3°C/min until it reached 240°C, maintaining this temperature for 5 min, with an injector temperature of 250°C, detector temperature of 280°C and split ratio of 1:30. GC/MS analysis was performed on equipment GCMS-QP2010 Plus with detector and AOC-5000 sample injection system (Shimadzu, Tokyo, Japan) operated by electronic impact with impact energy of 70 eV, scan speed of 1,000, scanning interval of 0.50 fragments sec^{-1}, and detected fragments from 29 to 400 m/z.

The identification of the chemical components of the oils was carried out by comparing their mass spectra with those available in the Willey 7, NIST 05, NIST 05s teak spectrum database with the co-injection of standards and by the retention indexes (RI). To calculate the RI, a mixture of linear n-alkanes (C7 to C40) was used as standard. The calculated RI for each compound was compared with values reported in the literature (Adams, 2007).

Preparation of mixtures of essential oils

The blends of the essential oils of *R. officinalis*, *M. piperita*, *M. alternifolia* and *C. myrrha* were made in the following proportion (Tab. 1), according to the methodology proposed by Pavela (2015).

TABLE 1. Proportions of mixtures of essential oils (v/v).

Mixtures	Proportions of mixtures	Essential oils
1	1:1:1:1	*R.officinalis + M.piperita + M.alternifolia + C.myrrha*
2	1:1	*C.myrrha + M.piperita*
3	1:1	*C.myrrha + M.alternifolia*
4	1:1	*C.myrrha + R.officinalis*
5	1:1	*M.alternifolia + R.officinalis*
6	1:1	*M.piperita + R.officinalis*
7	1:1	*M.piperita + M.alternifolia*

Residual contact toxicity test of essential oils and mixtures

The residual contact toxicity of essential oils was evaluated on adult females of *T. urticae* using the methodology adapted from Paes *et al.* (2015). Jack bean leaf discs with a 4.5 cm diameter were placed on a 6.5 cm diameter acrylic box with a 5 mm deep layer of a 1% ml L^{-1} agar-water solution; Tween 80 was diluted to a concentration of 0.05% (ml L^{-1}). To spray the treatments (control (distilled water + Tween 80), essential oil + distilled water + Tween 80, and mixtures of essential oils at a 1:1 proportion + distilled water + Tween 80 at a concentration of 2% (ml L^{-1})) an airbrush calibrated to a pressure of 15 N m^{-2} was used, at a distance of 30 cm. Each leaf disc was infested with 20 adult female mites aged 5 d, placed on the underside of the leaf. For each essential oil, five replicates were performed, totaling 100 mites per treatment. The concentration of treatments was in accordance with Ataide *et al.* (2020).

The test was conducted in an air-conditioned chamber (temperature of 25±1°C, relative humidity of 70±10% and photoperiod of 12 h). After 24 h, adult female mortality was assessed. To confirm mortality, the mites were lightly touched with a fine bristle brush (number 00) on the dorsal area. Immobile mites were considered dead.

Lethal concentration estimate

The lethal concentration (LC) was estimated with essential oils that reached a mortality rate above 80%, calculated by PROBIT analysis (Finney, 1971). Therefore, the lethal concentration of myrrh essential oil was estimated on adult females of *T. urticae*. For this, seven concentrations were used: 0.0, 0.3, 0.5, 0.8, 1.2, 1.5, and 2.0% (ml L^{-1}) of myrrh essential oil, with the lower limit (concentration that causes the death of about 10% of *T. urticae*) and higher (concentration that causes the death of about 90% of *T. urticae*) determined by preliminary tests. In the control, distilled water + Tween 80 was used; for the treatments, essential oil + distilled water + Tween 80 was used. This step of the test was performed according to the procedures adopted in the toxicity test. Mortality was assessed after 24 h.

Repellency test of essential oils and mixtures

The double choice chance method adapted from Aslan *et al.* (2004) was carried out using Petri dishes of 10 cm in diameter containing culture medium at 1% ml L^{-1} (water + agar). Two leaf discs of *C. ensiformis* (2.5 cm in diameter, treated and untreated) were placed in each Petri dish, joined by a glass coverslip (18 mm). The experiment was carried out in triplicate, with six replicates with 20 *T. urticae*, totaling 120 mites per treatment. Each treated leaf disc was immersed according to the proposed treatments: distilled water + essential oil + Tween 80 and the mixtures of essential oils at a 1:1 proportion + distilled water + Tween 80 at a concentration of 2% (ml L^{-1}). The untreated discs were immersed in distilled water + Tween 80. After immersion, the leaf discs were placed to dry for 20 min at room temperature. Subsequently, 20 *T. urticae* adult females of 5 d of age were placed on the underside of the leaf in the center of the coverslip with the aid of a fine-bristle brush (number 00). The treatments were placed in an air-conditioned room (temperature of 25±1°C, relative humidity of 70±10% and photoperiod of 12 h). After 72 h, the number of mites present in the treated and untreated discs was counted. The mites present on the untreated discs were considered to be repelled by the essential oil.

Toxicity test by residual contact in protected cultivation

The experiment was carried out in a protected cultivation at the Centro de Ciências Agrárias e Engenharia at Universidade Federal do Espírito Santo (CCAE-UFES) located in Alegre, ES, Brazil. The upper part of the greenhouse of the protected cultivation was covered with 150 µm plastic film and the side was covered with Optinet 50 mesh anti-aphid screen.

Jack beans (*Canavalia ensiformis* var. Coriacea (Biblioth. Robot) (Fabaceae) were sown manually and deposited in disposable cups (100 ml) containing Provaso® (Agrosolo, Conceição da Barra, ES, Brazil) organic substrate. Irrigation was performed daily, with each plant receiving the same amount of water for irrigation. When the *C. ensiformis* plants reached the age of 14 d, uniform plants were selected. After 10 d, the inoculation of 5,000 adult females of *T. urticae* was carried out. Ten d after inoculation, spraying was performed with an airbrush calibrated at a pressure of 15 N m^{-2} at a distance of approximately 30 cm from *C. ensiformis* plants, with a volume of 5 ml of the syrup of the aforementioned treatments.

Approximately 1 ml was sprayed on the upper and lower sides of each leaf of *C. ensiformis*; the treatments were as follows: distilled water + Tween 80 (control), *C. myrrha* essential oil + distilled water + Tween 80 (treatment) at LC_{95} and the acaricide fenpyroximate from the chemical group pyrazole + distilled water (positive treatment) at the commercial concentration.

The experimental design was in randomized blocks, with three treatments, five replicates and five blocks. Each block consisted of 15 plants, with five plants from each treatment arranged at random. After 24 h of exposure to treatments, the total number of live and dead mites was counted. To confirm mortality, the mites were lightly touched with a fine bristle brush (number 00); immobile mites were considered dead. The temperature of the protected cultivation varied between 25°C and 38°C, and the humidity between 40% and 85%.

Data analysis

For the acute toxicity test, an ANOVA was applied and then the means were compared by the Scott-Knott test ($P \leq 0.05$). For the pair test, the Pearson's chi-square test was applied. The concentration-response curves as well as the lethal concentration (LC_{50}) of the essential oil of myrrh were subjected to PROBIT analysis (Finney, 1971). In the test in protected cultivation, a randomized block design was used, with means compared by the Tukey's test ($P \leq 0.05$). All analyzes were performed using R software (R Development Core Team, 2010).

Results

Chemical characterization of essential oils

In the gas chromatography analysis with flame ionization detector (GC/FID), eight constituents were identified in the essential oil of *R. officinalis,* in which eucalyptol (49.66%), α-Pinene (17.08%) and camphor (14.79%) were the main ones. Fourteen constituents were identified in the essential oil of *M. alternifolia*, with terpinen-4-ol (48.93%) and γ-Terpinene (20.88%) being the main ones. Nine constituents were found in the essential oil of *M. piperita*, in which menthol (48.53%) and menthone (24.25%) were the main ones. Two constituents were identified in the essential oil of *C. myrrha*, with benzyl benzoate (97.71%) being the main one (Tab. 2).

Residual contact toxicity test of essential oils and mixtures

The residual contact toxicity of essential oils and their mixtures against adult females of *T. urticae* is shown in Figure 1. The essential oil *C. myrrha* exhibited 95% mortality ($F_{11, 48}$ = 119.12; P<0.001), and the mixtures *R. officinalis + M. piperita + M. alternifolia + C. myrrha, C. myrrha + M. piperita,* and *C. myrrha + M. alternifolia* showed 68%, 60% and 36% ($F_{11, 48}$ = 119.12; P<0.001).

TABLE 2. Chromatographic analysis of the essential oils of *R. officinalis, M. alternifolia, M. piperita,* and *C. myrrha*.

Name of compound	CRI	TRI	Relative area (%)			
			R. officinalis	*M. alternifolia*	*M. piperita*	*C. myrrha*
α-Pinene	930	932	17.08	2.41		
Camphene	943	946	4.63			
β-Pinene	972	974	4.76	0.63		
β-Myrcene	991	988		0.54		
α-Terpinene	1014	1014		8.24		
o-Cymene	1022	1022		4.11		
β-Phellandrene	1026	1025		1.58		
Eucalyptol	1028	1026	49.66	2.23		
γ-Terpinene	1057	1054		20.88		
Terpinolene	1086	1086		3.01		
Linalool	1100	1095	2.35			
Camphor	1141	1141	14.79			
Isopulegol	1143	1145			1.73	
Menthone	1153	1148			24.25	
Isoborneol	1154	1155	0.72			
Neomenthol	1163	1161			16.61	
Menthol	1172	1167			48.53	
Isomenthol	1182	1179			1.07	
Terpinen-4-ol	1178	1178		48.93		
α-Terpineol	1189	1186	3.94	3.51	0.78	
Piperitone	1252	1249			1.46	
Menthyl acetate	1293	1294			4.78	
trans-Caryophyllene	1414	1417			0.79	
Aromadendrene	1434	1439		1.24		
Viridiflorene	1491	1496		1.42		
δ-Cadinene	1520	1522		1.27		
Cubenol	1655	1645				2.29
Benzyl benzoate	1759	1759				97.71

The compounds were identified by LTPRI index (GC/FID) and mass spectrometry (GC/MS) using an Rtx®-5MS column. CRI - retention index calculated from data obtained by sampling of saturated n-alkanes (C7-C40). TRI - tabulated retention index (Adams, 2007). Compounds with relative areas >0.5% were identified.

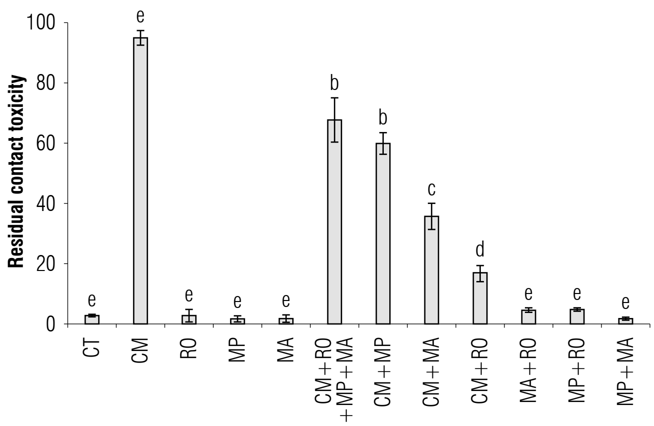

FIGURE 1. Means (± standard error) of mortality of *T. urticae* females at a temperature of 25±2ºC, relative humidity of 70±10%, and a photoperiod of 12 h by essential oil mixtures; CT - control, CM - *C. myrrha*, RO - *R. officinalis*, MP - *M. piperita*, MA - *M. alternifolia.* Equal letters do not indicate statistically significant differences from each other according to the Scott-Knott test at 5% probability.

Lethal concentration

The lethal concentration of the essential oil of *C. myrrha* showed an LC_{95} of 1.6 ml L^{-1} and an LC_{50} of 0.55 ml L^{-1} (Tab. 3).

Repellency test of essential oils and mixtures

The repellency effect of essential oils and their mixtures in adult females of *T. urticae* is shown in Figure 2. Among the essential oils and their mixtures, the essential oils of *C. myrrha* and *M. alternifolia* showed 95 and 70% of repellency, respectively ($F_{10, 89}$ = 4.020; P<0.001). Mixtures of *C. myrrha* + *M. alternifolia*, *C. myrrha* + *M. piperita,* and *C. myrrha* + *R. officinalis* provided repellency of 85, 74 and 73%, respectively ($F_{10, 89}$ = 4.020; P<0.001).

Toxicity test by residual contact in protected cultivation

The essential oil of *C. myrrha* showed 93% toxicity against adult females of *T. urticae* in protected cultivation, whereas

TABLE 3. Lethal concentration of *C. myrrha* oil on adult females of *T. urticae*.

Essential oil	N	DF	Slope±SE	X^2	*P*	Lethal concentration (ml L^{-1}) (LC 95%)	
						LC_{50}	LC_{95}
C. myrrha	300	3	3.54±0.36	2.02	0.56	0.55 (0.48±0.61)	1.6 (1.34±2.07)

N - Number of individuals; DF - degrees of freedom; Slope - slope of the line; X - Chi-square; P - *P*-value; SE - confidence interval.

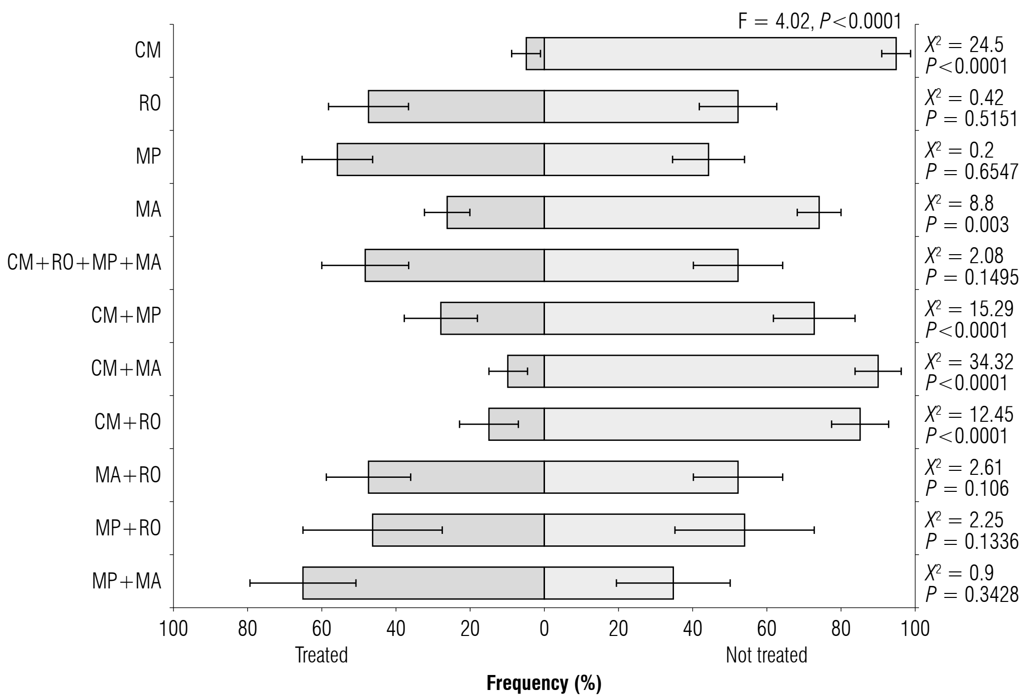

FIGURE 2. Frequency (%) (± standard error) of *T. urticae* adult females repelled at a temperature of 25±2ºC, a relative humidity of 70±10% and a photoperiod of 12 h on the application of essential oils and their mixtures; CM - *C. myrrha*, RO - *R. officinalis*, MP - *M. piperita*, MA - *M. alternifolia*.

the fenpyroximate showed 80% toxicity ($F_{2,8} = 545.24$; $P<0.001$) (Fig. 3).

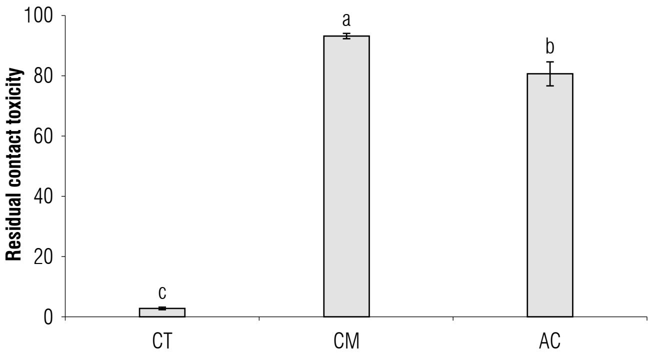

FIGURE 3. Means (± standard error) of mortality of females *T. urticae* in protected cultivation by the application of essential oil and acaricide; CT - control, CM - *C. myrrha* and AC - acaricide (fenpyroximate); equal letters indicate treatments that do not differ statistically from each other according to the Tukey's test at 5% probability.

Discussion

The major constituent identified in the essential oil of *C. myrrha* was benzyl benzoate (97.71%) which differed from the major constituents identified by Mohamed *et al.* (2014). The major constituents in the essential oil of *M. piperita* were menthol (48.53%) and menthone (24.25%), which were the same major constituents found by Kizil *et al.* (2010) and Moghaddam *et al.* (2013). The major constituents found in *M. alternifolia* were terpinen-4-ol (48.93%) and γ-Terpinene (20.88%), with the same major constituents found by Zhang *et al.* (2018) and Silva *et al.* (2019). In the essential oil of *R. officinalis,* the major constituents found were eucalyptol (49.66%), α-Pinene (17.08%) and camphor (14.79%); similar results were found by Borges *et al.* (2018) and Jardak *et al.* (2017) for constituents α-Pinene and camphor.

This study found that the essential oil of *C. myrrha* showed residual contact toxicity against adult females of *T. urticae*, exhibiting an LC_{50} of 0.55 ml L^{-1}. Araújo *et al.* (2012) found that the essential oil of *Piper aduncum*, Piperaceae showed an LC_{50} of 7.17 µl m^{-1} on adults of *T. urticae*. Ribeiro *et al.* (2019) evaluated four different *Citrus* sp. essential oils on *T. urticae*. Of these, the essential oil of *Citrus limon*, Rutaceae showed an LC_{50} of 25.18 µl ml^{-1} L. Essential oils that show efficiency in the control of pest arthropods by residual contact can penetrate the tegument layers (Enan, 2001). Consequently, since essential oils are made up of several substances, they can act in more than one place exhibiting a neurotoxic action (Isman, 2006).

The mixtures of *R. officinalis* + *M. piperita* + *M. alternifolia* + *C. myrrha* and *C. myrrha* + *M. piperita* at a 1:1 ratio showed promising results in terms of the toxic effect from residual contact on adult females of *T. urticae* with intermediate toxicity, which resulted in an additive interaction. Although we did not find synergistic effects, this type of interaction has been described by Mwaiko (1992) for mixtures of bark essential oils from two species of *Citrus* (*C. limon*, *C. aurantium*, Rutaceae) against *Culex pipiens* L. (Diptera: Culicidae) larvae. Benelli *et al.* (2017) evaluated the acute toxicity of binary mixtures of essential oils of the Apiaceae family on the larvae of *C. quinquefasciatus*, an important vector of filariasis, and found that *Trachyspermum ammi* Apiaceae + *Pimpinella anisum*, Apiaceae (1:2 ratio) and *S. olusatrum* + *P. anisum* (1:1 ratio) were the most toxic to the pest.

The repellent effect of essential oils in integrated pest management is important; as these substances keep pests away from the crop, damage is minimized (Da Camara *et al*., 2015). The results of the repellency tests suggest that the essential oils of *C. myrrha* and *M. alternifolia* and the mixtures of *C. myrrha* + *M. piperita*, *C. myrrha* + *M. alternifolia* and *C. myrrha* + *R. officinalis* showed a repellent effect on females of *T. urticae*. In previous studies, Araújo Júnior *et al.* (2010) found that the essential oils of *C. aurantium* and *C. sinensis* var. Mimo showed repellency of *T. urticae* at a concentration below 2.5%, while the essential oil of *C. sinensis* var. Pear had a neutral effect. Sararit and Auamcharoen (2020) observed that the essential oils of *Anethum graveolens* and *Allium sativum* exhibited repellency of adult females of *T. urticae* at concentrations of 15 to 20%. Farahani *et al.* (2020) noted that essential oils of *Thymus daenensis* (Lamiaceae)*, Satureja khuzestanica* (Lamiaceae), and *Satureja bakhtiarica* (Lamiaceae) showed repellency against adult *T. urticae*. The repelling action of essential oils triggers an escape behavior of mites, detected by the olfactory sensilla present in the legs (Missbach *et al*., 2014; Oliveira *et al*., 2018).

The tests in protected cultivation confirmed the laboratory observations that the essential oil of *C. myrrha* was toxic to adult females of *T. urticae*. In a study carried out to avoid the spread of mites in a greenhouse, Da Camara *et al.* (2015) observed that the essential oils of *C. sinensis* and *C. aurantium* were repellent to *T. urticae*. The essential oil of *C. aurantium,* in particular, prevented the dispersion of the pest for a period of 1 week. In a greenhouse trial, Potenza *et al.* (2006) observed toxicity by contact of the aqueous extract of *Allamanda cathartica* (Apocynaceae), *Dieffenbachia brasiliensis* (Araceae), *Cenchrus purpureus* (Schumach.) Morrone (Poaceae), *Annona squamosa* (Annonaceae), *Ruta graveolens* (Rutaceae), *Sonchus oleraceus*

(Asteraceae), *Spondias purpurea* (Anacardiaceae), *Lytechinus variegatus* (Euphorbiaceae), *Impatiens walleriana* (Balsaminaceae), *Stryphnodendron adstringens* (Fabaceae), *Solanum melongena* (Solanaceae), *Campsiandra angustifolia var. angustifolia* (Fabaceae), and *Allium* (Amaryllidaceae) on *T. urticae*. However, only plants with *D. brasiliensis, R. graveolens, A. squamosa*, *S. oleraceus*, *I. walleriana*, *A. angustifolia, S. adstringens, and S. melongena* promoted a significant reduction in the population of *T. urticae* of between 60 and 86%. In another study, EcoTrol (containing 10% rosemary oil), a pesticide based on the essential oil of rosemary, proved to be efficient in the control of *T. urticae* in a greenhouse (Miresmailli & Isman, 2006).

Conclusions

The essential oil of *C. myrrha* and the binary and quaternary mixtures *C. myrrha* + *M. piperita* and *R. officinalis* + *M. piperita* + *M. alternifolia* + *C. myrrha,* showed significant mortality in this study. The essential oil of *C. myrrha,* in turn, showed results in a protected cultivation similar to those obtained with the application of commercial acaricides for the control of *T. urticae* in orchards and in a protected system in Brazil. This study shows the feasibility of using a botanical acaricide with *C. myrrha* essential oil as an active ingredient for the management of *T. urticae*. However, for large-scale use, studies to reduce essential oil production costs and the effects on non-target organisms are needed.

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Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

HBZ and LM carried out the experiments. JOA, FDD, FGH, and AH carried out the laboratory and protected cultivation experiment and collected the data. JOA and FDD carried out the data analysis and writing of the manuscript. All the authors reviewed the manuscript.

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Relationships between mineral nutrients in banana (*Musa* AAA Simmonds cv. Williams) bunches fertilized with nitrogen in two production cycles

Relaciones entre nutrientes minerales en racimos de banano (*Musa* AAA Simmonds cv. Williams) fertilizados con nitrógeno en dos ciclos de producción

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ABSTRACT

The production of bananas (*Musa* AAA Simmonds cv. Williams) for export in Uraba, Colombia is an important source of income for the region, reflected in the offer of approximately 40,000 jobs. Production requires research into various subjects, with fertilization being a priority. In this study, the effect of N doses on the nutrient relationships existing in a banana bunch during two cycles was evaluated. A sixth-generation plantation was used and evaluated during the fruit filling and harvest stages. A multivariate approach was used for the analysis of variance of the design in repeated measures, with two factors between subjects (fertilization and repetitions), and one intra-subject associated with the cycle. The models were adjusted with the phenological stages. Synergy was found between Mg:Ca+N, Zn:B, K:Cu, K:P, and Ca:Cu, with K:P and K:Cu ratios being the only ones that showed a directly proportional behavior with the production variables. Optimal values of the interaction K:Cu+K:P (35.11-39.05) led to a greater accumulation of dry mass in the bunch (4.24 kg) in the treatments of 321.8 and 483 kg N ha^{-1} for the evaluated stages.

Key words: soil fertility, component analysis, Musaceae, perennials.

RESUMEN

La producción de banano (*Musa* AAA Simmonds cv. Williams) para exportación en Urabá, Colombia es una fuente importante de ingresos para la región, lo que se ve reflejado en la oferta aproximada de 40 000 puestos de trabajo. El proceso productivo requiere investigación en diversos temas, siendo la fertilización una prioridad. En el presente estudio se evaluó el efecto de dosis de N sobre las relaciones nutricionales existentes en el racimo de banano en dos ciclos. Se empleó un cultivo de sexta generación, el cual fue evaluado durante las etapas de llenado de fruto y cosecha. Se utilizó un enfoque multivariado para el análisis de varianza del diseño en medidas repetidas, con dos factores entre-sujetos (fertilización y repeticiones) y uno intra-sujetos asociado al ciclo. Se ajustaron los modelos con las etapas fenológicas. Se encontró sinergia entre Mg:Ca+N, Zn:B, K:Cu, K:P, y Ca:Cu siendo las relaciones K:P y K:Cu las únicas que presentaron un comportamiento directamente proporcional con las variables de producción. Valores óptimos de la interacción K:Cu+K:P (35.11-39.05) conllevaron a una mayor acumulación de masa seca en el racimo (4.24 kg) en los tratamientos de 321.8 y 483 kg ha^{-1} de N para las etapas evaluadas.

Palabras clave: fertilidad del suelo, análisis de componentes, Musaceae, plantas perennes.

Introduction

Bananas (*Musa*, Musaceae) represent an important role in the diet of millions of people in the tropical and subtropical regions of the world, and they are a staple food that contributes to food security and the reduction of malnutrition (Oyeyinka & Afolayan, 2019). This crop is considered to have some of the best phytochemical characteristics with rich sources of minerals, vitamins, carbohydrates, flavonoids, and phenolic compounds (Kookal & Thimmaiah, 2018). The consumption per capita of bananas varies between 50 and 150 kg, reaching 400 kg in the producing continents such as Africa (Brito *et al.*, 2015). The largest banana producer worldwide is India, while Colombia was ranked 11th among producers worldwide for the year 2019, with a production of 2.9 million t (FAOSTAT, 2020).

The technical management of fertilization in banana export production could represent approximately 40% of the production cost and is one of the most representative (Torres-Bazurto *et al.*, 2019). Several factors condition the absorption of mineral nutrients by the plants in which we can find physical-chemical properties of soil and physiological conditions associated with the plant phenological stages. Additionally, the distribution of assimilates towards

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the bunches plays an essential role (Espinosa & Mite, 2002), where it is important to highlight the decrease in root formation in the post-flowering stage (Galan *et al.*, 2018).

Nitrogen fertilization of bananas in the Caribbean region far exceeds the necessary amount that the crop extracts from the soil since other sources from which this element may come are not considered (Dorel *et al.*, 2008). The response of the banana crop to N fertilization is multifactorial and, therefore, the N content in soil, the dose, the source, and the time of N application are relevant to satisfy the needs of the plants and to guarantee optimal yield and quality of the commercial product (Sun *et al.*, 2020). Within perennial crops, antagonistic and synergistic relationships between nutrients can often be observed that are reflected in the yield (Espinosa & Mite, 2002). Regarding the relationship between K, Ca, and Mg in banana, when any of these elements is in high proportions, the absorption of other elements is reduced (IPNI, 2015a). Despite the above, there is very little information on the relevant results about the different associations between elements. There are approximately 200-300 banana cultivars; therefore, information on the nutrient aspects for growing cultivars on a large scale (such as Williams) are of the utmost importance (Ashokkumar *et al.*, 2018). This study sought, as a general objective, to evaluate the effect of N doses on the relationships between the mineral nutrients in the bunches of Williams banana during two production cycles in the Uraba region of Colombia, in order to adjust recommendations of N doses according to the needs of the plants.

Materials and methods

Location, plant material, and treatments

The study was carried out in the Uraba region in the department of Antioquia (Colombia), in the experimental field of the company Augura (municipality of Carepa), located at 7°46'46" N and 76°40'20" W at 20 m a.s.l. The soils of the study were classified according to the USDA taxonomic classification as fine Fluventric Eutrudepts, clay loam over clay Fluvaquentic Eutrudepts, and fine loam Vertic Endoaquepts (IGAC, 2007).

The climatic conditions at the study site were the following: mean relative air humidity of 87%, maximum air temperature of 32.3°C, minimum air temperature of 23.2°C, and mean air temperature of 26.7°C (regional mean is 27°C). The mean solar brightness was 5 h per d for a total of 1,700 h per year. The mean annual precipitation was 845 mm during the first production cycle (from August 2011 to April 2012) and 2,088 mm for the second production cycle (from February to December 2012). Each production cycle lasted 10 months.

A banana (*Musa* AAA Simmonds cv. Williams) crop of giant Cavendish subgroup, Williams clone, was used with a mean plant height of 3.5 m. This is one of the two most cultivated clones for export (Robinson & Galán Saúco, 2012). A sixth-generation banana plantation was used.

Fertilization plans were established as recommended by Cenibanano in the Uraba area, based on the soil analyses carried out and the proposals of Sánchez Torres and Mira Castillo (2013) who recommend not applying Fe, Mn and Cu since the soils contain these elements in appropriate quantities. The N doses (kg ha^{-1}) were as follows: 1) absolute control without fertilization; 2) 0; 3) 161; 4) 321.8, and 5) 483. All treatments, except the absolute control, received the following fertilization (kg ha^{-1}): 87.1 P_2O_5, 678.8 K_2O; 50.5 CaO; 117.5 MgO; 64.2 S; 1.4 B; and 9.3 Zn. The sources of the mineral fertilizers were as follows: urea (46% of N), KMag (22% K_2O, 18% MgO, and 22% S), fertiboro (10% B), solufos (30% P_2O_5, 36% Ca, 5% S, and 8% Si), ZnO (80% Zn), potassium sulfate (50% K_2O and 18% S), and KCl (60% K_2O).

Four replicates (blocks) were established per treatment and the treatments were randomized. Each of these replicates (blocks) was distributed according to the types of soil in the experiment. Each treatment corresponded to the area units, called "botalones". Each "botalon" had an area of 1,563 m^2 and 250 banana plants, of which 15 plants were selected with a height between 1.0 m and 1.5 m. These plants formed an experimental unit and were evaluated according to the number of weeks and growth stages of development (Tab. 1).

TABLE 1. Sampling times of banana cv. Williams according to the number of weeks and stages of development.

Phase of growth	Sampling time	Weeks of growth from sucker emergence
Productive	Bunch filling	43-44
Productive	Harvest	50-51

The agronomic management in the area was similar to that of the commercial banana plantations in the Uraba region, except for the doses of fertilizers. Fertilizer doses were divided within each production cycle for a total of 17 fertilizer applications per year, with approximately 3 weeks between applications. The sampling time was selected considering the phase and the development time of the plant, with at least two weeks after the application of the respective fertilizer dose.

Production variables

The following production variables were measured in the bunch in the last two selected stages of development.

Banana bunch dry mass (BDM): the whole bunch was weighed fresh, taking the rachis on top of the first hand until the last scar, packing 300 g in a paper bag. The total dry weight was obtained using a rule of three, relating the total fresh weight to the fresh and dry weight of the sample.

The number of hands (NHPB) and total number of fingers per bunch (TNFB) were counted before separating the rachis from the fruits.

The average number of fingers per bunch (ANFB) was determined by averaging the TNFB of each of the treated samples.

For the fruit sample, the two central fingers of hands 1, 3, and 5 were selected since they are the ones used to measure the commercial quality of bananas in the study area, separating the peel from the pulp from the six fingers, weighing them separately, and packing them in paper bags.

Subsequently, we sent pulp and peel separately to the Soil and Water Laboratory of the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Bogota campus, to determine their mineral nutrient content. We determined the total N, P, Ca, K, Mg, Cu, Fe, Mn, Zn and B concentrations in pulp and peel using the methodology of IGAC (2006). For S concentration, we used the method described by Tabatabai and Bremner (1970).

Finally, we added the concentrations of the elements in pulp and peel to obtain the total concentration of the fruit that was the one used in this study.

Statistical analysis

We performed statistical analyses using both descriptive and inferential procedures. Regarding the descriptive methods, the averages of the variables obtained were used to generate tables and combined line and bar diagrams for the variables derived from the different procedures performed. As for the inferential method, we used a principal component analysis (PCA) to decrease the dimensionality of each of the nutrients associated with the bunch, regardless of the experimental design used. This relates variables that obtained a similar trend across the components and/or a high degree of significance in any of the components shown in the rotated component matrix using the Varimax method with Kaiser normalization (Kaiser, 1958; Aldás & Uriel, 2017).

We obtained possible relationships between bunch nutrients considering what models were generated based on linear regressions by corroborating their relationship (synergistic or antagonistic). After this, we sought to associate the available production variables with the nutrient relationships found, following the same procedure mentioned above. The relationship between the different responses and the different levels of the factors was modeled using a linear model. Such a model was associated with the design of

TABLE 2. Principal component matrix rotated for the nutrient contents in banana bunches.

Nutrient content in banana bunches	Rotated component matrix				
	Components				
	1	2	3	4	5
N	0.825	0.382	0.262	0.148	0.100
Mg	0.800	0.329	0.065	0.268	0.364
Ca	0.711	0.296	0.252	0.388	0.377
Cu	0.670	0.316	0.446	0.311	0.295
K	0.623	0.350	0.444	0.402	0.294
P	0.559	0.292	0.419	0.457	0.373
Zn	0.271	0.890	0.162	0.139	0.200
B	0.379	0.823	0.200	0.245	0.103
Mn	0.226	0.201	0.895	0.220	0.214
S	-0.336	-0.255	-0.273	-0.820	-0.224
Fe	0.368	0.213	0.308	0.245	0.800

Highlighted values have a component greater than 0.7 and/or a similar trend.

repeated measures with a factor between subjects (N dose), depending on the effect of the blocks (fixed factor) and an intra-factor-subject (production cycle). The data were processed by the SPSS 23 program (IBM Corp., Armonk, NY, USA), the free software Rstudio, and the MS Excel 2017 program (Microsoft Office Inc., Seattle, WA, USA).

Results and discussion

Statistical analysis of nutrient relationships

We analyzed the results obtained in the rotated component matrix from the PCA (Tab. 2), decreasing the dimensionality of the data.

Data were chosen so that any of the components was greater than 0.7 and/or had a similar trend. Although the analysis indicates that only one of the components should be used since it is the one that explains most of the variability of the data (75.395%), five components were considered to facilitate the analysis.

We found significance in the first component of N (0.825), Ca (0.800) and Mg (0.711), in the second component of Zn (0.890) and B (0.823), and trends of similar and near increase through the components of K and Cu, K and P, Ca, and Cu.

Linear regression models of each of the interactions mentioned above were proposed and, from them, we determined whether the relationship was antagonistic or synergistic. Since there were two independent variables, in the case of Mg, Ca, and N, the model with the lowest variance inflation factor (VIF) was used to avoid collinearity of the data. Also, linear equations were generated to give a glimpse of the relationship obtained.

Mg:Ca+N ratio in the fruits

As for the Mg:Ca+Mg ratio, the model with Ca and N as predictors had an adjusted R squared of 0.859, which indicated a high positive correlation (synergy) between Ca and N (independent), and Mg (dependent), as Equation 1 shows:

$$\textit{Bunch Mg concentration } (\%) = (1.294 + (0.382 \times \textit{Bunch Ca concentration } (\%)) + (0.056 \times \textit{Bunch N concentration } (\%))) \pm 1.805 \quad (1)$$

Calcium has structural characteristics in plant cells because it is a fundamental part of cell walls and membranes; therefore, it is essential for plant growth and fruit development (Díaz *et al.*, 2007). The allocation of Ca occurs unevenly within the reproductive organs due to the rate of expansion of the fruit and the distribution of the vascular bundles. In bananas, the allocation of Ca occurs in the central area of the convex part of the fruit, where the nutrient deficiencies of Ca manifest themselves. Magnesium is essential for plant nutrition since it participates in various metabolic functions. Its deficiency accelerates premature ripening and, in certain cases, causes the abortion of fruits before reaching their physiological maturity (Marschner, 2012). Nitrogen has a great influence on the flowering and fruiting processes and is directly related to crop yield. Its deficiencies are associated with decreased BDM (Sun *et al.*, 2020).

The ratio between Ca and Mg has been widely documented since they are cationic bases. If they are balanced in the soil, no imbalance will occur that negatively affects the intake of the other elements (White & Brown, 2010). Due to the low mobility of Ca in the phloem, it is not well redistributed from the leaves to the bunches. There is a very important relationship of Ca with mobile elements due to its structural functions that guarantee the post-harvest quality of the fruit. On the other hand, N and Mg have greater phloem mobility in the plant than Ca that favors their accumulation in the bunches due to the filling process and their role in the functioning of the enzymes necessary for fruit formation.

Zn:B ratio in the fruits

The regression model used for the Zn:B ratio had an adjusted squared R of 0.747, indicating a high positive correlation between Zn and B (synergy), given by the following equation:

$$\textit{Bunch Zn concentration } (\text{mg kg}^{-1}) = (-7.141 + (0.774 \times \textit{Bunch B concentration } (\text{mg kg}^{-1})) \pm 69.875 \quad (2)$$

In bananas, B is essential for the functioning of the plasma membrane since this nutrient is involved in the activity of the enzyme ATPase, while Zn plays an important role in redox reactions as part of the superoxide dismutase, promoting the protection of the membranes, interfering in the oxidation of NADPH and radical removal. Boron deficiency in some plant tissues promotes a decrease in ATPase activity and its ion absorption rate, while high amounts of this element are toxic to bananas. Zinc plays a fundamental role in decreasing the accumulation of B, reducing its negative effects (Kirkby & Römheld, 2008a; 2008b; Sánchez Torres & Mira Castillo, 2013). Plants fertilized with the supplement of Zn and B obtained 400 commercial boxes of fruits (41 lb) ha^{-1} per year (IPNI, 2015b). This value is far higher than that obtained from plants under basic recommended fertilizer management.

K:Cu ratio in the fruits

The regression model used for the K:Cu ratio had an adjusted R squared of 0.848, with variables positively correlated with each other indicating their synergy (Eq. 3).

$$\textit{Bunch K concentration} (\%) = (-121.025 + (13.682 \times \textit{Bunch Cu concentration} (\text{mg kg}^{-1}))) \pm 81.779 \quad (4)$$

Potassium plays a fundamental role in various metabolic processes, including photosynthesis and respiration. The accumulation of K in banana fruits varies between 21% and 24% at the stage of harvest in the bunch for the Prata variety, because of the source-sink relationship (Deus *et al.,* 2020). Copper in banana plants is part of the enzymatic processes involved in respiration and photosynthesis. Also, Cu-related proteins participate in lignification, anaerobic metabolism, cellular defense, and hormonal metabolism (Espinosa & Mite, 2002).

Jivan and Sala (2014) found a high correlation ($R^2 = 0.702$) between the contents of K and Cu in apple trees as observed in this study. However, there is no explanation for the ratio. On an equivalent chemical basis, plants tend to maintain constant amounts of total cations, a fact that leads to very complex nutrient relationships that are not characterized (Ranade-Malvi, 2011). Still, the K:Cu ratio is associated with the plant's defense system.

K:P ratio in the fruits

The K:P ratio has an adjusted R squared of 0.904 and the variables are positively correlated with each other, indicating that there is synergy between them (Eq. 4).

$$\textit{Bunch K concentration} (\%) = (-109.422 + (22.606 \times \textit{Bunch P concentration} (\%)) \pm 65.158 \quad (4)$$

K is very important for the growth and development of bananas due to its multiple functions, but P is relevant at the morphological and root levels. Likewise, P is a component of ATP, necessary in various reactions at the molecular level, such as photosynthesis, respiration, and carbohydrate metabolism (Leonel *et al*., 2020). It is also related to the flowering and fruit formation (Marschner, 2012).

Both K and P tend to accumulate (24% and 35%, respectively) in the bunch at the harvest stage, showing a strong correlation in their accumulation (Deus *et al.,* 2020). In oil palms, K and P tend to accumulate up to 45% in bunches, showing a synergistic relationship like in this study (Herrera Peña, 2015). We can infer that the ratio of these two nutrients affects the formation and filling of the bunch, due to their metabolic role.

Ca:Cu ratio in the fruits

The regression model used for the Ca:Cu ratio has an adjusted R squared of 0.788, explaining that the variables are positively correlated (synergy) (Eq. 5).

$$\textit{Bunch Ca concentration} (\%) = (-2.249 + (0.611 \times \textit{Bunch Cu concentration} (\text{mg kg}^{-1}))) \pm 4.513 \quad (5)$$

In oil palms, Cu shows the highest accumulation in the bunch among all micronutrients; approximately 46% of the total Cu absorbed is concentrated in this organ (Owen, 1992). This behavior is similar to that shown by Ca whose accumulation amounts to 25%, demonstrating that both nutrients tend to accumulate in the bunch efficiently and synergistically, perhaps associated with structural and defense functions.

Influence of N doses on nutrient relationships in the fruits

For the filling stage, the multivariate analysis showed differences between cycles (P<1.407e-09), treatments (P<0.01220) and in the cycle*treatment interaction (P<0.01674). In contrast, in the univariate analysis the only ratio that generated differences for the factor treatment was the Mg:Ca+N ratio (P<0.0811). Therefore, multiple comparison tests were carried out, demonstrating a significantly greater effect in the dose with 0 kg N ha^{-1} than in the treatment with 483 kg N ha^{-1}, while in the other treatments there were no significant differences.

During the harvest stage, the multivariate analysis showed differences between cycles (P<0.002867), treatments (P<0.001595) and the cycle*treatment interaction (P<0.006214). Regarding the univariate analysis, differences were observed for the Mg:Ca+N (P<0.0005091) and K:P (P<0.01723) ratios. Therefore, multiple comparison tests were performed finding that for Mg:Ca+N a significant difference was observed between the treatment with 0 kg N ha^{-1} and the other treatments (absolute control without fertilization and the doses with 161, 321.8, 483 kg N ha^{-1}).

While the K:P ratio was influenced by the N content, showing differences between the treatments of 0 and 483 kg N ha^{-1}, there were no differences between the other treatments.

The trends found in the Mg:Ca+N ratio in both stages are due to the fact that the accumulation of N shows an increase according to the dose used. This agrees with data by Keshava and Iyengar (2000), who find high levels of N accumulation in the Robusta banana when a higher dose of N was used.

The K:P ratio was explained by the increasing N fertilization levels and, as a result, the absorption of all nutrients together was expected to be more efficient and balanced. This agrees with a report by Sun *et al.* (2020), who state that the higher the amounts of metabolized nitrate, the higher the concentration of nutrients such as N, P, and K.

The other nutrient relationships were not negatively or positively affected by N doses in the two phenological stages studied.

Statistical analysis of the interaction between the nutrient relationships and production variables

From the PCA, the results obtained in the rotated component matrix (Tab. 3) were processed, reducing the dimensionality of the data. Any of the components considered was greater than 0.7.

TABLE 3. Rotated principal component matrix of the proposed nutrient relationships and production variables of banana bunches.

Rotated component matrix			
	Component		
Proposed nutrient relationships / production variables	1	2	3
Mg:Ca+N	-0.317	0.676	0.032
K:Cu	0.942	0.118	-0.032
Zn:B	0.138	0.441	0.037
K:P	0.818	-0.242	0.256
NHPB	0.341	-0.651	0.524
TNFB	0.271	-0.338	0.845
ANFB	-0.061	0.410	0.820
Ca:Cu	0.563	0.593	-0.109
BDM	0.855	-0.380	0.192

NHPB - Number of hands per bunch; TNFB - Total number of fingers per bunch; ANFB - Average number of fingers per bunch; BDM - Bunch dry mass. Highlighted values have a component greater than 0.7.

As the analysis indicated, three components were used since these explained most of the variability of the data (71.994%).

Significance was found in the first component of the K:Cu (0.942) and K:P (0.818) ratios and of the production variable BDM (0.855). There were no significant variables in the second component, while in the third there was significance for the variables TNFB (0.845) and ANFB (0.820). Despite this, these variables were not considered to continue the analysis because they were not associated with a ratio.

As shown in Figure 1, the comparison between CP1 and CP2 and CP3 showed there was a relationship between the variables K:P, K:Cu and BDM. Regarding the comparison between CP2 and CP3, although K:P and BDM are in the same quadrant, K:Cu is a little further apart despite having the same trend. This is explained because CP1 explains most of the variability of the data.

Therefore, the comparison between CP2 and CP3 does not reflect a spatial distribution as marked as the comparison between CP1 and CP2 and CP3.

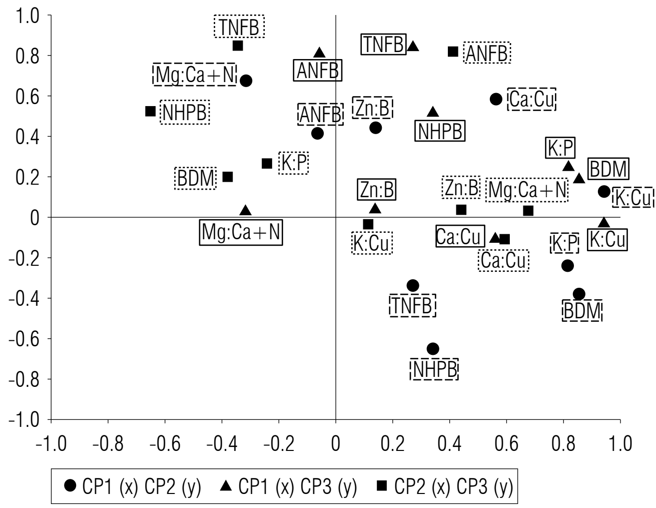

FIGURE 1. Comparison between the three components that explained most of the data variability of the proposed nutrient relationships and production variables of banana bunches.

A linear regression model was performed between the significant variables of the first component (BDM, K:Cu, K:P) to observe their relationship. A model was obtained with a variance inflation value (VIF) of <10, showing low data collinearity.

Interaction between BDM and K:Cu and K:P ratios

The association of the K:Cu and K:P ratios with the BDM production has an adjusted R squared of 0.713 showing a direct correlation between the variables (Eq. 6).

$$BDM = (-2.537 + (0.229 \times K{:}Cu\ ratio) + (0.175 \times K{:}P\ ratio)) \pm 0.752 \quad (6)$$

For the filling period, the multivariate analysis showed differences between cycles (P<9,128e-05), treatments (P<0.0002102) and the cycle*treatment interaction (P<0.0020895). Regarding the univariate analysis of the K:Cu+K:P regression model, no significant differences were obtained, but for the BDM they were found between treatments (P<1.69e-05).

The greatest accumulation of BDM was obtained in the 321.8 and 483 kg N ha^{-1} N treatments for the bunch filling

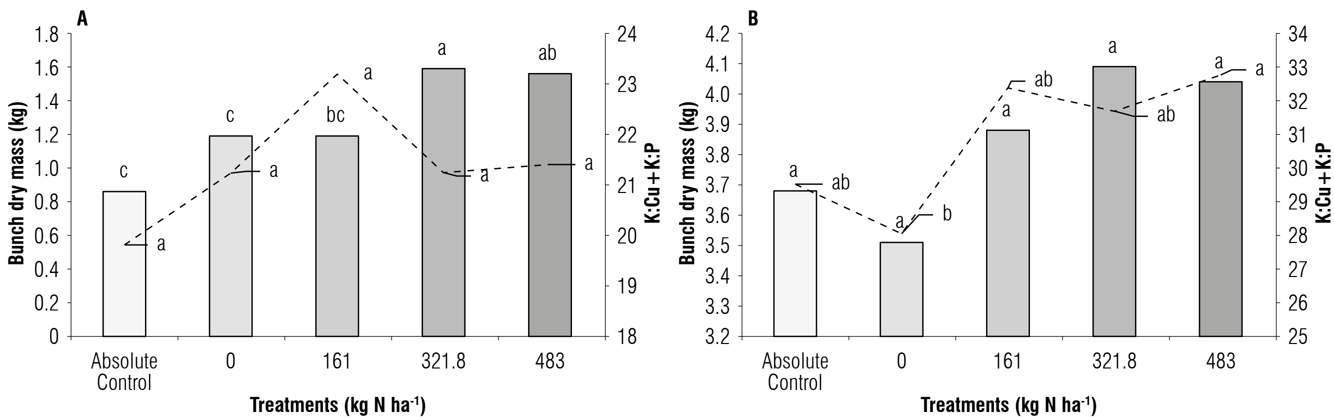

FIGURE 2. Influence of different nitrogen doses on the bunch dry mass (BDM) and the K:Cu+K:P model at the phenological stages of A) bunch filling and B) harvest.

stage (Fig. 2A). At the harvest stage, there was an increasing trend despite not showing significant differences between treatments (Fig. 2B).

At both stages, a similar behavior of the K:Cu+K:P model and the BDM was observed, with a tendency of the two variables to be greater according to the increase in the N dose. This was because N is involved in plant growth, development, and dry mass production (Yang *et al.*, 2013). Greater amounts of metabolized nitrate increase the concentration of other nutrients that, in the case of K, Cu and P, are involved in different metabolic processes that promote the formation of fruits and their subsequent filling (Espinosa & Mite, 2002; Sun *et al.*, 2020).

Proposed ranges for the K:Cu+K:P model, relative to the BDM

The general behavior of K implies a considerable translocation towards the bunch after the flowering stage because of fruit filling (Torres Bazurto *et al.,* 2017). Banana plants do not require a large dose of P compared to the other primary macronutrients. However, continuous extraction of P after flowering is required, intervening in fruit formation (Leonel *et al*., 2020).

Regarding Cu, the content of this element in the soils was sufficient for the development of the crop, which influenced the effect of the K:Cu ratio with an increase in BDM. This is because Cu plays a fundamental role as a cofactor of various enzymes and is part of the metabolism of carbohydrates in the plant (Espinosa & Mite, 2002).

Higher accumulations of K together with P and Cu resulted in a greater amount of BDM, both for the filling and harvest stages (Tab. 4).

Conclusions

A significant and synergistic relationship was found between mineral nutrients, with the main ones being Mg:Ca+N; Zn:B; K:Cu; K:P, and Ca:Cu. However, the Mg:Ca+N; Zn:B and Ca:Cu ratios in the fruits showed no effect on production variables of Williams bananas.

The high doses of N showed that the nutrient relationships in the fruits that influenced the production were those of K:P and K:Cu.

The proposed ranges of the interaction K:Cu+K:P can be used to make comparisons with data obtained from nutrient analyses of banana cv. Williams bunches to determine their nutrient status in relation to their dry matter content, finding possible problems in the accumulation of K, P or Cu. This becomes a useful tool for planning and decision making in mother plants or their suckers.

TABLE 4. Proposed ranges for the K:Cu+K:P interaction with the bunch dry mass (BDM) at the phenological stages of bunch filling and harvest.

	Bunch filling		Harvest	
Level	**K:Cu+K:P**	**Average dry mass of the bunch (kg)**	**K:Cu+K:P**	**Average dry mass of the bunch (kg)**
Low	9.22 - 14.04	0.58	23.24 - 27.29	3.32
Medium	14.05 - 18.88	0.89	27.20 - 31.14	3.69
Medium-high	18.89 - 23.71	1.37	31.15 - 35.10	4.08
High	23.72 - 28.55	1.50	35.11 - 39.05	4.24

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

SDGG and SCMR worked on the conceptualization and methodology of the study and wrote the article. SDGG carried out the implementation of the computer code and supporting algorithms, performed the formal analysis, data curation, preparation and creation of data presentation. JTB verified the results and supervised and managed the project. SDGG, SCMR and JTB carried out the investigation process. JDS provided the resources and acquired the financial support for the research. All authors reviewed the manuscript.

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Effect of volcanic rock dust and Fe-EDTA on the root nodule bacteria and the growth and yield of broad bean plants

Efecto del polvo de roca volcánica y Fe-EDTA sobre las bacterias de los nódulos de las raíces y el crecimiento y rendimiento de plantas de haba

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ABSTRACT

The experiment was conducted during the 2018 season at the research station of the College of Agriculture and Forestry (University of Mosul, Iraq) to investigate the effect of volcanic rock dust (VRD) (0, 125, 250 g m^{-2}) and the fertilizer chelated iron Fe-EDTA (0, 100, 200 mg L^{-1}) on the bacteria *Rhizobium fabae*, and the growth and yield of two broad bean (*Vicia faba* L.) varieties, Histal and Aquadulce. A randomized complete block design was used with three replicates and least significant difference (LSD) to compare means at a significance level of 0.05. Results showed that VRD 250 g m^{-2} obtained the highest significant mean for the following traits: leaf area index (LAI), number of branches per plant, leaf contents of chlorophyll and total iron, number of effective bacteria nodules per plant, 100-seed weight, plant yield, and percentage of protein in the seeds. On the other hand, 200 mg L^{-1} Fe-EDTA obtained the highest significant mean of all the traits for both varieties, except for LAI and number of branches per plant. The application of Fe-EDTA per plant had no significant effect on the leaf content of chlorophyll for both varieties and the percentage of protein in the seeds for variety Aquadulce. The interaction of VRD 250 g m^{-2} with 200 mg L^{-1} Fe-EDTA achieved the highest significant average for all the traits, except for plant height.

Key words: *Vicia faba* L., iron fertilizers, chelated Fe.

RESUMEN

El experimento se llevó a cabo durante la temporada 2018 en la estación de investigación del College of Agriculture and Forestry (Universidad de Mosul, Irak), para investigar el efecto del polvo de rocas volcánicas (PRV) (0, 125, 250 g m^{-2}) y el fertilizante hierro quelado Fe-EDTA (0, 100, 200 mg L^{-1}) sobre la bacteria *Rhizobium fabae* y el crecimiento y rendimiento de dos variedades de haba (*Vicia faba* L.), Histal y Aquadulce. Se utilizó un diseño en bloques completos al azar con tres repeticiones y la diferencia mínima significativa (DMS) para hacer una comparación entre las medias a un nivel de significancia de 0.05. Los resultados mostraron que PRV 250 g m^{-2} obtuvo la media significativa más alta para las siguientes características: índice de área foliar (IAF), número de ramas por planta, contenidos foliares de clorofila y hierro, número de nódulos bacterianos efectivos por planta, peso de 100 semillas, rendimiento de la planta, y porcentaje de proteína en las semillas. Por otra parte, 200 mg L^{-1} Fe-EDTA obtuvo la media significativa más alta de todas las características para ambas variedades, excepto para el IAF y el número de ramas por planta. La aplicación de Fe-EDTA por planta no tuvo un efecto significativo sobre el contenido foliar de clorofila para ambas variedades ni sobre el porcentaje de proteína en las semillas para la variedad Aquadulce. La interacción del PRV 250 g m^{-2} con 200 mg L^{-1} Fe-EDTA logró la media significativa más alta para todas las características, a excepción de la altura de la planta.

Palabras clave: *Vicia faba* L., fertilizantes de hierro, hierro quelatado.

Introduction

The broad bean is one of the old-world crops; it was one of the first plants cultivated in the Mediterranean basin (Albala, 2007) and was an important part of the nutrition system of the countries located to the east of the Mediterranean 6000 years B.C. (Smartt, 1990). It is a winter crop that belongs to the Fabaceae family (Stuessy, 2009). The seeds of broad beans contain about 11% water, 50-58% carbohydrates, 25-50% protein, 2% lipids and mineral nutrients including manganese, phosphorus, magnesium, and iron (Tull, 1997; Lewis *et al*., 2005; Duke, 2012). It is a rich source of nutrition for millions of people around the world (Nadal *et al*., 2005), particularly low-income societies. Broad bean plants enhance soil fertility as their roots are infected with nitrogen-fixing bacteria (Avila *et al*., 2005). The bacteria contain a leghemoglobin pigment (de Bruijn, 2015) that provides beans with effective iron, which is scarce in alkaline soils (Havlin *et al*., 2005).

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Volcanic rocks can be exploited as an alternative for soil fertilization and a more sustainable agriculture (Plata *et al.,* 2021). The primary oxides detected by X-ray fluorescence in the samples of volcanic rock dust were calcium oxide, silicon dioxide, aluminum oxide, iron oxide, and potassium oxide, with phosphorus oxide found at lower concentrations. The use of volcanic rocks solves an environmental issue associated with rock exploitation, providing an alternative for soil fertilization and a more sustainable agriculture (Ramos *et al.,* 2017). Ramos *et al.* (2020) found that the addition of 3625 and 7251 kg ha^{-1} of volcanic rock dust significantly increased the dry weight of corn leaves and incremented the absorption of phosphorus, potassium, calcium, and magnesium. Fadhil and Jader (2020) showed that chelated iron sprayed on the vegetative parts of peas at concentrations of 150 mg L^{-1} generated a significant increase in the plant height (cm), number of branches per plant, total number of leaves, number of pods per plant, length of the pods (cm), weight of the pod (g), number of seeds per pod, 100-seed weight (g), and total seed yield for the broad bean. This research aimed to study the effect of volcanic rock dust (VRD) on the growth and yield of broad bean to compare it with the most common method of iron fertilizer (chelated iron Fe-EDTA).

Materials and methods

The experiment was conducted at the research station of the College of Agriculture and Forestry, at the University of Mosul (Iraq) in December 2018. This experiment consisted in adding 0, 125, and 250 g m^{-2} of VRD in broadcast application to rows in the soil one month after planting. The VRD was obtained from Plan "B" Organics (Medford, OR, USA). The chelated iron Fe-EDTA fertilizer was added by spraying the plants until they were completely wet with concentrations of 0, 100, and 200 mg L^{-1} one month after planting. These treatments were applied to two varieties of broad beans, Histal and Aquadulce. The area of the experimental unit was 6 m^2. Cultivation was carried out on 4 rows at a spacing of 75 cm between rows and 20 cm between plants. Information about the physical and chemical properties of the soil used for cultivation and the rainfall rate and temperature during the growing season is shown in Table 1. The following variables were studied: plant height, leaf area index (LAI), number of branches per plant, contents of total chlorophyll and total iron (measured following the methodology of AOAC (1980)) in the leaves (at the beginning of the flowering stage), number of effective bacteria (*Rhizobium fabae*) nodules per plant, 100-seed weight, plant yield, and percentage of protein in seeds (measured at maturity).

TABLE 1. Physical and chemical properties of soil, rainfall rate, and temperature during the growing season.

Variable		Unit
Soil reaction degree	7.65	
Electrical conductivity	2.4	dS m^{-1}
Average temperature	24	°Celsius per d
Rainfall rate	118	mm per month
Available Nitrogen	12.1	mg kg^{-1}
Available Phosphorous	38.6	mg kg^{-1}
Available Potassium	14.1	mg kg^{-1}
Organic matter	0.473	g kg^{-1}
Volume distribution of soil particles		
Clay	15.2%	
Loam	61.75%	
Sand	23.05%	
Soil texture	Silty clay loam	

Data analysis

The statistical analysis was conducted using a randomized complete block design with three replicates. The significant differences between the means of the treatments were tested using the least significant difference (LSD) with a probability level of 0.05.

Results and discussion

Plant height

The highest significant mean for plant height was achieved without adding VRD 0 g m^{-2} with values of 82.7 and 87.5 cm (Tab. 2) for varieties Histal and Aquadulce, respectively. Adding 125 and 250 g m^{-1} of VRD resulted in a significant decrease in plant height for both varieties, with no significant difference between them. When no Fe-EDTA was added, the highest significant average was achieved with values of 82.1 and 86.3 cm for varieties Histal and Aquadulce, respectively. Adding 100 or 200 mg L^{-1} Fe-EDTA led to a significant decrease in plant height, with no significant difference between these doses. This fact might be due to the effects of VRD and Fe-EDTA that stimulate an increase in the number of branches per plant (Tab. 4) and, consequently, lead to a decrease in the main branch length (Ali *et al.*, 2020). The interaction of VRD 0 g m^{-2} and 0 mg L^{-1} Fe-EDTA obtained the highest significant mean with values of 84.2 and 89.3 cm for varieties Histal and Aquadulce, respectively. The interaction of VRD 250 g m^{-2} and 200 mg L^{-1} Fe-EDTA provided the least significant mean with values of 80.5 and 83.9 cm for varieties Histal and Aquadulce, respectively.

TABLE 2. Effect of volcanic rock dust (VRD) and Fe-EDTA and their interaction on the mean plant height (cm) of broad beans.

	Fe-EDTA 0 mg L^{-1}	Fe-EDTA 100 mg L^{-1}	Fe-EDTA 200 mg L^{-1}	VRD
Variety Histal				
VRD 0 g m^{-2}	84.2	82.1	81.8	82.7
VRD 125 g m^{-2}	81	80.25	80.21	80.5
VRD 250 g $^{m-}$2	81.1	80.37	80.5	80.7
Fe-EDTA	82.1	80.9	80.8	
$LSD_{0.05}$	VRD = 1.9	Fe-EDTA = 1.2		VRD*Fe-EDTA = 1.0
Variety Aquadulce				
VRD 0 g m^{-2}	89.3	86.7	86.5	87.5
VRD 125 g m^{-2}	85.3	84.4	84.5	84.7
VRD 250 g m^{-2}	84.2	84.2	83.9	84.1
Fe-EDTA	86.3	85.1	85.0	
$LSD_{0.05}$	VRD = 2.5	Fe-EDTA = 1.0		VRD*Fe-EDTA = 0.8

LSD – least significant difference test.

Leaf area index (LAI)

The highest significant mean for LAI was 4.2 and 4.8 for varieties Histal and Aquadulce, respectively, which was obtained by the interaction with VRD 250 g m^{-2}. There was no significant difference from VRD 125 g m^{-2}, while the treatment with VRD 0 g m^{-2} obtained the lowest significant mean with values of 3.3 and 4.4 for varieties Histal and Aquadulce, respectively. These results obtained when VRD was not applied are because it increases the leaf area (Tab. 3). Chelated iron Fe-EDTA application had no significant effect on the LAI for varieties Histal and Aquadulce. The highest mean was achieved by the interaction between VRD 250 g m^{-2} and 200 mg L^{-1} Fe-EDTA with values of 4.3 and 4.8 for varieties Histal and Aquadulce, respectively. The findings showed that the interactions between VRD 250 g m^{-2} and all Fe-EDTA levels were not significant; VRD 0 g m^{-2} with 0 mg L^{-1} Fe-EDTA showed the lowest significant interaction with values of 3.1 and 4.3 mg L^{-1} for varieties Histal and Aquadulce, respectively. The increase in the leaf area index may be due to the higher number of branches, as evidenced by Ali *et al.* (2020).

Number of branches per plant

VRD 250 g m^{-2} provided the highest significant means for number of branches with values of 5.1 and 5.6 for varieties Histal and Aquadulce, respectively, but it was not significantly different from VRD 125 g m^{-2} with values of 4.7 and 5.3 for varieties Histal and Aquadulce, respectively. The

TABLE 3. Effect of volcanic rock dust (VRD) and Fe-EDTA and their interaction on mean leaf area index (LAI) in broad beans.

	Fe-EDTA 0 mg L^{-1}	Fe-EDTA 100 mg L^{-1}	Fe-EDTA 200 mg L^{-1}	VRD
Variety Histal				
VRD 0 g m^{-2}	3.1	3.4	3.5	3.3
VRD 125 g m^{-2}	3.8	3.8	4.0	3.9
VRD 250 g m^{-2}	4.1	4.3	4.3	4.2
Fe-EDTA	3.7	3.8	3.9	
$LSD_{0.05}$	VRD = 0.5	Fe-EDTA = N.S.		VRD*Fe-EDTA = 0.3
Variety Aquadulce				
VRD 0 g m^{-2}	4.3	4.4	4.4	4.4
VRD 125 g m^{-2}	4.6	4.7	4.8	4.7
VRD 250 g m^{-2}	4.8	4.8	4.8	4.8
Fe-EDTA	4.6	4.6	4.7	
$LSD_{0.05}$	VRD = 0.3	Fe-EDTA = N.S.		VRD*Fe-EDTA = 0.2

LSD – least significant difference test; N.S. – Not significant.

least significant means were obtained when no Fe-EDTA was added with values of 4.4 and 4.9 for varieties Histal and Aquadulce, respectively. This was due to the significant impact of VRD on LAI (Tab. 3), leaf chlorophyll and iron content (Tabs. 4 and 5), and the number of active bacteria nodules (Tab. 6). As for the application of Fe-EDTA, there were no significant difference levels in terms of the number of branches formed by the plant. The reason behind this may be that the quantity of iron used by the plant is lower compared to VRD (Tab. 6). Also, the interaction between VRD 250 g m^{-2} and 200 mg L^{-1} Fe-EDTA provided the highest means, 5.2 and 5.6, for Histal and Aquadulce, respectively. There was no significant difference for the interaction between VRD 0 g m^{-2} and all Fe-EDTA levels, and the lowest significant interaction for varieties Histal and Aquadulce showed values of 4.3 and 4.7, respectively. An increasing number of active bacterial nodules and nitrogen content in plants may lead to a higher number of branches, as nitrogen enters the formation of auxins (de Bruijn, 2015).

Leaf chlorophyll content

The addition of VRD 250 g m^{-2} resulted in a significant increase of the chlorophyll content in the leaves, with mean values of 33.5 and 34.5 μg cm^{-2} for varieties Histal and Aquadulce, respectively (Tab. 5). There were no significant differences with VRD 125 g m^{-2}, with values of 33.2 and 34.1 μg cm^{-2}. When VRD was not added, the least significant

TABLE 4. Effect of volcanic volcanic rock dust (VRD) and Fe-EDTA and their interaction on the mean number of branches per plant in broad bean.

	Fe-EDTA 0 mg L^{-1}	Fe-EDTA 100 mg L^{-1}	Fe-EDTA 200 mg L^{-1}	VRD
Variety Histal				
VRD 0 g m^{-2}	4.3	4.3	4.5	4.4
VRD 125 g m^{-2}	4.6	4.6	4.8	4.7
VRD 250 g m^{-2}	5.0	5.0	5.2	5.1
Fe-EDTA	4.6	4.6	4.8	
$LSD_{0.05}$	VRD = 0.5	Fe-EDTA = N.S.	VRD*Fe-EDTA = 0.3	
Variety Aquadulce				
VRD 0 g m^{-2}	4.7	4.9	5.0	4.9
VRD 125 g m^{-2}	5.3	5.3	5.4	5.3
VRD 250 g m^{-2}	5.6	5.6	5.6	5.6
Fe-EDTA	5.2	5.3	5.3	
$LSD_{0.05}$	VRD = 0.6	Fe-EDTA = N.S.	VRD*Fe-EDTA = 0.4	

LSD - least significant difference test; N.S. Not significant.

TABLE 5. Effect of volcanic rock dust (VRD) and Fe-EDTA and their interaction on the leaf chlorophyll content (μg cm^{-2}).

	Fe-EDTA 0 mgL^{-1}	Fe-EDTA 100 mg L^{-1}	Fe-EDTA 200 mg L^{-1}	VRD
Variety Histal				
VRD 0 g m^{-2}	32.6	32.6	32.8	32.7
VRD 125 g m^{-2}	33.1	33.2	33.2	33.2
VRD 250 g m^{-2}	33.4	33.5	33.5	33.5
Fe-EDTA	33.0	33.1	33.2	
$LSD_{0.05}$	VRD = 0.5	Fe-EDTA = N.S.	VRD*Fe-EDTA = 0.3	
Variety Aquadulce				
VRD 0 g m^{-2}	33.6	33.7	33.8	33.7
VRD 125 g m^{-2}	33.9	34.1	34.3	34.1
VRD 250 g m^{-2}	34.5	34.6	34.5	34.5
Fe-EDTA	34.0	34.1	34.2	
$LSD_{0.05}$	VRD = 0.6	Fe-EDTA = N.S.	VRD*Fe-EDTA = 0.3	

LSD - least significant difference test; N.S. - Not significant.

means were 32.7 and 33.7 μg cm^{-2} for varieties Histal and Aquadulce, respectively. This might be because VRD contains magnesium, which is a component of chlorophyll. The addition of Fe-EDTA had no significant effect on the chlorophyll content in the leaf as it does not contain magnesium. The highest significant averages were obtained by the interaction of VRD 250 g m^{-2} and 200 mg L^{-1} Fe-EDTA with a value of 33.5 μg cm^{-2} for both varieties. There was no significant difference for the interaction between VRD 250 g m^{-2} and 100 mg L^{-1} Fe-EDTA with a value of 33.5 for variety Histal, while the interaction of VRD 250 g m^{-2} with 100 mg L^{-1} Fe-EDTA obtained the highest significant mean with a value of 34.6 μg cm^{-2} for variety Aquadulce. No significant differences were observed for the interaction between VRD 250 g m^{-2} and all the Fe-EDTA levels for both varieties. The least significant mean for the interaction between VRD 0 g m^{-2} and 0 mg L^{-1} Fe-EDTA was 32.6 and 33.6 μg cm^{-2} for varieties Histal and Aquadulce, respectively. The higher content of chlorophyll in leaves may be due to the increment in nitrogen produced by the root nodes, as nitrogen increases the formation of chlorophyll (Havlin *et al.*, 2005).

Total leaf iron content

The highest means of leaf total iron content were obtained with VRD 250 g m^{-2} with values of 2.103 and 2.642 g kg^{-1} for varieties Histal and Aquadulce, respectively; the treatment without Fe-EDTA obtained the lowest mean values of 0.382 and 0.493 g kg^{-1} when VRD 0 g m^{-2} was added to the varieties. Also, the highest significant mean values of 1.312 and 1.707 g kg^{-1} were obtained by using Fe-EDTA 200 mg L^{-1} for varieties Histal and Aquadulce, respectively, and it was not significantly different from Fe-EDTA 100 mg L^{-1} that obtained a mean value of 1.256 g kg^{-1}. The least significant means of 1.142 and 1.503 g kg^{-1} were obtained when Fe-EDTA 0 mg L^{-1} was added. The increase in the iron content in the leaf was due to the VRD and Fe-EDTA content (Tab. 6). The interaction between VRD 250 g m^{-2} and Fe-EDTA 200 mg L^{-1} obtained the highest significant means of 2.166 and 2.711 g kg^{-1} for varieties Histal and Aquadulce, respectively. There were no significant differences for the interaction between VRD 250 g m^{-2} and all the Fe-EDTA levels for both varieties. The lowest means were 0.238 and 0.301 g kg^{-1} and resulted from the interaction between VRD 0 g m^{-2} and Fe-EDTA 0 mg L^{-1} for Histal and Aquadulce, respectively. The significant effect of VRD on the leaf content of total iron may be due to the reduction in the degree of soil reaction due to its acidic effect in the soil (Ramos *et al.,* 2020) increasing iron availability in the alkaline soil (Jensen, 2010).

Number of effective bacteria nodules per plant

The highest significant means of the number of effective bacteria nodules per plant were 16.7 and 16.0 for varieties Histal and Aquadulce, respectively (Tab. 7), when VRD 250 g m^{-2} was added. This treatment was not significantly different from VRD 125 g m^{-2}, which obtained a mean value of 15.1 mg L^{-1} for the variety Aquadulce. When VRD was not added, the least significant means with values of 7.3 and 9.6 were obtained for varieties Histal and Aquadulce, respectively. The concentration of 200 mg L^{-1} Fe-EDTA obtained the highest significant means for the effective bacteria with values of 13.8 and 14.1 for varieties Histal and Aquadulce, respectively. There was no significant difference from 100 mg L^{-1} Fe-EDTA, which obtained an average value of 13.8

TABLE 6. Effect of volcanic rock dust (VRD) and Fe-EDTA and their interaction on the total leaf iron content (g kg^{-1}).

	Fe-EDTA 0 mg L^{-1}	Fe-EDTA 100 mg L^{-1}	Fe-EDTA 200 mg L^{-1}	VRD
Variety Histal				
VRD 0 g m^{-2}	0.238	0.420	0.487	0.382
VRD 125 g m^{-2}	1.147	1.244	1.284	1.225
VRD 250 g m^{-2}	2.041	2.103	2.166	2.103
Fe-EDTA	1.142	1.256	1.312	
$LSD_{0.05}$	VRD = 0.811	Fe-EDTA = 0.291	VRD*Fe-EDTA = 0.159	
Variety Aquadulce				
VRD 0 g m^{-2}	0.301	0.570	0.607	0.493
VRD 125 g m^{-2}	1.702	1.790	1.804	1.765
VRD 250 g m^{-2}	2.505	2.710	2.711	2.642
Fe-EDTA	1.503	1.690	1.707	
$LSD_{0.05}$	VRD = 0.713	Fe-EDTA = 0.201	VRD*Fe-EDTA = 0.110	

LSD - least significant difference test.

mg L^{-1} for variety Aquadulce compared to the treatment without addition of Fe-EDTA, which gave the lowest mean values of 11.5 and 12.8 for varieties Histal and Aquadulce, respectively. The reason is that VRD and Fe-EDTA contain iron that is a constituent of leghemoglobin that exists in bacteria nodules (Johnston, 2004). The use of VRD 250 g m^{-2} with 200 mg L^{-1} Fe-EDTA showed the highest significant means of effective bacteria nodules, 17.1 and 16.3, for varieties Histal and Aquadulce, respectively. There was no significant difference for the interaction between VRD 250 g m^{-2} and all Fe-EDTA levels or for the interaction between VRD 125 g m^{-2} and 200 mg L^{-1} Fe-EDTA. The least significant means were 6.1 and 8.2 for varieties Histal and Aquadulce, respectively, which resulted from the interaction between VRD 0 g m^{-2} and 0 mg L^{-1} Fe-EDTA.

Weight of 100 seeds

Significant differences were observed in the weight of 100 seeds; VRD 250 g m^{-2} obtained the highest means of 35.4 and 34.5 g for varieties Histal and Aquadulce, respectively (Tab. 8). The treatment without addition of VRD obtained the least significant means of 33.0 and 31.2 g respectively. This is due to the positive impact of VRD on LAI and chlorophyll and iron contents in the leaf, and on the number of effective bacteria nodes (Tabs. 3, 5, 6 and 7). The application of 200 mg L^{-1} Fe-EDTA showed the highest significant means of 34.7 and 33.1 g for varieties Histal and Aquadulce, respectively. There was no significant difference with 100 mg L^{-1} Fe-EDTA, which obtained values of 34.4 and 33.0 g compared to the treatment without the addition of Fe-EDTA, which showed the lowest means of 33.8 and 32.7 g

TABLE 7. Effect of volcanic rock dust (VRD) and Fe-EDTA and their interaction on the number of effective bacteria nodules per plant.

	Fe-EDTA 0 mg L^{-1}	Fe-EDTA 100 mg L^{-1}	Fe-EDTA 200 mg L^{-1}	VRD
Variety Histal				
VRD 0 g m^{-2}	6.1	7.2	8.6	7.3
VRD 125 g m^{-2}	12.1	15.5	15.7	14.4
VRD 250 g m^{-2}	16.2	16.8	17.1	16.7
Fe-EDTA	11.5	13.2	13.8	
$LSD_{0.05}$	VRD = 1.7	Fe-EDTA = 0.5	VRD*Fe-EDTA = 0.3	
Variety Aquadulce				
VRD 0 g m^{-2}	8.2	10.2	10.4	9.6
VRD 125 g m^{-2}	14.6	15.2	15.6	15.1
VRD 250 g m^{-2}	15.5	16.1	16.3	16.0
Fe-EDTA	12.8	13.8	14.1	
$LSD_{0.05}$	VRD = 1.3	Fe-EDTA = 0.7	VRD*Fe-EDTA = 0.4	

LSD - least significant difference test.

TABLE 8. Effect of volcanic rock dust (VRD) and Fe-EDTA and their interaction on the weight of 100 seeds (g) of broad beans.

	Fe-EDTA 0 mg L^{-1}	Fe-EDTA 100 mg L^{-1}	Fe-EDTA 200 mg L^{-1}	VRD
Variety Histal				
VRD 0 g m^{-2}	32.3	33.4	33.4	33.0
VRD 125 g m^{-2}	34.1	34.7	34.9	34.6
VRD 250 g m^{-2}	35.1	35.2	35.8	35.4
Fe-EDTA	33.8	34.4	34.7	
$LSD_{0.05}$	VRD = 0.6	Fe-EDTA = 0.4	VRD*Fe-EDTA = 0.2	
Variety Aquadulce				
VRD 0 g m^{-2}	31.0	31.2	31.4	31.2
VRD 125 g m^{-2}	32.8	33.1	33.3	33.1
VRD 250 g m^{-2}	34.4	34.6	34.6	34.5
Fe-EDTA	32.7	33.0	33.1	
$LSD_{0.05}$	VRD = 0.4	Fe-EDTA = 0.4	VRD*Fe-EDTA = 0.3	

LSD - least significant difference test.

at 0 g m^{-2} Fe-EDTA. This is due to the positive impact of Fe-EDTA in terms of the number of active bacteria (Tab. 7). A positive relationship between the 100-seed weight, leaf area, and leaf area index was observed (Ali *et al.*, 2020). The interaction between VRD 250 g m^{-2} and 200 mg L^{-1} Fe-EDTA obtained the highest significant means of 35.8 and 34.6 g for varieties Histal and Aquadulce, respectively. There was no significant difference regarding the interaction between VRD 250 g m^{-2} and all Fe-EDTA levels. The lowest significant averages were 32.2 and 31.0 g for varieties Histal and Aquadulce, respectively, for the interaction between VRD 0 g m^{-2} and 0 mg L^{-1} Fe-EDTA (Ali, 2020), showing the variation in the 100-seed weight trait.

Plant yield

The highest plant yield values were 33.6 and 33.6 g, which resulted from adding VRD 250 g m^{-2} to varieties Histal and Aquadulce, respectively; the lowest significant mean values of 28.9 and 30.8 g for both varieties (Tab. 9), resulted from not adding VRD. This result is due to the significant effect of VRD on LAI, number of branches per plant, number of effective bacteria, and 100-seed weight (Tabs. 3, 4, 7, and 8). VRD interaction with 200 mg L^{-1} Fe-EDTA obtained the highest significant mean with values 32.4 and 32.5 g for Histal and Aquadulce, respectively, with no significant difference with 100 mg L^{-1} Fe-EDTA, which showed values of 31.8 and 32.0 g. The lowest significant means of 30.8 and 31.5 g for varieties Histal and Aquadulce, respectively, were obtained when no Fe-EDTA was added. This is due to the significant effect of Fe-EDTA on the number of effective bacteria and 100-seed weight (Tabs. 7 and 8). A positive relationship between plant yield, leaf area, leaf area index, and leaf chlorophyll content was observed (Ali *et al.*, 2020). The interaction between VRD 250 g m^{-2} and 200 mg L^{-1} Fe-EDTA showed the highest significant mean for plant yield with values of 34.1 and 33.9 g for varieties Histal and Aquadulce, respectively, and there was no significant difference for the interaction between VRD 250 g m^{-2} and all Fe-EDTA levels. The lowest means were 28.2 and 30.4 g for varieties Histal and Aquadulce, respectively, resulting from the interaction between VRD 0 g m^{-2} and 0 mg L^{-1} Fe-EDTA.

Protein contents in seeds

VRD 250 g m^{-2} showed the highest significant mean for the percentage of protein in seeds with values of 26.4 and 27.5% for varieties Histal and Aquadulce, respectively (Tab. 10) compared to the least significant mean for the application of VRD 0 g m^{-2} with values of 24.8 and 25.4% for both varieties. This result is due to the positive impact of VRD in terms of the number of effective bacteria (Tab. 7). The application of 200 mg L^{-1} Fe-EDTA obtained the highest significant mean of 25.8% and was not significantly different from 100 mg L^{-1} Fe-EDTA. The least significant mean (25.4%) resulted from the treatment 0 mg L^{-1} Fe-EDTA to Histal, but variety Aquadulce was not significantly affected by Fe-EDTA application. This may be because the increase of carbohydrates was higher than proteins and eventually affected the contents of proteins. The interaction between VRD 250 g m^{-2} and 200 mg L^{-1} Fe-EDTA obtained the highest significant mean values of 26.5 and 27.5% for varieties Histal and Aquadulce, respectively. There was no significant difference for the interaction between VRD 250 g m^{-2} and all the levels of Fe-EDTA. The least significant means from the interaction between VRD 0 g m^{-2} and

TABLE 9. Effect of volcanic rock dust (VRD) and Fe-EDTA and their interaction on plant yield (g).

	Fe-EDTA 0 mg L^{-1}	Fe-EDTA 100 mg L^{-1}	Fe-EDTA 200 mg L^{-1}	VRD
Variety Histal				
VRD 0 g m^{-2}	28.2	29.1	29.5	28.9
VRD 125 g m^{-2}	31.4	32.4	33.5	32.4
VRD 250 g m^{-2}	32.9	33.8	34.1	33.6
Fe-EDTA	30.8	31.8	32.4	
$LSD_{0.05}$	VRD = 1.1	Fe-EDTA = 0.9	VRD*Fe-EDTA = 0.7	
Variety Aquadulce				
VRD 0 g m^{-2}	30.4	30.8	31.1	30.8
VRD 125 g m^{-2}	30.9	31.6	32.6	31.7
VRD 250 g m^{-2}	33.2	33.7	33.9	33.6
Fe-EDTA	31.5	32.0	32.5	
$LSD_{0.05}$	VRD = 1.2	Fe-EDTA = 0.7	VRD*Fe-EDTA = 0.5	

LSD - least significant difference test.

TABLE 10. Effect of volcanic rock dust (VRD) and Fe-EDTA and their interaction on protein contents (%) in seeds of broad bean plants.

	Fe-EDTA 0 mg L^{-1}	Fe-EDTA 100 mg L^{-1}	Fe-EDTA 200 mg L^{-1}	VRD
Variety Histal				
VRD 0 g m^{-2}	24.3	25.1	25.1	24.8
VRD 125 g m^{-2}	25.7	25.7	25.8	25.7
VRD 250 g m^{-2}	26.2	26.4	26.5	26.4
Fe-EDTA	25.4	25.7	25.8	
$LSD_{0.05}$	VRD = 0.5	Fe-EDTA = 0.3	VRD*Fe-EDTA = 0.3	
Variety Aquadulce				
VRD 0 g m^{-2}	25.1	25.4	25.6	25.4
VRD 125 g m^{-2}	26.3	26.4	26.7	26.5
VRD 250 g m^{-2}	27.4	27.5	27.5	27.5
Fe-EDTA	26.3	26.4	26.6	
$LSD_{0.05}$	VRD = 0.6	Fe-EDTA = N.S.	VRD*Fe-EDTA = 0.3	

LSD - least significant difference test.

0 mg L^{-1} Fe-EDTA were 24.3 and 25.1% for Histal and Aquadulce, respectively. This significant effect was observed by Ali *et al.* (2020) in most of the variables studied.

Conclusions

Volcanic rock dust (VRD) was more effective than chelated iron Fe-EDTA in terms of increasing effective bacteria nodules, growth (except for the plant height) and yield of both varieties of broad beans. The application of chelated iron had no significant effect on LAI, number of branches per plant, and chlorophyll content for both varieties. Additionally, it did not have a significant impact on the percentage of protein in the seeds of variety Aquadulce. The interaction of Fe-EDTA and VRD showed significant effects on all the variables.

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

OAA and MAA designed and carried out the field experiments, OAA carried out the laboratory experiments, and MMA contributed to the data analysis. Both authors wrote the article.

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Soil fertility management practices for faba bean (*Vicia faba* L.) production in Wolaita zone, Southern Ethiopia

Prácticas de manejo de la fertilidad del suelo para la producción de habas (*Vicia faba* L.) en la zona de Wolaita, sur de Etiopía

Bekalu Abebe[1*], Nigussie Dechassa[2], Tamado Tana[3], Fanuel Laekemariam[1], and Yibekal Alemayehu[2]

ABSTRACT

Faba bean is an important food security crop in Southern Ethiopia. Understanding the soil fertility management practices of faba bean farmers could aid in finding a method to replenish soil fertility. However, information on the type and extent of soil fertility management practiced by smallholder faba bean farmers is scarce. Therefore, a study was conducted in the districts of Damot Gale and Sodo Zuria in Wolaita zone in Southern Ethiopia to assess soil fertility management practiced by farmers for faba bean production. In the 2019 main crop season, 310 farmers were purposively selected by using Yamane's simplified formula to calculate the sample size and a short structured questionnaire was used to elicit information. The results revealed that faba bean production in the districts studied was constrained by scarcity of arable land that resulted in extensive exploitation of soil nutrients, poor inherent soil fertility, and soil acidity. Poor soil fertility limited grain yield productivity about 57.4%. Additionally, 36.5% of the farm yield was constrained by soil acidity. However, only 27.7% of farms managed the soil by using mineral fertilizers, 32.3% applied farmyard manure, 3.5% used liming, and 2.9% used fallowing. Consequently, the average grain productions of both fertilized and unfertilized faba bean farms were far less than the national average yield of 2.1 t ha^{-1}. The study concluded that soils of the study districts are managed inadequately to enhance their fertility and improve crop yield.

Key words: fertilizer, liming, production constraints, national average yield.

RESUMEN

Las habas son un cultivo importante para la seguridad alimentaria en el sur de Etiopía. Comprender las prácticas de manejo de la fertilidad del suelo de los agricultores de habas podría ser de ayuda para encontrar un método para reponer la fertilidad del suelo. Sin embargo, la información sobre el tipo y el alcance del manejo de la fertilidad del suelo practicado por los pequeños agricultores de habas es escasa. Por lo tanto, se realizó un estudio en los distritos de Damot Gale y Sodo Zuria en la zona de Wolaita en el sur de Etiopía para evaluar el manejo de la fertilidad del suelo practicado por los agricultores para la producción de habas. En la temporada agrícola principal de 2019, se seleccionaron intencionalmente 310 agricultores mediante el uso de la fórmula simplificada de Yamane para calcular el tamaño de la muestra y se utilizó un breve cuestionario estructurado para obtener información. Los resultados revelaron que la producción de habas en los distritos estudiados estaba restringida por la escasez de tierra cultivable que resultó en una explotación extensiva de los nutrientes del suelo, la pobre fertilidad inherente del suelo y la acidez del mismo. La mala fertilidad del suelo limitó la productividad del rendimiento de grano alrededor del 57.4%. Además, el 36.5% del rendimiento de las granjas se vio limitado por la acidez del suelo. Sin embargo, sólo el 27.7% de las granjas manejó el suelo utilizando fertilizantes minerales; el 32.3% aplicó estiércol de corral, el 3.5% utilizó encalado y el 2.9% utilizó barbecho. En consecuencia, la producción promedio de granos de las granjas de habas fertilizadas y no fertilizadas fue mucho menor que el rendimiento promedio nacional de 2.1 t ha^{-1}. El estudio concluyó que los suelos de los distritos del estudio se manejan de manera inadecuada para mejorar su fertilidad y el rendimiento de los cultivos.

Palabras clave: fertilizante, encalado, restricciones de producción, rendimiento promedio nacional.

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Introduction

Faba bean (*Vicia faba* L.) is one of the major pulse crops grown in the highlands of Ethiopia (Fedaku *et al.*, 2019). Currently, it occupies 31% of the area cultivated to pulses (1,863,445 ha) in the country (CSA, 2019). The crop plays significant roles in human and livestock feed and the improvement of soil fertility (Mulugeta *et al.*, 2019). However, the productivity of the crop in the country is low (2.12 t ha^{-1}) compared to the average yield (3.7 t ha^{-1}) obtained in major faba bean producing countries in the world (FAOSTAT, 2017; CSA, 2019). In Wolaita zone, faba bean occupies 96.4% of the area of land cultivated to pulses (1,074.91 ha) (CSA, 2019), while the farmers harvest a lower average yield (1.2 t ha^{-1}) than the national average yield obtained in the country (CSA, 2018). The major factors usually mentioned for the low yield of faba bean in Wolaita zone include climatic conditions, edaphic factors (soil fertility and acidity), biotic factors (diseases, pests, and weeds), scarcity of improved varieties, and poor agronomic practices (Buraka *et al.,* 2016).

Extensive exploitation and depletion of nutrients occur in Ethiopia due to continuous cropping, limited fallowing and crop rotation, complete removal of crop residues, and minimum or no use of mineral fertilizers and lime on acidic soil (Haileslassie *et al.*, 2006; Abera & Belachew, 2011). The loss of nitrates, phosphates, and potassium in the soils results in macronutrient imbalance (Ayalew & Dejene, 2011; Shanka *et al.*, 2018). Kassa Colbe *et al.* (2020) indicate that the soil management intervention of Wolaita zone farmers is also inadequate to improve soil fertility and produce high yield. The cumulative effect of nutrient deficiency in the soils has resulted in less productivity of faba bean in Wolaita zone (Buraka *et al.,* 2016; Belete *et al.*, 2019).

Several researchers studied soil fertility management practices for faba bean production in different parts of Ethiopia (Agegnehu & Yirga, 2009; Fedaku *et al.*, 2019; Mesfin *et al.*, 2020). Those reports revealed that significant improvements in the yield of faba bean can be brought about by proper soil fertility management like crop rotation, crop residue management, fallowing, application of balanced fertilizer, and use of lime on acidic soils. However, in Wolaita zone, limited research is done on the soil fertility management practices of faba bean producing farmers, and little information is available. Concrete information is required about farmer soil fertility management for faba bean production to rate the potential and limitations of the soils for faba bean productivity in the farming area of Wolaita zone. In the meantime, the findings enable the formulation of strategies for soil fertility management and forward directions to enhance the crop production of smallholder farmers. Therefore, this study aimed to investigate the effects of farmer soil fertility management on faba bean productivity.

Materials and methods

Study area

The study was conducted in Damot Gale and Sodo Zuria woredas (districts), Wolaita zone, in Southern Ethiopia (Fig. 1) during the 2019 growing season. The districts were selected based on their high faba bean production potential. Ethiopia is located from 3°00’00” to 14°08’00” N, and 33°00’00” to 48°00’00” E in Eastern Africa. Damot Gale district is located from 6°55’22” to 7°05’00” N and 37°45’31” to 37°59’58” E. The elevation of Damot Gale district ranges from 1501 to 2950 m a.s.l. (Mota *et al.*, 2019). Sodo Zuria district is located from 6°46’60” to 6°56’45” N and 37°38’10” to 37°50’60” E at an elevation from 1500 to 3500 m a.s.l. (Bashe *et al.*, 2018). According to MOA (1998) classification, faba bean producing areas of both Damot Gale and Sodo Zuria districts are predominantly characterized by cool sub-humid climates (Woinadega). The total annual rainfall of Damot Gale district in the last ten years (2011-2020) was 1,181 mm and that of Sodo Zuria district was 1,426 mm. Both districts have a bimodal rainfall pattern, which consists of *Belg* (short rainy season) and *Meher* (long main rainy season) (FAO, 2020). The *Belg* rainfall in the Zone occurs mainly during March, April, and May and the *Meher* rain occurs during June, July, and August. In Damot Gale district, about 32.9% and 38.2% of the precipitation occurred during the *Belg* and *Meher*, respectively. The last ten-year mean monthly temperature of Damot Gale district ranged from 13.8 to 24.9°C with an average of 19.4°C. In Sodo Zuria district, about 31% and 40% of precipitation occurred during the *Belg* and *Meher*, respectively. The mean monthly temperature of Damot Gale district in the last ten years ranged from 15.4 to 25.8°C with an average of 20.6°C. The agricultural practices are predominantly small-scale mixed subsistence farming. The cropping system is mainly based on continuous cultivation without any fallow periods (Laekemariam *et al.*, 2016). The colors on the map indicate six sub-districts of the study.

Sampling method and sample size

The sample size for each district was fixed according to Yamane’s (1967) simplified formula to calculate the sample size:

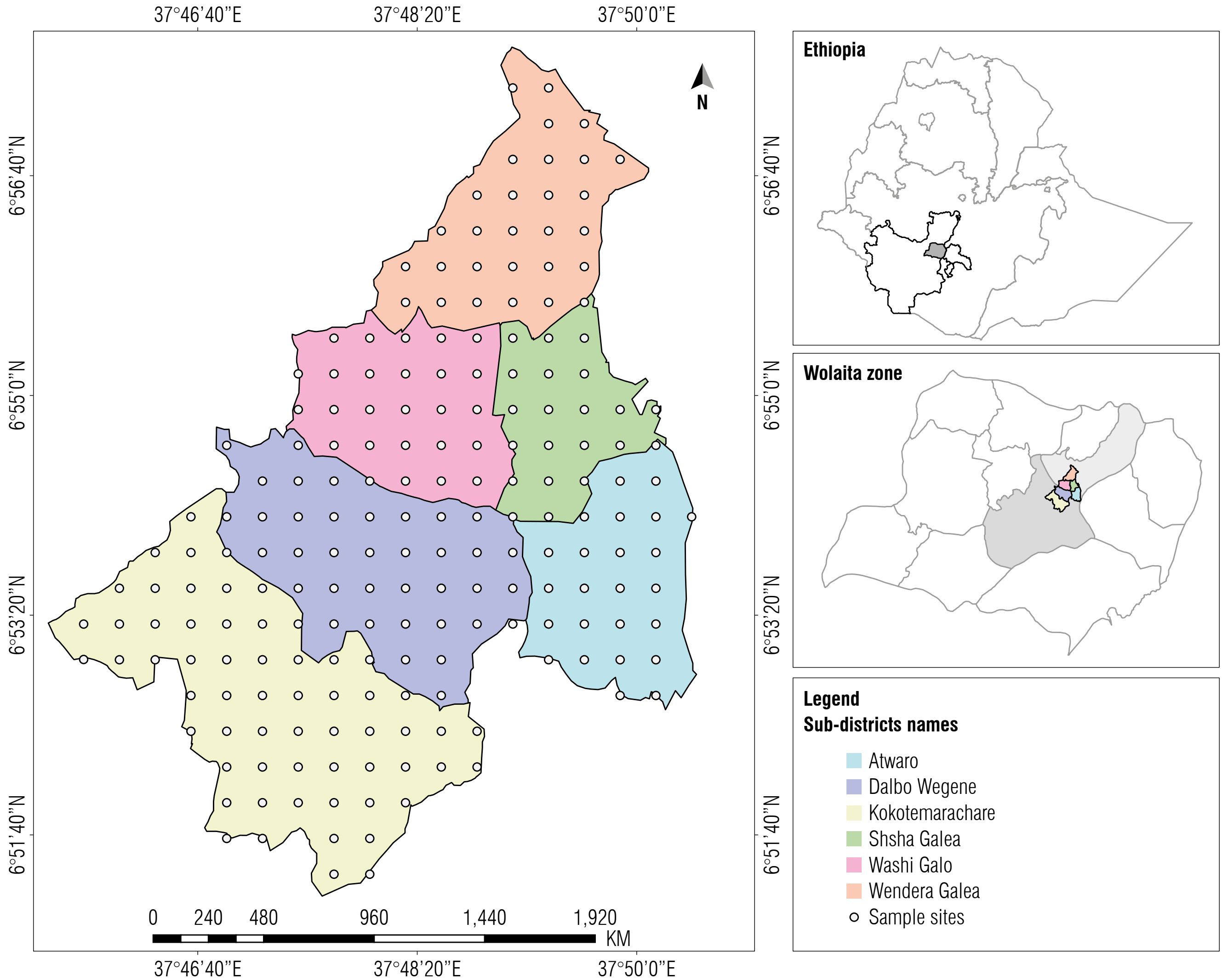

FIGURE 1. Map of the study area.

$$n = \frac{N}{1+N(e^2)} \quad (1)$$

where n is the sample size, N is the population size, and e is the level of precision at 95% confidence level. The number of samples varied in the sub-districts depending on the number of the human population residing in the areas. Hence, 310 household heads (163 from Damot Gale and 147 from Sodo Zuria) were interviewed about the soil management practices for faba bean production.

Data collection

The short-structured questionnaire (Supplementary material 1) used to record the soil fertility management practices included cropping history, crop rotation practices, fallowing, cropping intensity, production constraints, soil fertility management practices, crop residue management, seed use (local seed purchased from the local market and improved seed obtained freely from a research center), and fertilizer use (types and rates). The altitude and latitude of each farm was recorded by using the global positioning system (GPS).

Statistical analysis

To analyze the data, descriptive statistics were employed. Mean and percentage were computed for different variables. Pearson chi-square, t, and F tests also were calculated. Data analysis was carried out using the statistical package for social sciences (SPSS) software version 20 (SPSS, 2011).

Results and discussion

Faba bean farmer soil classification

In the study area, the faba bean producing farmers classified and assigned the local names to the soils to manage these accordingly. Farmers in both Damot Gale and Sodo Zuria districts used similar parameters to classify and name the soils. These farmers characterized and named seven faba bean growing soil types by using *bita as* a suffix, which connotes the term "soil" in the Wolaitia language. According to this, the soils were *Arradabita* (Eutric Nitosols), *Lada bita* (Haplic Alfisols), *Kareta bita* (Humic Nitisols), *Zo'o bita* (Vertisols), *Gobo bita* (Vertisols), *Chare bita* (Orthic Luvisols), and *Talla bita* (Haplic Alisols) (Fig. 2). The first six soils are common in both districts, except for *Talla bita,* which is found in Damot Gale district only. Among the soil types, *Arrada bita* is predominant in both districts, followed by *Lada bita*. *Zo'o bita* is less represented in both districts (Fig. 2).

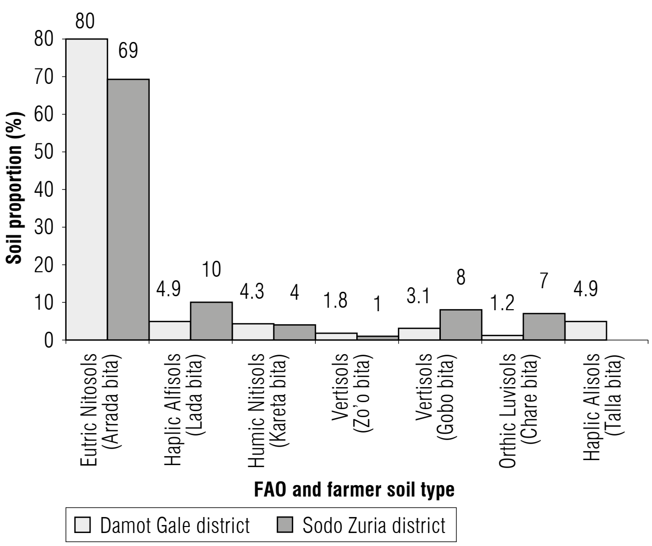

FIGURE 2. FAO and farmer soil types for samples collected from Damot Gale and Sodo Zuria districts in Wolaita zone, Southern Ethiopia.

Most farmers used soil color, soil fertility, workability, and water permeability as the criteria for classification (Tab. 1). Accordingly, farmers considered black/dark soil as fertile and suitable for faba bean productivity, while the brown/red soil is considered of low fertility and less productive. The perception of farmers was also reported by Bobo *et al.* (2017) and Corbeels *et al.* (2000) who stated that the dark soil is more fertile than brown. In general, farmers ranked *Arrada bita* in the highest to medium fertility status. Similarly, most faba bean farmers preferred *Arrada bita* for enhanced productivity.

On the contrary, *Lada*, *Gobo*, *Zo'o* and *Chere bita* were ranked as of low fertility and were the less preferred soils for faba bean production. The farmers ranked *Talla* and *Kareta bita* under medium to low soil fertility class (Tab. 1). Overall, as noticed from the interview, the majority of the farmers planted faba bean in fertile soil (*Arrada bita*).

Cropping history of faba bean farms

Crop intensity

The number of crops grown each year on a faba bean production field varies from one to three, depending on the soil fertility and amount of rainfall in the cropping season. The cropping intensity varied significantly among districts ($\chi^2 = 22.93$, $P \leq 0.001$) (Tab. 2). Also, the intensity was in the order of two crops>one crop>three crops per land per year (Tab. 1). About 54% and 63.3% of the interviewed farmers have grown two successive crops a year in the same field at Damot Gale and Sodo Zuria districts, respectively. On the other hand, 42.3% and 22.5% of the sampled farmers have grown only one crop per year on a plot of land at Damot Gale and Sodo Zuria districts, respectively. However, very few Damot Gale (3.7%) and Sodo Zuria district (14.2%) farmers had grown three successive crops a year in the same field (Tab. 2). The majority (58.4%) of the interviewed farmers had grown more than one crop each year (Tab. 2).

TABLE 1. Classification and commonly perceived soil characteristics by faba bean farmers at Damot Gale and Sodo Zuria districts in Wolaita zone, Southern Ethiopia.

Common soil name (FAO, 1984)	Soil classification	Textural class (Laekermariam *et al.*, 2016)	Farmer's parameter for classification			
			Color	Fertility	Workability	Permeability
Eutric nitosols	*Arrada bita*	Silty clay	Black/dark	High to medium	Easy	High
Haplic alfisols	*Lada bita*	Clay	Red	Low	Moderate	Moderate
Haplic alisols	*Talla bita*	Clay	Reddish brown	Medium to low	Difficult (sticky)	Low
Humic nitisols	*Kareta bita*	Silty clay loam	Black/dark	Medium	Difficult	Moderate
Vertisols	*Zo'o bita*	Clay	Brown	Low	Moderate	Moderate to low
Vertisols	*Gobo bita*	Clay	Red	Low	Moderate	Moderate to low
Orthic luvisols	*Chere bita*	Silty clay	Brown	Low	Difficult	Very low

"*Bita*" means soil in Wolaita language.

TABLE 2. Faba bean farmer cropping practices in the sampled agricultural fields at Damot Gale and Sodo Zuria districts of Wolaita zone, Southern Ethiopia.

Source of variations	DF	Sampled farmers	Districts		
			Damot Gale (%)	Sodo Zuria (%)	Total (%)
Crop intensity per year	1	χ^2 value	N = 163	N = 147	N = 310
One			42.3	22.5	32.9
Two		22.93***	54	63.3	58.4
Three			3.7	14.2	8.7
Varieties planted	1		N = 163	N = 147	N = 310
Local		0.98 NS	65.6	64	64.8
Improved variety			34.4	36	35.2

χ^2 - Chi-square value; N - number of sampled farmers involved in faba bean cropping practices; ***Significant at $P<0.001$; NS - not significant; DF - degree of freedom.

The sampled smallholder farmers indicated poor soil fertility, inorganic fertilizer prices and a limited labor force as challenges to grow more than one crop each year on a field. Hence, most of the study area farmers were economically poor, leading a hand to mouth lifestyle. Similarly, soil fertility constraints, limited rainfall, and financial problems were reported in different parts of Ethiopia (Headey *et al.*, 2014; Kemaw & Fentahun, 2018). In general, cultivating only faba bean per plot each year would reduce over-exploitation of soil nutrients and would increase grain production. Nevertheless, multiple cropping had a relative yield and economic advantage to single cropping. Increased cropping intensity requires better soil fertility management (Kemaw & Fentahun, 2018). In this regard, the sampled faba bean fields were not managed adequately. Therefore, increased cropping intensity results in large nutrient exploitation, unless proper soil management practices are applied to balance required nutrients.

Faba bean varieties used in studied districts

The number of farmers who grow improved varieties did not show statistical differences between both districts (Tab. 2). The local faba bean variety was dominant over-improved varieties and covered about 65.6% and 64% at DamotGale and Sodo Zuria districts, respectively (Tab. 2). Different research studies conducted on acidic soils in Ethiopia showed that the improved varieties had a significantly higher grain yield over the local variety (Agegnehu & Yirga, 2009; Belachew & Stoddard, 2017). However, the results in this study indicated that very few farmers grow improved varieties (Tab. 2). As noticed from the discussion, farmers used only 'Dosha' as the improved faba bean variety. Thus, the predominant growth of the local variety might be among the reasons for far lower productivity in the studied districts.

Faba bean production constraints

Land shortage

The number of faba bean farmers challenged by land shortage did not significantly vary between Damot Gale and Sodo Zuria districts (Tab. 3). The survey recognized that most of the farmers have less than a "Timad" or 0.25 ha of land per household. In line with the findings of this study, the Wolaita Zone Administration (2019) indicated that 60% of households in Wolaita zone possessed less than 0.25 ha of farmland, which is smaller than the national average of 1.01 ha (Milas & Aynaoui, 2004). Overall, 85.3% and 78.9% of farmers interviewed in Damot Gale and Sodo Zuria districts, respectively, indicated farm size as a constraint for faba bean production (Tab. 3). Consequently, the abandonment of fallowing in the studied districts was recorded, which resulted in low soil fertility and faba bean productivity. Different studies conducted in Ethiopia also indicated small farmland size as a reason for continuous cultivation and less crop productivity (Headey *et al.*, 2014; Kemaw & Fentahun, 2018). In general, continuous cultivation without fallowing due to small farm size affected the soil fertility and faba bean productivity in the studied districts. Hence, maintenance of the soil fertility status through fertilizer application and the use of different soil management interventions are required to restore the soil and faba bean productivity.

Poor soil fertility

Farmers used the soil color, workability, water permeability, and water holding capacity as criteria to judge the soil fertility status. Bobo *et al.* (2017) and Corbeels *et al.* (2000) also reported similar perceptions of farmers to classify the soil fertility status. Thus, the dark soil, which has high water retention and is easy to plow, is classified

TABLE 3. Major faba bean production constraints in the sampled agricultural fields at Damot Gale and Sodo Zuria districts of Wolaita zone, Southern Ethiopia.

Source of variations	DF	Sampled farmers	Districts		
			Damot Gale (%)	Sodo Zuria (%)	Total (%)
Land shortage	1	χ^2	N = 163	N = 147	N = 310
Yes		2.15 NS	85.3	78.9	82.3
No			14.7	21.1	17.7
Low soil fertility	1	χ^2	N = 163	N = 147	N = 310
Yes		1.12 NS	54.6	60.5	57.42
No			45.4	39.5	42.58
Soil acidity	1	χ^2	N = 163	N = 147	N = 310
Yes		0.11 NS	35.6	37.4	36.5
No			64.4	62.6	63.5
Soil erosion	1	χ^2	N = 163	N = 147	N = 310
Yes		4.93**	39.9	27.9	33.9
No			60.1	72.1	66.1

N represents the number of farmers involved in cropping practices; ** Significant at $P<0.01$; NS - not significant; DF - degree of freedom.

as fertile by faba bean growers in both districts. Accordingly, the farmer perception about the soil fertility status did not significantly vary between Damot Gale and Sodo Zuria districts (Tab. 3).

About 54.6% and 60.5% of the interviewed farmers at Damot Gale and Sodo Zuria districts complained about the poor soil fertility as one of the faba bean production constraints (Tab. 3). In line with the findings of this study, Buraka *et al.* (2016) indicated poor soil fertility as one of the faba bean production constraints in Wolaita zone. Though poor soil fertility constrains faba bean growers, farmers have limited capacity to improve the soil fertility by applying adequate mineral fertilizer, liming and managing residues. Thus, soil fertility interventions require special attention.

Soil acidity

The short-structured questionnaire was used to verify whether soil acidity is a constraint for faba bean production in the studied districts. Most of the interviewed farmers did not know what soil acidity means. However, those few who indicated soil acidity as a constraint used the wilting of leaves, stunted growth, and inadequate fertilizer application conditions as the criteria to judge. The chi-square statistic revealed a non-significant difference between Damot Gale and Sodo Zuria districts in the number of farmers who perceive soil acidity as a constraint (Tab. 3). About 35.6% of Damot Gale and 37.4% of Sodo Zuria district farmers complained about soil acidity as a constraint for faba bean production (Tab. 3). Buraka *et al.* (2016) also reported soil acidity as a serious constraint for faba bean production in Wolaita zone. Hence, soil acidity is a problem for optimal faba bean productivity, even with fertilized soil. Therefore, identifying the proper lime rate and timely application is required to reduce the soil fertility problems and to improve faba bean productivity.

Soil erosion

The number of farmers who complained about soil erosion as a constraint for faba bean production showed significant variation ($\chi^2 = 4.93$, $P \leq 0.01$) between both districts (Tab. 3). Out of the interviewed farmers, 39.9% and 27.9% indicated soil erosion as a production constraint at Damot Gale and Sodo Zuria districts, respectively (Tab. 3). This implied that soil erosion is among the major constraints for faba bean production.

The erosion problem is more serious at Damot Gale than at Sodo Zuria. The higher erosion in Damot Gale district may be due to steeper slopes than in Sodo Zuria district (Laekemariam, 2015). In general, intensive crop cultivation, complete crop residue removal, and high nutrient depletion may intensify erosion in the soils under faba bean cultivation (Buraka *et al.*, 2016).

Soil fertility management practices for faba bean production

Mineral fertilizer application

The mineral fertilizer application practice significantly varied between districts ($\chi^2 = 0.50$, $P \leq 0.05$) (Tab. 4). Only

TABLE 4. Faba bean farmer soil management practices in the sampled agricultural fields at Damot Gale and Sodo Zuria districts of Wolaita zone, Southern Ethiopia.

Management practices	DF	Sampled farmers	Districts		
			Damot Gale (%)	Sodo Zuria (%)	Total (%)
Number of plow	2	χ^2	N = 163	N = 147	N = 310
Plough once		0.18 NS	35.6	35.4	35.4
Plough twice			33.1	31.3	32.3
Plough three times			31.3	33.3	32.3
Mineral fertilizer application		χ^2	N = 163	N = 147	N = 310
Yes		0.50*	29.5	25.9	27.7
No			70.5	74.1	72.3
Type of fertilizer applied	2	χ^2	N = 48	N = 38	N = 86
DAP		0.46 NS	48	52.3	51.1
Urea			0	0	0
DAP + Urea			52	47.7	48.9
Amount of fertilizer applied	1	F-test	N = 48	N = 38	N = 86
DAP (kg ha^{-1})		0.66 NS	81.5±3.5	84.5±1.0	82.7±2.3
Urea (different kg ha^{-1}) + DAP (100 kg ha^{-1})		4.22*	147.1±2.9	144.0±2.9	146±3.0
Farmyard manure (FYM)	1	χ^2	N = 163	N = 147	N = 310
Yes		0.48 NS	31.3	33.3	32.3
No			68.7	66.7	67.7
Amount of FYM applied	1	F-test	N = 51	N = 49	N = 100
FYM applied (t ha^{-1})		23.65***	1.4±0.2	1.2±0.1	1.3±0.3
Lime applied	1	χ^2	N = 163	N = 147	N = 310
Yes		0.23 NS	3.1	4.1	3.5
No			96.9	95.9	96.5
Amount of lime applied	1	F-test	N = 5	N = 6	N = 11
Lime application (t ha^{-1})		0.21 NS	1.6±0.1	1.5±0.1	1.6±0.6
Faba bean residue management	2	χ^2	N = 163	N = 147	N = 310
Remain on field		0.02 NS	1.8	2	1.9
Incorporated into the soil			0	0	0
Clearing			98.2	98	98.1
Faba bean rotation	1	χ^2	N = 163	N = 147	N = 310
Yes		3.49 *	31.3	41.5	36.1
No			68.7	58.5	63.9
Fallowing	1	χ^2	N = 163	N = 147	N = 310
Yes		0.25 NS	2.5	3.4	2.9
No			97.5	96.6	97.1

N represents the number of farmers involved in cropping practices; *, *** significant at $P \leq 0.05$, 0.001, respectively; NS - non-significant difference; DAP- diammonium phosphate; FYM - farmyard manure; DF - degree of freedom.

29.5% and 25.9% of the sampled farmers applied mineral fertilizer in Damot Gale and Sodo Zuria districts, respectively (Tab. 4).

The farmers mentioned limited financial capacity and increasing price of mineral fertilizer as the reasons for limited fertilizer application. Other researchers also mentioned these factors as a challenge for applying adequate amounts of fertilizers (Abebe & Abebe, 2016; Guteta & Abegaz, 2016). A significant number of farmers are skeptical of the application of mineral fertilizers. Those farmers believed that a crop does not require inorganic fertilizer. However, the previous study in the neighboring district of Boloso Sore, Wolaita zone by Buraka *et al.* (2016) indicated a significant yield improvement in faba beans when fertilizers were applied. Farmer perception of fertilizer use should be corrected to improve faba bean yield. Identifying the type of fertilizer and defining the best rate is required for optimum economic return of the faba bean.

Type of fertilizer applied

The type of inorganic fertilizers used among farmers did not show statistical differences between both districts. The mineral fertilizer applied by farmers was either diammonium phosphate (DAP) alone and/or DAP and urea together at a different time (Tab. 4). Farmers in both districts did not apply urea fertilizers alone for faba bean production.

Overall, about 48% and 52.3% of the sampled faba bean fields were managed with DAP fertilizer alone at Damot Gale and Sodo Zuria districts, respectively. The remaining 52% (Damot Gale) and 47.7% (Sodo Zuria) sampled fields were managed with DAP and urea fertilizers together (Tab. 4). Those farmers who used urea and DAP in combination applied DAP at the time of sowing and urea at the active vegetative stage.

Amount of fertilizer applied

The amount of combined application of urea and DAP fertilizer significantly varied (4.22, $P<0.05$) between districts (Tab. 4). The sampled farmers applied 100 kg ha^{-1} DAP and 50 kg ha^{-1} urea at planting and active vegetative stage, respectively. This rate is in line with EIAR (2018) blanket recommendations of 100 kg ha^{-1} DAP and 50 kg ha^{-1} for all legume crops. However, due to the differences in inherent soil properties and spatial variation, the blanket management approach could not address yield-limiting nutrients in the soils to enhance faba bean productivity. Thus, adequate site-specific fertilizer recommendation is required for improved crop productivity (Mulugeta *et al.*, 2019).

The rate of combined application of urea and DAP was significantly higher in the Damot Gale (147.1±2.9) than in Sodo Zuria district (144.0±2.9) (Tab. 4). Laekemariam *et al.* (2016) also reported that more urea and DAP were applied for crop production in Damot Gale than in Sodo Zuria district. Generally, the use of low amounts and non-balanced nutrients leads to nutrient depletion and reduced faba bean productivity.

Farmyard manure application

The use of cow farmyard manure (FYM) for faba bean did not show significant variation between both districts (Tab. 4). Only 31.3% of Damot Gale and 33.3% of Sodo Zuria district farmers applied farmyard manure for faba bean production (Tab. 4).

Most farmers who applied FYM did so near to their residence rather than to distant faba bean plots. Additionally, growers indicated the farm distance from their residence and a shortage of manure as the major reasons for lower manure application. Corbeels *et al.* (2000) also reported for the Tigray regional state in Northern Ethiopia higher FYM rate near residence than on distant plots due to the difficulty in transportation. However, the application of an adequate amount of FYM for faba bean is of substantial importance to improve the productivity of small-scale farmers (Fedaku *et al.*, 2019).

Farmyard manure application rate

The FYM applied rate for faba bean indicated significant (23.65***) variations among the studied districts (Tab. 4). The FYM application rate was significantly greater in Damot Gale than in Sodo Zuria district (Tab. 4).

The amount of FYM applied both in Damot Gale and Sodo Zuria ranges from 1 to 2 t ha^{-1}. Among FYM applying farmers, 59.2% and 81.6% applied 1 t ha^{-1} in Damot Gale and Sodo Zuria districts, respectively, and the remaining farmers applied 2 t ha^{-1}. The average application rate for faba bean varies between 1.4±0.2 t ha^{-1} and 1.2±0.1 t ha^{-1} in Damot Gale and Sodo Zuria districts, respectively (Tab. 4). Buraka *et al.* (2016), in a study in the neighboring district of Boloso Sore in Wolaita zone, revealed that faba bean required 4 t ha^{-1} for optimum growth. Yield improvement of faba bean was reported in different parts of Ethiopia due to FYM application, in which pH, available P, and cation exchange capacity of the soil increased (Agegnehu & Yirga, 2009; Fedaku *et al.*, 2019). Thus, the FYM rate used in the studied districts is very low and negatively affects the soil fertility status, requiring replenishment of nutrients.

Lime application

Faba bean farmers who applied lime in the study used $CaCO_3$ as the liming material. Lime application had no significant statistical differences between both districts (Tab. 4). About 3.1% and 4.1% of the farmers applied lime in Damot Gale and Sodo Zuria districts, respectively (Tab. 4). Similarly, Ayalew and Dejene (2011) and Buraka *et al.* (2016) showed the limited knowledge of farmers to apply lime in Woliata zone. However, different researchers have reported soil acidity as a serious problem for crop productivity in Wolaita zone (Ayalew & Dejene, 2011; Buraka *et al.*, 2016; Shanka *et al.*, 2018; Kassa Colbe *et al.*, 2020). For instance, Shanka *et al.* (2018) reported pH values of 4.4 and 4.6 at Kokate and Areka in Wolaita zone, which are very low (Landon, 1991). Though soil acidity is a serious constraint, most farmers do not solve the problem adequately. Therefore, soil acidity might lead to further grain yield reduction; additional research must be conducted to identify the optimum lime rate and soil acid-tolerant faba bean variety.

The rate of lime application

The rate of lime application in faba bean farms did not show significant variation between both districts (Tab. 4). The lime applied in faba bean farms varied from 1 to 2 t ha^{-1} with an average rate of 1.6±0.1 t ha^{-1} and 1.5±0.1 in Damot Gale and Sodo Zuria districts, respectively (Tab. 4). Kassa *et al.* (2014) indicated the soil of Wolaita zone requires 4 t ha^{-1} for improved common bean production. Overall, the average lime applied rate (1.6±0.6 t ha^{-1}) on the faba bean fields in the studied districts is not sufficient to mitigate soil acidity. Therefore, to secure a higher faba bean production in the studied districts, further research is required to reclaim the soil by using optimum lime and fertilizer balance.

Faba bean residue management

Faba bean residue management did not show significant differences among the districts (Tab. 4). Farmers were not aware of the advantage of retaining and incorporating faba bean residues into the soil. Faba bean residues were removed in 98.2% of Damot Gale and 98% in Sodo Zuria districts farms (Tab. 4). Laekemariam *et al.* (2016) also reported that crop residues were removed for varied purposes in Wolaita zone. Furthermore, southeastern Ethiopia farmers clear the crop residues for construction material, fuel, and animal feed (Abera & Belachew, 2011). Hence, faba bean residue retention and/or incorporation into the soil require special attention to restore soil fertility and improve crop productivity in the studied area.

Faba bean rotation

Faba bean rotation with different crops significantly varied (χ^2 = 3.49, P<0.05) between districts (Tab. 4). Sodo Zuria (41.5%) showed significantly higher faba bean rotation than Damot Gale district (31.3%) (Tab. 4). Thus, in Sodo Zuria, the soil fertility is significantly better than in Damot Gale district.

Faba bean is often rotated with cereals and infrequently rotated with roots and tubers (potato, sweet potato, and yam). Most farmers implement the rotations as maize - faba bean - cereals and/or root and tubers - faba bean - cereals. Pound and Jonfa (2005) also reported similar faba bean rotation practices in Wolaita zone. On the other hand, crop rotation in Tigria regional state in Northern Ethiopia is dominated by cereals (Corbeels *et al.*, 2000). However, including legumes at least once in the rotation cycle influences the soil microbial activities (Abera & Belachew, 2011). These practices enable the soil to increase soil organic matter (OM), creating an ideal condition for crop productivity (Aschi *et al.*, 2017). Thus, including grain legumes in crop rotation provides multiple environmental, agricultural, and economic benefits, such as fixing the atmospheric nitrogen, releasing high-quality OM in the soil, and facilitating soil nutrient circulation and water retention. Moreover, the type of legume species used for rotation purposes affects the mineralization process and the amount of fixation. Faba bean is the preferred legume for rotation purposes, due to its powerful nitrogen-fixing (177-250 kg ha^{-1} per crop) capacity (Mulugeta *et al.*, 2019). In line with this, Aschi *et al.* (2017) reported faba bean-rape-wheat rotation

TABLE 5. Grain yield production of faba bean in the sampled agricultural fields at Damot Gale and Sodo Zuria districts of Wolaita zone, Southern Ethiopia.

Year of production	Mean yield (t ha^{-1})			
	Fertilizer application	Damot Gale (N = 163)	Sodo Zuria (N = 147)	F-test
Production in 2016	No	0.52±0.2	0.50±0.1	4.05*
	Yes	1.80±0.6	1.72±0.7	1.73*
	t-test	***	***	
Production in 2017	No	0.77±0.3	0.71±0.3	5.9**
	Yes	1.88±0.7	1.74±0.6	0.01**
	t-test	***	***	
Production in 2018	No	0.63±0.3	0.61±0.4	6.73*
	Yes	1.84±0.12	1.84±0.11	0.84 NS
	t-test	***	***	

N represents the number of farmers involved in cropping practices; *,**,*** significant at $P\leq$0.05, 0.01, and 0.001, respectively; NS - not significant.

as a suitable crop rotation to improve soil fertility status. In general, farmers are aware of the benefits of faba bean rotation with other crops to improve soil fertility. Most farmers indicated their preference for faba bean rotation than fallowing, due to the limited farmland size.

Fallowing

The interviewed farmers revealed that the practice of fallowing did not significantly vary within the studied districts (Tab. 4). In this regard, only about 2.5% of Damot Gale and 3.4% of Sodo Zuria farmers practiced fallowing. Overall, the fallowing practice is very limited (2.9%) (Tab. 4). The farmers are aware of the significant role of fallowing in reclaiming soil fertility. However, the small farmland size in the studied districts forces farmers to limit fallowing. Thus, the problem of fallowing abandonment is common in Wolaita zone (Pound & Jonfa, 2005; Laekemariam *et al.*, 2016). Similarly, the limited practice of fallowing due to small farmland size was reported in different parts of Ethiopia (Corbeels *et al.*, 2000; Abera & Belachew, 2011; Mamuye *et al.*, 2020). Thus, the abandonment of fallowing negatively affects soil fertility and grain yield productivity (Mamuye *et al.*, 2020). The soil fertility constraints due to continuous cropping requires immediate attention for sustaining faba bean production in the studied districts.

Faba bean grain yield production

From 2016 to 2018, faba bean grain yield production showed statistically significant differences (P<0.001) between fertilizer application and non-aplication. In all three years, fertilized and unfertilized faba bean farms had an average grain yield of 1.8±0.8 t ha^{-1} and 0.62±0.3 t ha^{-1}, respectively (Tab. 5). In line with this, CSA (2018) reported average grain production of 1 t ha^{-1} in Wolaita zone, which is far less than the national average (2.1 t ha^{-1}).

In 2016, the yield (t ha^{-1}) of fertilized faba bean was 1.80±0.6 and 1.72±0.7 in Damot Gale and Sodo Zuria districts, respectively. However, in the same year, the yield of an unfertilized farm was very low in both Damot Gale (0.52±0.2 t ha^{-1}) and Sodo Zuria (0.50±0.1 t ha^{-1}) districts (Tab. 5). A fertilized faba bean farm in 2017 yielded 1.88±0.7 t ha^{-1} and 1.74±0.6 t ha^{-1} in Damot Gale and Sodo Zuria districts, respectively.

The yields in an unfertilized farm in 2017 were lower in Damot Gale (0.77±0.3 t ha^{-1}) and Sodo Zuria (0.71±0.3 t ha^{-1}) districts (Tab. 5). The fertilized field in 2018 yielded about 1.84±0.12 t ha^{-1} and 1.84± 0.11 t ha^{-1} in Damot Gale and Sodo Zuria districts, respectively. In 2018, the yield of an unfertilized farm was lower at Damot Gale (0.63±0.3) and Sodo Zuria (0.61±0.4) districts (Tab. 5). Scarcities of arable lands, poor soil fertility, erosion, soil acidity, limited fallowing and poor residue management are the reasons for reduced faba bean yield.

Conclusions

The results of this study demonstrate that the productions of faba bean in the studied districts are constrained mainly by poor soil fertility, soil acidity, erosion, and lack of soil acidity tolerant varieties as well as small landholdings. In general, the soil management practices by farmers were inadequate to improve the soil fertility status and to enhance faba bean productivity. Consequently, the grain yield productivity of unfertilized farms was below 1 t ha^{-1}. Thus, adequate soil fertility management practices are necessary. Intensive soil fertility management interventions including faba bean residue management, crop rotation, application of sufficient and balanced organic and mineral fertilizers, adequate lime application, and use of soil acidity tolerant varieties are required to improve faba bean productivity in the study area.

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Conflict of Interest statement

The authors declare that they have no conflict of interest regarding the publication of this article.

Author's contributions

BA, ND, TT, and Fl designed the experiments, BA and TT contributed to the data analysis, and BA wrote the article. All authors reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

1. Survey questionnaire

Project title: Soil fertility management practices for faba bean (*Vicia faba* L.) production in Wolaita zone, Southern Ethiopia.

Part A: structured interview guide

Part I: informed oral consent

Hello, my name is Bekalu Abebe and I am a student at Haramaya University. This is my colleague [name]. We are conducting a study of Faba bean (*Vicia faba* L.) as part of my education at the university.

Do you grow faba bean? ☐ Yes ☐ No

▸ If yes, the brief objective of this study is indicated as follows.

The purpose of this study is to understand faba bean management by farmers and evaluate soil and plant nutrient status.

I would like to ask you questions about faba bean. Of course, your participation is entirely voluntary. These interviews require less than one hour. I do not anticipate that the interview will pose any risks to you. Because I am a student, I cannot pay you.

We hope that our research will benefit farmers in Wolaita by promoting the diversity of faba bean and giving direction for agronomic management. If you agree to an interview, you do not have to answer all my questions, and you can tell me at any time if you would like to stop. I will record your answers to my questions using my cell phone and my notebook. I will include this information in my dissertation, and it will be shared with other researchers who are interested in faba bean. Do you have any questions? If you have any questions in the future, here is my contact information. Do you agree to participate in this study of faba bean?

▸ If yes, let us proceed to the following interview.

Part II: structured Interview

SECTION 1: basic Information (complete prior to interview)

Date of interview:	Start time of interview:
Name of interviewer:	
Wereda/district:	Kebele:
Latitude (in decimal degrees N):	Longitude (in decimal degrees):
Altitude (in meters):	
Agro-ecological	
Relative wealth on farmers' association list: ☐ Low income ☐ Middle income ☐ High income	

SECTION 2: general Information about the informant

Name of informant:
Language spoken by informant during interview: ☐ Wolaitigna ☐ Amharic ☐ Other (specify): _ _ _ _ _ _ _ _ _ _
Age (observed): ☐ 18 to 30 ☐ 30 to 45 ☐ 45 to 60 ☐ 60+
Gender (observed): ☐ Male ☐ Female
Is the informant the household head? ☐ Yes ☐ No
☐ If no, household-head gender: ☐ Female-headed ☐ Male-headed

SECTION 3: inter-specific diversity of legume crops

Are you growing any legume this year? ☐ Yes ☐ No

For each of the legumes you listed, how many hectares of land did you plant in 2018 (June to September including any areas under intercropping).

SECTION 4: cropping and crop management practices

1. What is your perception about the soil fertility status of farmland you used for faba bean?
A. Highly fertile B. Moderately fertility C. Poor in fertility

2. Local soil name (type) of farm used for faba bean:
Reasoning evidence (major classification criteria)
1. Color: dark/red/brown 2. Fertility: fertile/infertile 3. Workability: hard/easy to plough in dry and wet 4. Water retention: high/low 5. Other:
_ _

3. Number of crops per year growing land used for faba bean farm:
_ _

4. What are the major production constraints for faba bean in your community? (check all that apply) ☐ Land shortage ☐ Drought ☐ Poor soil fertility ☐ Soil acidity ☐ Diseases ☐ Insect pests ☐ Weeds ☐ Lack of seeds ☐ Flooding ☐ Erratic rain ☐ Low market value ☐ High price of inputs ☐ Other (specify): _ _ _ _ _ _ _ _ _ _ _ _

5. Do you observe change in the fertility status of the agricultural soils in your village? ☐ Yes ☐ No

6. If yes, what are the indicators compared to the past
A. Reduced crop growth (e.g., non-healthy color, plant height)
B. Reduced yield C. Farm does not respond without fertilizer
D. Other: _

7. What are the driving forces that resulted in those changes in soil fertility?
A. Soil erosion B. Inadequate fertilizer application (organic and chemical)
C. Absence of soil conservation structure D. Lack of response after applying fertilizer E. Continuous cropping/no fallowing F. Complete residue removal
G. Other _

8. Do you maintain soil fertility status for better productivity of faba bean?
☐ Yes ☐ No

▸ If yes, what are the major practices you implement to improve soil fertility?

9. Do you use chemical fertilizers for faba bean production? ☐ Yes ☐ No

10. Fertilizer rate for the crop grown on sampling plot
1. Type: N = NPS, U = Urea, C = Compost, FYM = Biomass
2. Rate (kg): N------------ U------------ C------------ FYM ------------

▸ If no, why? _

11. Do you apply both organic and chemical fertilizer together at a time:
☐ Yes ☐ No

▸ If no, your reason:

A. I don't know the advantage of using both together
B. Tried but not found the benefit
C. I know the advantage, but to share fertilizer sources to individual fields
D. Other:

12. Do you know about soil acidity? ☐ Yes ☐ No

▸ If yes, what do you do to alleviate soil acidity problem for faba bean?
☐ Lime ☐ Ash ☐ Other (specify): _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

13. Do you rotate faba bean with other crops? ☐ Yes ☐ No

▸ If yes, with which crops do you rotate with faba bean? _ _ _ _ _ _ _ _ _
_ _

▸ If yes, how often do you plant faba bean within the crop sequence? _ _ _ _
_ _

▸ If no, why? _

14. Do you intercrop faba bean with other crops? ☐ Yes ☐ No

▸ If yes, with which crops do you plant faba bean in the same field?
_ _

▸ If no, why? _

15. What varieties of faba bean have you grown in the past three years? _ _ _
_ _

16. What is the estimated yield in the plot of land you planted (Qt ha-1)

17. Do you fallow the land? ☐ Yes ☐ No

▸ If Yes,
Duration/at what time interval: i.e. Every year _ _ _ _ _ _ _ _ _ _ _ _

▸ If No,
Reason: _

18. Crop residue management on bean growing farm?
a. Burned b. Cleared c. Remain in the field d. Other _ _ _ _ _ _ _ _

19. Do you control insect pests on faba bean? ☐ Yes ☐ No
▸ If yes, which type of control? ☐ Chemical ☐ Other (specify) _ _ _ _

20. Do you control weeds? ☐ Yes ☐ No
▸ If yes, which type of control on faba bean?
☐ Chemical ☐ Other (specify) _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

21. Do you control diseases on faba bean? ☐ Yes ☐ No
▸ If yes, which type of control?
☐ Chemical ☐ Other (specify) _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

22. What is the yield you gained on plot of land for last three years and your estimate for this year? _
_ _

Consumer profile and factors determining the purchase of agroecological products. A case study: UNIMINUTO Agroecological Fair and Minuto de Dios Solidarity Market, Colombia

Perfil del consumidor y factores que determinan la compra de productos agroecológicos. Un caso de estudio: Feria Agroecológica UNIMINUTO y Mercados Solidarios Minuto de Dios, Colombia

Adriana María Chaparro-Africano[1*] and Juan Diego Garzón-Méndez[1]

ABSTRACT

This research sought to characterize consumers and consumption in the UNIMINUTO Agroecological Fair and the Minuto de Dios Solidarity Market, which both have a low number of consumers and sales. A survey was designed and implemented through a Google form and in person, between September and October 2019. The total sample was 146 consumers (90% reliability, 5% error). The results were analyzed through descriptive statistics and comparison with other studies. The consumer's profile is mostly university students of natural sciences, young adults of low and low-middle income strata from small households, and women; the age, gender, and educational level are common features with similar studies. The consumer lacks knowledge about the products, the participatory certification, and pricing, which can be remedied through consumer education. The supply and quality of agroecological products must also be improved.

Key words: agroecology, agroecological markets, agroecological consumer, sustainable consumption.

RESUMEN

Esta investigación buscó caracterizar los consumidores y el consumo en la Feria Agroecológica UNIMINUTO y el Mercado Solidario Minuto de Dios, que cuentan con un bajo número de consumidores y ventas. Se diseñó y aplicó una encuesta a través de un formulario de Google y presencialmente, entre septiembre y octubre de 2019. La muestra total fue de 146 consumidores (99% confiabilidad, 5% de error). Los resultados se analizaron mediante estadística descriptiva y comparación con otros estudios. El perfil de los consumidores es en su mayoría estudiantes universitarios de ciencias naturales, adultos jóvenes, de estratos bajo y medio-bajo de hogares pequeños, y mujeres; la edad, el género, y el nivel educativo son características comunes con estudios similares. El consumidor carece de conocimiento sobre los productos, la certificación participativa, y los precios, lo que se puede resolver con educación al consumidor. La oferta y la calidad de los productos agroecológicos son aspectos que también se deben mejorar.

Palabras clave: agroecología, mercados agroecológicos, consumidor agroecológico, consumo sostenible.

Introduction

Agroecological markets are formally or informally established organizations made up of producers, consumers and/or promoters, who seek the sustainability of agri-food systems and society in general. Such sustainability may be achieved through the distribution of agroecological products in various short marketing circuits: farms, fairs, home markets, and shops. The main characteristic of these markets is their alternative rationality focused on strong economic, social, environmental, and political sustainability of traditional markets (Chaparro-Africano, 2019). Some types of agroecological markets are fairs, home delivery, and stores.

The UNIMINUTO Agroecological Fair (UAF) of Colombia was officially introduced in 2012 with the pedagogical objective of providing a learning environment for students of the Agroecological Engineering program as well as for students of other programs and the public. The UAF seeks to generate new knowledge and offer a space that contributes to the well-being of the UNIMINUTO community and the city of Bogota, which, in turn, offer an alternative of economic inclusion for agroecological producers. The Minuto de Dios Solidarity Market (MDSM) emerged as a complement to UAF in 2018 and works as a point of sale and home delivery service between Mondays and Saturdays of each week.

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A framework of sustainability indicators was designed in 2016 to manage the sustainability of UAF and other agroecological markets of the Agroecological Markets Network of Bogota - Region (AMNBR). The framework includes four sustainability attributes (productivity, stability, resilience and reliability, equity, and autonomy) and twelve indicators: sales per producer, income/cost ratio of the market, producers, consumers, frequency, local market, agroecological production, distribution of benefits, prices, participation, financing, and pedagogy (Chaparro-Africano, 2019).

Some of the UAF's main shortcomings in 2018, according to the evaluation of sustainability indicators, were the number of consumers per fair (359) and total sales ($26,449,600 Colombian pesos (COP) annually, with an average per producer/day of $132,248 COP and consumer/day of $9,209 COP) which show few consumers (of 1,377 visitors on average only 359 are consumers, 26%), and few sales per producer and consumer. The same occurs in other agroecological markets (Chaparro-Africano, 2019), including the MDSM.

Previous research determined that Colombian consumers did not yet recognize the benefits of organic products, although there is no equivalent information on agro-ecological products, so it is relevant to identify the consumer profile and the elements that influence purchases (Sánchez Castañeda, 2017) to draw up sustainable marketing (Kamiński, 2016) that allows progress in the scaling of agroecology.

Sustainable marketing emerged to protect the environment and promote responsible economic and social development. It was preceded by green marketing in the 1970s that focused on pollution and depletion of natural resources, and environmental marketing in the 1980s that focused on the development of "clean" technologies (Hunt, 2011).

Since it was not possible to identify any studies that develop the concept of sustainable marketing in agroecology, the authors propose sustainable marketing to be understood as the set of actions necessary to ensure that a product reaches those who need it. This process must be carried out while ensuring the sustainability of production and use/consumption of the product, negotiation of fair prices, and ethical promotion, sale and distribution mechanisms that do not promote consumerism.

For the UAF and MDSM, an agroecological product is a fresh, processed food, personal care, medicinal, or cosmetic product manufactured without the use of chemical synthesis and external inputs harmful to human or environmental health. An agroecological product promotes the resilience of the system through the conservation of common goods (soil, water, biodiversity, air), so that the sustainability of the agroecosystem can be ensured. This way, agroecological products link the environmental, social, and economic dimensions of production considering people, their culture, and their ancestral and new knowledge. They also focus on processes rather than inputs to avoid dependencies and promote the general welfare of both producing and consuming families (Chaparro-Africano & Naranjo, 2020).

Agroecological products, the production systems from which they are obtained, and the markets in which they are exchanged focus on the pursuit of strong sustainability, which refers to the promotion of social welfare while common goods are preserved. Strong sustainability proposes increasing the efficiency of resource consumption so that the use of resources does not exceed their regeneration capacity, and production of waste does not exceed the environment's assimilation capacity. Additionally, non-renewable resources are exploited at a rate equal to the creation of renewable substitutes (Daly, 1991).

To advance towards strong sustainability, the UAF and MDSM promote backward agroecological production and forward sustainable consumption through actions of resistance, cooperation, and social mobilization (Chaparro Africano & Calle Collado, 2017). Sustainable consumption reduces the negative impacts and increases the positive effects of consumption, with these two aspects having a strong ethical component (Hinton & Goodman, 2010). From this perspective, consumption is not positive or negative per se.

Additionally, organic/ecological/biological production and agroecological production are understood as different processes. According to the Ministry of Agriculture and Rural Development of Colombia (MADR), an organic agricultural, aquaculture or fisheries production system conserves biodiversity and the biological cycles of ecosystems, excluding synthetic inputs and reducing external inputs (MADR, 2006). Agroecology proposes that this ecological management of common goods occurs through collective action, not only in the production phase but also in the alternative circulation (Sevilla Guzmán, 2006) and consumption phases (Calle Collado *et al.*, 2013). Therefore, the proposal for the sustainability of organic production falls short, becoming a conventional (Darnhofer *et al.*, 2010; González de Molina *et al*., 2017) and unfair system (Kröger & Schäfer, 2014).

Regarding market research, the study of supply and demand began after the World War ll, when initially and due to shortages, many goods were sold out. However, supply gradually increased, and market research was needed to reduce uncertainty and guide production decisions. A market study can be general or specific, aiming to understand the supply and demand status of a product or sector in each context as well as its trends to achieve effective and efficient management. With the emergence of information and communication technologies, in addition to globalization, companies have invested more in market studies, and methodologies were refined (Dos Santos, 2017). While market studies are fallible because reality is complex, marketing based on market research has helped to achieve well-being (Lim, 2015).

In the 1970s and 1980s, the social and environmental consequences of marketing were questioned. Marketing was blamed for hyper-risk (Beck, 1992), hyper-reality and related psychological problems (Baudrillard, 1998), the promotion of a consumerist and materialistic society, and the use of resources in an unsustainable and unethical way (Alexander *et al*., 2011). This scenario led to a debate in the critical discipline of marketing (McDonagh & Prothero, 2014), resulting in the emergence of Societal Marketing, Ecological Marketing, Green Marketing, Environmental Marketing, and Sustainable Marketing (Kamiński, 2016). This allowed agroecology to rely on evolved marketing, avoiding repeating the mistakes of the past.

Almost all the studies identified by researchers that characterize consumers and consumption focus on organic products. A few of them focus on green products (Arroyave, 2015; Escobar-Moreno *et al*., 2015; Vargas Restrepo & Valencia Bitar, 2015) and only one on agroecological products (Prada, 2017).

Some studies have been dedicated to assigning all participants evaluated into a category of greater or less radicality regarding sustainable consumption: effective, potential and/or non-potential consumers (Andrade Ortiz & Flores, 2008; Henryks *et al*., 2014; Arroyave, 2015). Others have been focused on a particular product category (vegetables) (Díaz Pinilla, 2012) or on a single marketing aspect (price) (Rödiger & Hamm, 2015).

Most studies sought to identify the consumer by including the knowledge of characterized concepts, their preferences and/or market variables (Higuchi, 2015; Muhammad *et al*., 2016; Vietoris *et al*., 2016; Kranjac *et al*., 2017; Krishna & Balasubramanian, 2018), while others focused only on the market (Henryks *et al*., 2014).

These studies were conducted in Colombia, Ecuador, Argentina, Mexico, Canada, Australia, Peru, Spain, Serbia, India, United Arab Emirates, and Romania. The most widely applied data collection methods were surveys, followed by interviews and other methodologies such as focus groups and review of other research studies. This review of the state of the art evidences the lack of information on consumers and consumption of agroecological products in Colombia and the world.

In this context of lack of knowledge and unsustainability of some agroecological markets, this research aimed to characterize the consumer and the consumption of agroecological products in UAF and MDSM, to understand the consumer's motivations and limitations and extract elements that allow promoting sustainable consumption and agroecology. The research questions this study sought to answer were: what is the consumer's profile? what are their perceptions of agroecological products? and what are the processes of promotion, sale, and distribution of the evaluated agroecological markets?

Materials and methods

This study consisted of descriptive mixed (quantitative and qualitative) research (Hernández-Sampieri & Mendoza Torres, 2018). Primary information was collected in the second half of 2019 through a face-to-face and Google-formatted survey of consumers of agroecological products from UAF and MDSM, before, during, or after the purchase process.

The population corresponded to UAF and MDSM consumers. The sample contained 146 consumers, with a reliability level of 90% and a maximum error level of 5%. The sampling was accidental, not probabilistic.

The survey included 24 questions: eight to characterize consumers, four to evaluate concepts and media, and 12 to characterize consumption in UAF and MDSM (Supplementary material 1). The questions were designed to build a consumer profile and evaluate the perception of the agroecological product and of the processes of promotion, sale, and distribution of the evaluated agroecological markets. The survey was applied between September and October 2019.

The analysis of the results was based on descriptive statistics supported by Excel spreadsheets to calculate and plot frequencies, and comparison with other results reported in the literature. No discrimination was made between reports of agroecological, green, or organic products since no studies were found on the consumption of agroecological products.

Results and discussion

Characterization of UAF and MDSM consumers

Age of participants

The ages of the participants were classified into five groups, following the methodology of Martín Ruiz (2005): young people (<20 years), young adults (20 and 39 years), middle aged adults (40 and 49 years), mature adults (50 and 60 years) and elderly adults (>60 years), as shown in Figure 1:

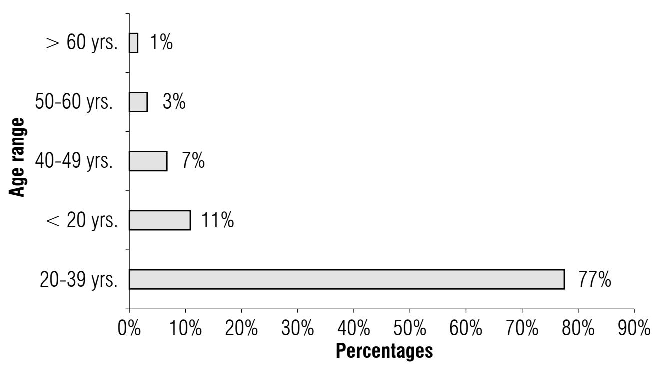

FIGURE 1. Ages of consumers at the UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM).

Most of the surveyed consumers were students (61%), who made up the young people segment and, partially, the young adult age segment (UNIMINUTO, 2020). These results can also be explained because young and middle-aged adults have grown up in the period of development of organic production in Colombia, which, according to Sánchez (2017), started in 1980. The results show a similar population to that in a study conducted in several agroecological markets in Colombia, where most buyers were older than 30 years (Prada, 2017); Escobar-Moreno *et al.* (2015) also report an average of 30-40 years for the "green" consumer in Antioquia (Colombia).

Genders

Fifty two percent of the agroecological consumers were female and 48% male, paralleling that of gender in students: 53% women and 47% men (UNIMINUTO, 2020). This proportion differs from the "green" consumers reported by Escobar-Moreno *et al.* (2015) in Bogota, where 80% were women. Similar results were obtained in Norway, where 68% of organic consumers were women (Storstad & Bjørkhaug, 2003) and Serbia, where 42.7% were women (Kranjac *et al.*, 2017).

This higher proportion of female buyers in the markets evaluated is because in Colombia, as in other countries, women spend more time doing the household shopping (Puente & Dakduk, 2011). Moreover, since the beginning of ecofeminism, more actions of environmental protection have been started by women than by men (Hosseinnezhad, 2017).

Academic level

The results in Figure 2 reflect that the study was carried out in two university markets. In comparison, a study in Mexico (Pérez-Vázquez *et al.*, 2012) reports a lower educational level for consumers in organic markets: 45% indicated having a primary or high school diploma, 50% have a university degree and 5% a graduate degree.

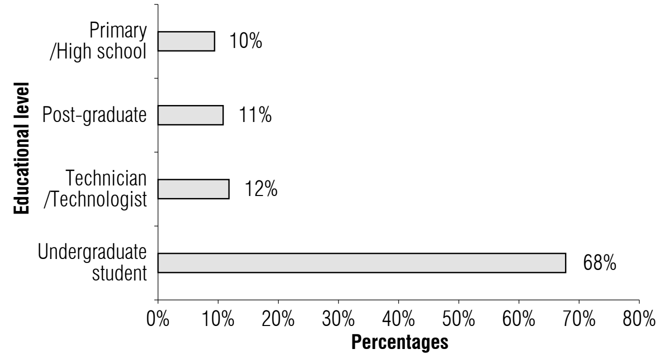

FIGURE 2. Educational level of consumers at the UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM).

According to studies in Norway (Storstad & Bjørkhaug, 2003), Colombia (Arroyave, 2015; Vargas Restrepo & Valencia Bitar, 2015; Prada, 2017), United Arab Emirates (Muhammad *et al.*, 2016), and Serbia (Kranjac *et al.*, 2017), most consumers of organic products have a university education degree or higher.

Consumers of agroecological products, especially those with a higher academic level, are presumed to have received environmental education or better-quality education. According to Maldonado Hernández *et al.* (2007), education is the main explanatory variable of organic consumer behavior, especially in aspects such as human-nature orientation, ecological knowledge, perceived control, personal consequences, and environmental consequences.

Profession

Responses regarding the professions of participants were divided into eight categories (SENA, 2017), as shown in Figure 3.

The increased participation of the natural sciences in the results is likely because the two agroecological markets evaluated are coordinated by the agroecological engineering program, the only academic program in natural sciences at UNIMINUTO. These results differ from studies conducted in Mexico (Pérez-Vázquez *et al*., 2012), where consumers belonged to the following categories: administration (13%), biology or natural sciences (8%), communication (7%), pedagogy (6%) and other programs such as psychology, music, education, marketing, and systems with 3%.

Occupation

The activity to which respondents dedicate most time was considered as the current occupation. In total, 51% of the respondents were students, 32% were employed, and 18% were self-employed. These results reflect that these are university agroecological markets.

Household stratum

The household economic strata in Colombia are classified as follows: 1 and 2 are low, 3 and 4 are medium, and 5 and 6 are high strata (DANE, 2020a). Most respondents are in strata 3 and 2, due to the fact that the UAF and MDSM are in a university and neighborhood where most of professors, students, and staff belong to those strata (UNIMINUTO, 2020). This access to agroecological products by people from low and low-middle household strata agrees with the mission of the UAF and MDSM (Fig. 4).

Number of people in the consumer's household

The results in Figure 5 coincide with those reported by DANE (2020b) for Bogota households in 2018, although they differ from those reported in Argentina by Gentile and Rodríguez (2002), where half of the consumer households are made up of three people, 35% are two-person households, and 15% are single-person households.

According to Vargas Restrepo and Valencia Bitar (2015), Bogota shows higher purchasing potential in married women with children, while in Ecuador, having a child is

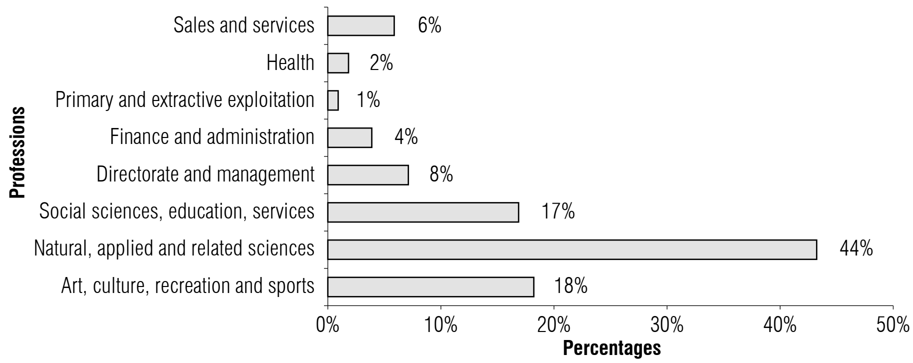

FIGURE 3. Professions of consumers at the UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM).

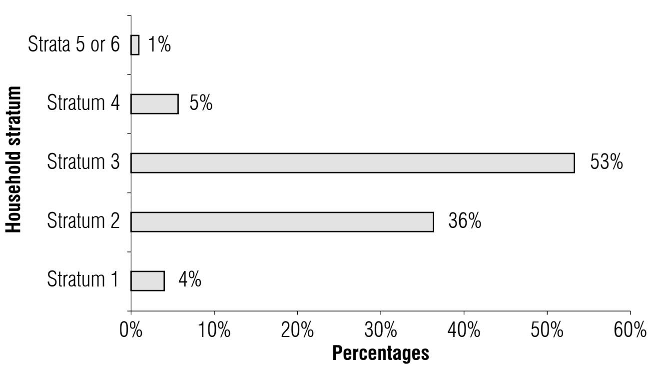

FIGURE 4. Household stratum of consumers at the UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM).

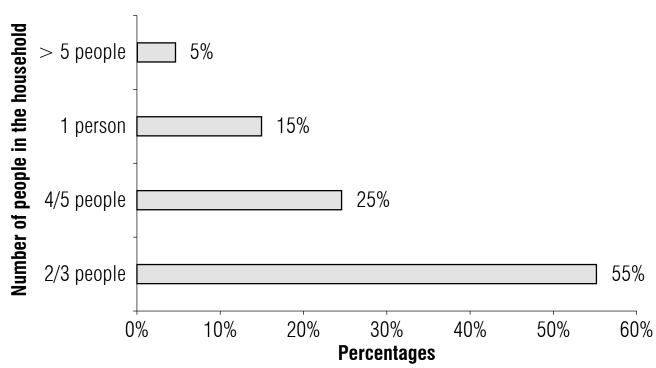

FIGURE 5. Number of people in the household of consumers at the UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM).

not a determining factor for purchasing (Andrade Ortiz & Flores, 2008). According to these findings, the size of the household is not conclusive in terms of the consumption of agroecological or organic products.

Link with UNIMINUTO

The UAF takes place in the Plazoleta Verde of UNIMINUTO's campus in Bogota on the first Thursday of each month; MDSM also has a permanent point of sale there. This explains why 83% of the consumers surveyed have links with the university (Fig. 6). People without a link to UNIMINUTO attend because both markets are open to people from the neighborhood and town; however, the low percentage may be due to the presence of bars around the Plazoleta Verde and an MDSM point of sale.

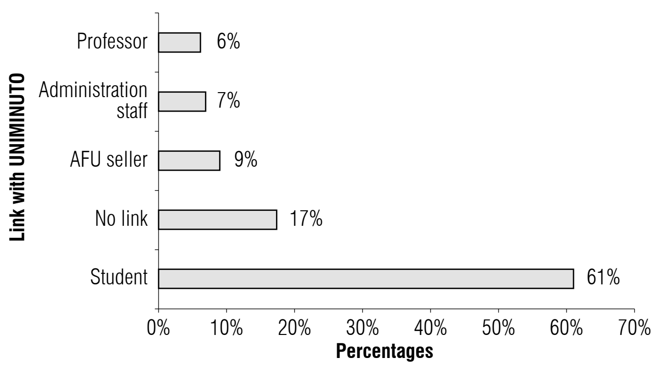

FIGURE 6. Link between consumers at the UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM) and UNIMINUTO.

The difference between the proportion of those whose occupation is student (51%) and those who state that they are linked with UNIMINUTO as students (61%) may be because not all of them consider being a student as their main occupation.

The profile of the consumer of agroecological products from UAF and MDSM, their age, gender, educational level, profession, occupation, stratum, relationship with UNIMINUTO and size of their households derive from most of the characteristics of the population that coordinates the agroecological markets evaluated or of the population of UNIMINUTO and Bogota. Some consumer characteristics, such as being young adults, women, and having a higher education, especially in natural sciences, are the most frequent in this and other similar consumer studies (Storstad & Bjørkhaug, 2003).

UAF and MDSM concepts and media

Knowledge of the date, location, and times of the UAF and MDSM

From the total consumers surveyed, 77% said they knew the date, location, and times of the UAF and MDSM. This result is considered low, given that to promote these two markets, posters and flyers are printed, emails are sent, and the information is disseminated in radio programs and social networks. However, every semester new students, professors, and administrative staff enter UNIMINUTO and may not receive the information. Moreover, other events are held at the same tent and on the same site, which causes confusion. Additionally, the number of emails sent and the number of events held at UNIMINUTO are very high, making effective communication difficult.

UAF and MDSM media

The media most consulted by UAF and MDSM consumers is Instagram (@feriaagroecologicauniminuto and @ mercadosolidariosminutodedios), followed by Facebook (FeriaAgroecologicaUniminuto) (Fig. 7). This is contrary to user statistics in Colombia, where in January 2019 there were nearly 32 million users on Facebook and 12 million on Instagram, with the majority women between 25 and 34 years of age (Shum, 2019). These demographic characteristics coincide with those of the respondents in this research. The preference for Instagram over Facebook

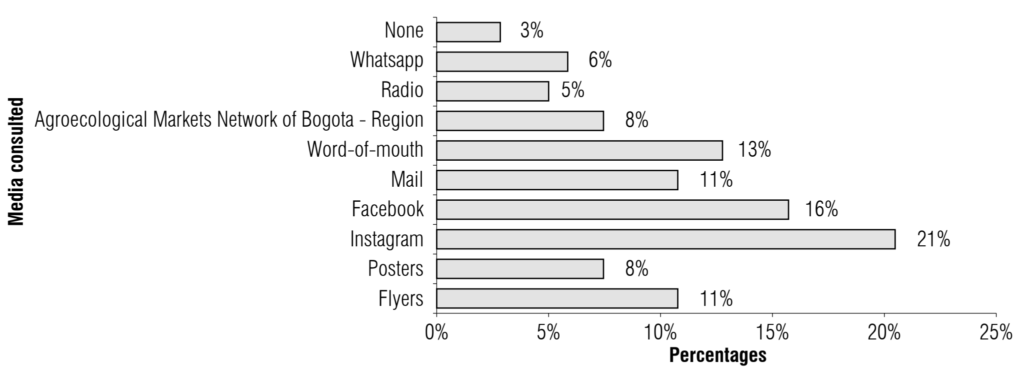

FIGURE 7. UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM) media consulted by consumers.

may be beneficial according to other studies (Belanche *et al.*, 2019) that found that Instagram stories increase loyalty among millennial users of both genders and non-millennial women more effectively than the Facebook wall.

Topics of interest to the UAF and MDSM

Most respondents want to know about the products (Fig. 8), which indicate that the information disseminated by producers in person and virtually by social networks is insufficient. Secondly, the selection of information on dates, times, and places of the fair indicates that the promotion of the fair is insufficient, possibly due to the complexity of keeping a university population of about 18,000 people, as well as the residents of the neighborhood, well informed.

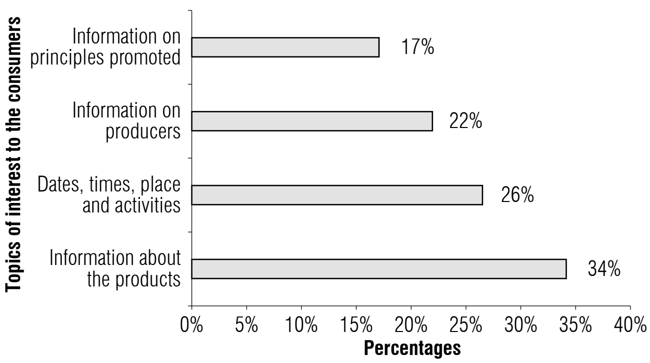

FIGURE 8. Topics of interest to the consumers at the UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM).

Knowledge of UAF and MDSM principles

Figure 9 shows the principles promoted by UAF and MDSM, which are intended to contribute to building sustainable agri-food systems. We expected that most consumers would identify agroecology as the main principle promoted since the name of the fair refers to it. Principles such as fair trade, social and solidarity economy, and sustainable consumption are common in the university's research and social projection work, while the little-known principles such as food sovereignty are mostly worked on only in the Agroecological Engineering program and, in some cases, in the Faculty of Communications. Finally, the principle of radical democracy is almost unknown; this may be due to the newness of concept (Calle Collado, 2011) and to the scarce political formation of the citizenry in general. Only 13% of those surveyed recognize all the principles promoted by the UAF and MDSM, which shows a significant need for education in these areas.

Characterization of consumption in UAF and MDSM

Type of buyer in the UAF and MDSM

In total, 68% of respondents confirmed to be occasional buyers; most of these are students (61%) who usually do not decide on purchases for their homes. Only 32% of the respondents are loyal buyers. This shows that there is a lot of work to be done to build loyalty among buyers.

Purchase of organic products in conventional markets

The results reveal that 46% of respondents buy organic products in conventional markets (49% do not and 5% did not respond), especially supermarkets and hypermarkets, which are more convenient to households. However, many of these products are not locally produced (i.e., https://mah.com/pages/nosotros organic baby food UK brand and present in Colombian supermarkets) or do not use biodegradable or reusable packaging (*i.e.*, https://www.taeq.com.co/productos/organico organic food brand from the Éxito Group, owned by the Casino Group, France). The fact that these products are not agroecological is not recognized by consumers; this corresponds to the criticized "conventionalization" of the organic (Köger & Schäfer, 2014; González de Molina *et al.*, 2017).

The low proportion of loyal buyers (32%) compared to a higher proportion of respondents who buy in other markets (46%) may be because other markets offer similar products

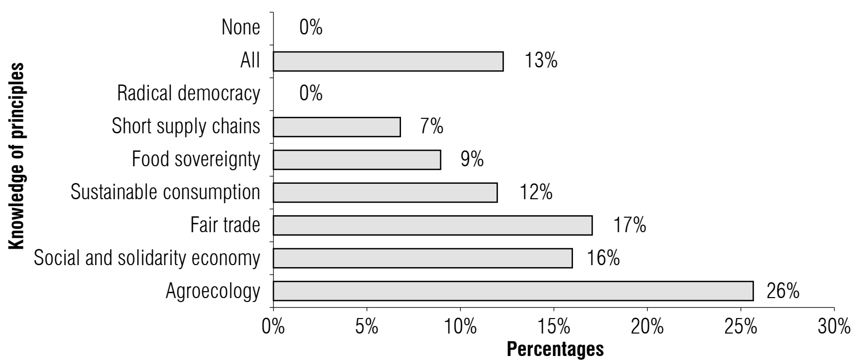

FIGURE 9. Knowledge of the UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM) principles.

(organic), with better access alternatives (frequency, proximity, etc.).

Products missing from the UAF and MDSM

The results shown in Figure 10 are similar to those of a study carried out in Andalucia, where most consumers expressed interest in buying other products in addition to those they currently buy. However, the two studies differ in the products demanded; for Andalucia, vegetables (mainly lettuce, tomato, and onion) and fruits (mainly oranges, pears, apples) (Ipsos Insight, 2007) are the most demanded products, whereas for the UAF and MDSM fast food or snacks were the most requested products. This is explained by the fact that UAF and MDSM are university markets, and their buyers are mostly students.

UAF and MDSM frequency

Sixty six percent of respondents believed that the monthly frequency of UAF and MDSM Monday through Saturday is sufficient, while 32% said that UAF should take place twice a month, and 2% did not respond. These results may be caused by the fact that since May 2018, MDSM supplemented the UAF that takes place only eight months a year. The approval of the frequency of MDSM is because most consumers are not in the neighborhood on Sundays, making it unnecessary to open the market on this day.

Motivations to buy agroecological products

These results show equal evaluations for local production, organoleptic characteristics, health, and contribution to a social system and fairer trade (Fig. 11). The results differ somewhat from the findings of other research also in Bogota, where the main motivation for purchasing was health (23% medical recommendation) (Vargas Restrepo & Valencia Bitar, 2015). Similar results are also reported in Spain (Ipsos Insight, 2007), Argentina (Gentile & Rodríguez, 2002), Serbia (Kranjac *et al*., 2017), and India (Krishna & Balasubramanian, 2018). More people are aware of the negative impacts of conventional foods on their health as these effects are discovered and recognized. Consequently, they choose to consume organic foods, as reported by Raigón Jiménez (2008) which are healthier because they do not receive insecticides, herbicides, synthetic fertilizers, and additives (in the case of processed products) and contain more nutrients. Additionally, organic foods are of better quality in terms of vitamins, minerals, antioxidants, proteins, and fats. However, there may be some confusion among consumers regarding these products. According

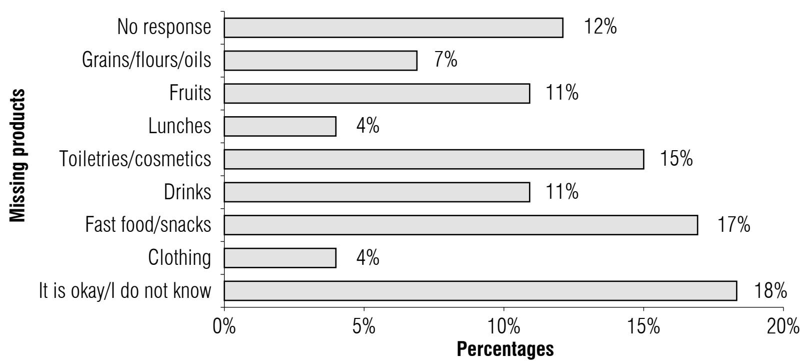

FIGURE 10. Missing products from the UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM).

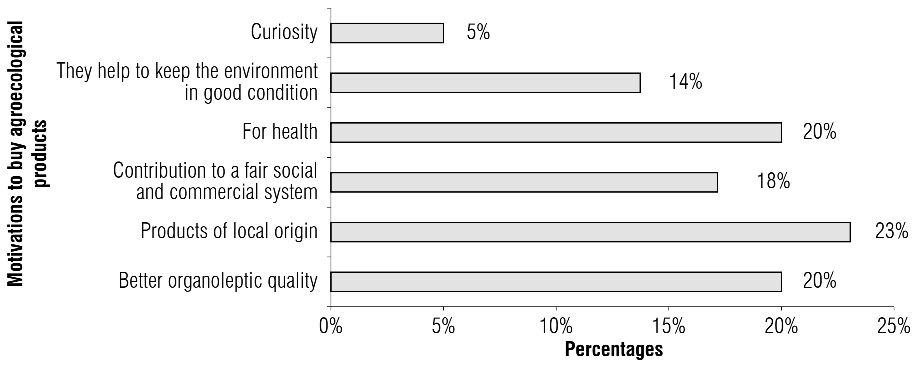

FIGURE 11. Motivations to buy agroecological products.

to Higuchi (2015), more than half of all organic consumers in the world believe that natural products are organic; therefore, consumer education remains necessary.

The difference in consumer motivations found in this study with that of other studies may be that most respondents are young adults (77%) and students (61%) who, therefore, may not suffer from serious illnesses or may not be aware of the social injustice experienced by the peasants, especially as they belong to a university focused on social projection.

Reasons not to buy more products from the UAF and MDSM

The results (Fig. 12) are the same as those reported in the study in Andalucia, in which prices were recorded as the main constraint to purchase, both for new consumers to arrive and for existing ones to remain faithful (Ipsos Insight, 2007). According to Aschemann-Witzel and Zielke (2017), price is the most significant perceived barrier to purchase and depends on the role that the consumer plays at the time of the purchase. A positive role means that the higher price is a sign of quality, and a negative role indicates that a sacrifice must be made to acquire the product. A person's income defines the role; if their income is low, the consumer takes a negative role, and if their income is high, the product gains a positive role, which was expected given the consumer's stratum of the UAF and MDSM. However, checking if the sale prices of some agroecological products can be corrected is not ruled out (Chaparro-Africano & Salazar Soto, 2020).

The second most frequent response (25% do not see the difference between one agroecological product and another) suggests that the positive role is achieved if there is better education, communication, and marketing of agroecological products.

Education also helps buyers to better understand the prices of agro-ecological products, an aspect of great relevance highlighted by other authors (Rödiger & Hamm, 2015). Although price was the most frequent response possibly because most respondents are low- or middle-income students, it did not obtain an overwhelming proportion, possibly because most respondents have university training, especially in natural sciences, that allows them to value rural agricultural work more.

Price is also the most important criterion when buying any food in Colombia (El Tiempo, 2019), although it is combined with criteria such as the appearance at the time of the purchase (Henryks *et al.*, 2014). Therefore, these findings must be analyzed with an integrative approach.

Money invested in UAF and MDSM purchases

The little money invested in each purchase of agroecological products (Fig. 13) may be due to the low purchasing power of most respondents (89% of strata 2 and 3). Most of those respondents do not shop from home (61% are students) and do not make important purchases to avoid carrying packages over long distances. This result is decisive from the geomarketing theory, according to which buyers acquire products at the point of sale closest to their homes (Ramadani *et al.*, 2018), although for UNIMINUTO it is crucial to offer agroecological products to the middle and lower strata of the city.

Willingness to pay extra

We found that 63% of the consumers are willing to pay up to 10% premium, 24% would accept between 11 and 20%, 9% of the customers would pay 0%, 2% would pay over 20% and 2% did not respond. This result (89% willing to pay extra) differs slightly from data reported in Figure 12, where 55% considered the products expensive or did not know the differences compared to non-agricultural products. These findings suggest that the respondents recognize a superior quality in agroecological products (Fig. 11) but do not have greater purchasing power, or require more information about their benefits.

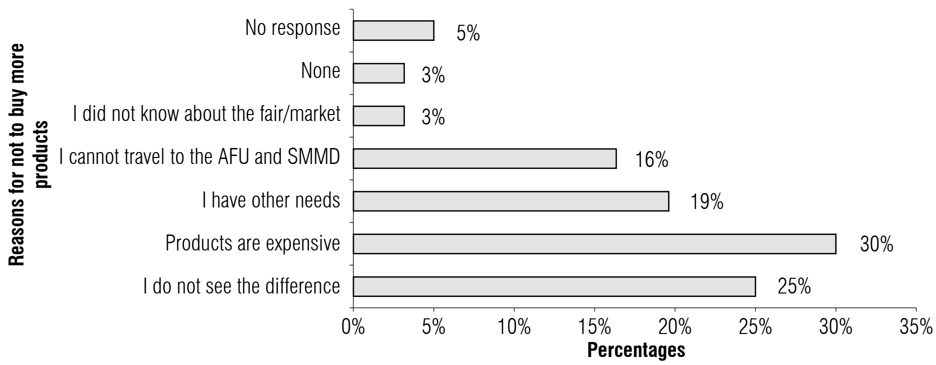

FIGURE 12. Reasons for not to buy more products from the UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM).

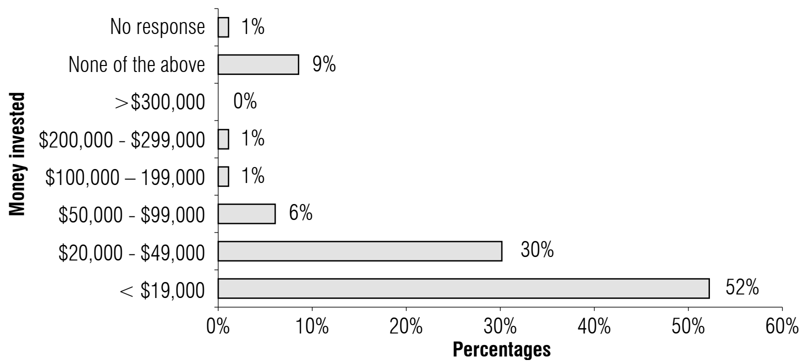

FIGURE 13. Money invested in purchases at the UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM).

The results in this study are like those reported in Spain (Ipsos Insight, 2007), Bogota (Vargas Restrepo & Valencia Bitar, 2015), and Romania (Vietoris *et al*., 2016), where the highest percentage of respondents were willing to pay 10% premium. However, in Serbia this premium increases to 20%, (Kranjac *et al*., 2017). Escobar-Moreno *et al*. (2015) also report that only 10% would not be willing to pay a premium in a study carried out in Antioquia.

Packaging preferred by respondents in UAF and MDSM

Eighty percent of respondents prefer biodegradable packaging, reusable packaging such as glass, or no packaging (Fig. 14), reflecting their interest to the environment. These results coincide with other studies (Orzan *et al*., 2018), since these preferences will be maintained if the product's final price responds to the quality demanded by the consumer.

The UAF and the MDSM replaced plastic packaging with biodegradable or reusable packaging and have done reverse logistics of reusable packaging since 2012. These practices were implemented well before the issuance of Resolution 1407 of 2018 by the Ministry of the Environment and Sustainable Development that encourages the use, innovation

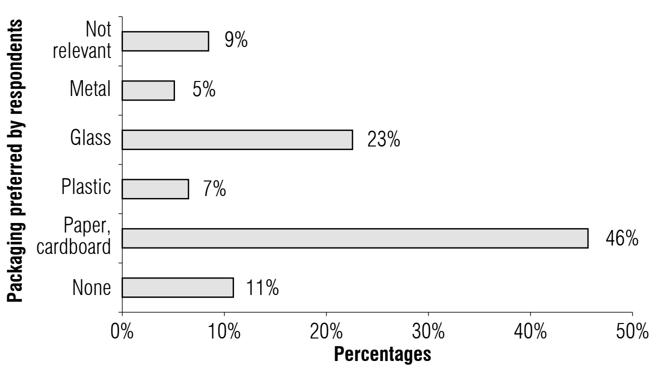

FIGURE 14. Packaging preferred by respondents at the UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM).

and eco-design of packaging placed on the market (Ministerio de Ambiente y Desarrollo Sostenible, 2018).

Identification of agroecological products

Thirty four percent of respondents identified agroecological products by appearance, 29% by the seal or certificate, 23% based on their trust in the UAF and its producers, and 14% by the label. The proportion of respondents who believe that they can identify an agroecological product by its appearance is surprising since this does not depend only on its agroecological quality but also on elements related to the variety, irrigation, crop or animal nutrition, and processing (Raigón Jiménez, 2008). According to Gentile and Rodríguez (2002), most consumers (60%) identify an organic product by the brand, the certification seal, and/or the label, while the remaining 40% based on their trust in the producer.

The trust generated by the UAF and MDSM, their seal, their product labels, and their producers is positive (23% respondents). However, the results also evidenced that consumers have little knowledge of the Participatory Guarantee System (PGS) of the AMNBR (Chaparro-Africano & Naranjo, 2020), a mechanism recognized by the MADR through Resolution 464 of 2017 (MADR, 2017) that seeks to overcome multiple deficiencies of third-party certification such as cost, the non-participation of those who produce, distribute, and consume the products, and its lack of adaptation to the needs of agroecological production. Low consumer recognition of PGS was also reported by other authors (Hamzaoui Essoussi & Zahaf, 2008). This should be corrected as it reinforces the prior information received by consumers.

Regarding the organic seal, in Colombia the legislation includes Resolution 0148/2004 (MADR, 2004), Resolution 00036/2007 that modifies Resolution 0148 (MADR, 2007), Resolution 187/2006 about organic production (MADR, 2006) and its regulation, and Resolution 199/2016 that modifies Resolution 199 (MADR, 2016). Altogether, these resolutions determined that organic products should be identified as such by the seal issued by the respective certifier, and the organic food seal of the MADR.

Information of interest on labels

The results about information of interest on labels (Fig. 15) differ from those of the National Institute of Health, where only 28% of respondents read the labels, 39% read the nutritional table and 33% the ingredients, versus 82% of respondents who read labels according to this study. These last two elements (nutritional table and ingredients) are the third and fourth criteria to be considered when buying food, only after the price and brand (El Tiempo, 2019).

Many people do not read labels due to lack of time, small print size and technical language (El Tiempo, 2019). For this reason, the UAF as a producer market invests time in disseminating this information and supports a proposal to renew labels (Semana, 2019).

Elements that affect the purchase of agroecological products in the UAF and MDSM Product appearance is always a determining indicator for consumers of organic products (Henryks *et al*., 2014). This result was also observed in this (Fig. 16) and other studies (Rojas Ramírez & Cuéllar Rojas, 2014). For this reason, the appearance of the product should reinforce the positive consumer perception of agroecological products and is, therefore, no less important than safety, superior nutritional content, or fair price.

Conclusions

The profile of consumers of agroecological products from the two university agroecological markets evaluated and the characteristics of their consumption are closely related to the surveyed population, who are mostly university students from middle and lower strata; however, age, gender, and educational level are common features with similar studies.

Most UAF and MDSM consumers are occasional because these markets do not meet their expectations regarding the

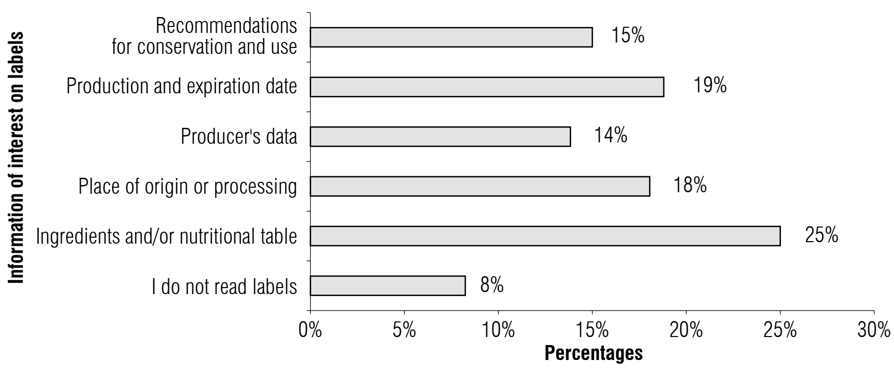

FIGURE 15. Information of interest on labels of agroecological products.

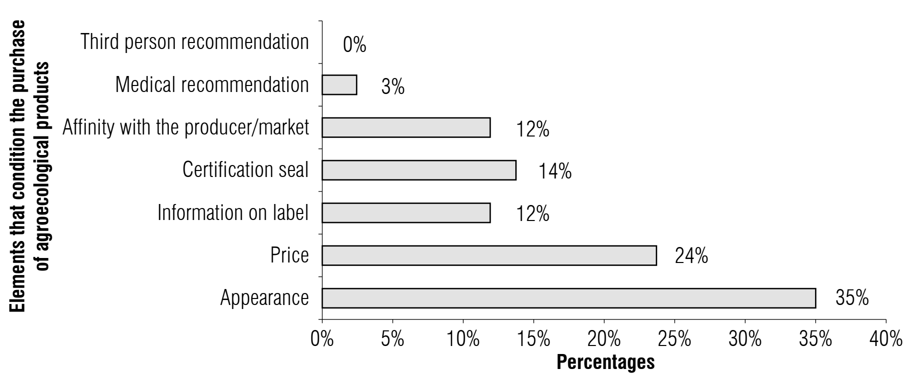

FIGURE 16. Elements that condition the purchase of agroecological products at the UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM).

type of product (many hope to find fast food and agroecological snacks), the frequency of the UAF (many prefer a fortnightly frequency), and prices (products are perceived as expensive due to low purchasing power of consumers). This is observed because of better alternatives regarding location, frequency, hours, etc. offered by conventional markets, insufficient information campaign, and the consumer's profile.

The motivations for the purchase of agroecological products combine aspects of collective well-being such as local production and the contribution to a fairer socioeconomic system. They also include aspects related to individual well-being such as health and organoleptic characteristics of the products. These motivations cover socioeconomic but not environmental aspects, which was an unexpected finding.

The main limitation for the purchase of agroecological products is appearance and the second is price. Therefore, if the appearance were improved, consumers would recognize pricing as fairer. This highlights the need to simultaneously improve the appearance of the products by producers and education by markets.

Additionally, the main limitation for not buying more agroecological products is pricing, which seems to be contradictory since there is a great willingness to pay some premium. Consumers consider that the pricing of the products is fair, but they may not have enough purchasing power or require more information to avoid perceiving the negative side of prices.

The educational effort of these university agroecological markets (UAF and MDSM) is insufficient. Consumers do not know the difference between agroecological products and other kinds of products, think that an agroecological product can be recognized only by its appearance, do not understand the role of participatory certification, and directly express interest in knowing more about the markets, products, producers, and principles promoted by UAF and MDSM. Despite this, the large proportion of respondents who read the labels can be considered a great educational achievement of the UAF and MDSM.

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

JDGM collected and analyzed the data and prepared the first draft of this article. AMCA conceptualized the research, managed the funding, designed the methodology, analyzed the data, prepared the first draft and the final version of this article, and made the adjustments requested by the journal.

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SUPPLEMENTARY MATERIAL 1. Survey for the characterization of consumers and consumption in UNIMINUTO Agroecological Fair (UAF) and Minuto de Dios Solidarity Market (MDSM).

Email
Name
ID
Date

Characterization of UAF and MDSM consumers		
1	How old are you?	• < 20 years old • 20 to 39 years old • 40 to 49 years old • 50 to 60 years old • >60 years old
2	What is your gender?	• Male • Female
3	What is your educational level?	• Primary/high school • Technician/technologist • Undergraduate student • Post-graduate
4	What is your profession?	• Art, culture, recreation, and sports • Natural, applied, and related sciences • Social sciences, education, services • Directorate and management • Finance and administration • Primary and extractive exploitation • Health • Sales and services
5	What is your occupation?	• Student • Independent • Employed • Retired
6	What is your household stratum?	• 1 • 2 • 3 • 4 • 5 or 6
7	How many people make up your household?	• 1 • 2/3 • 4/5 • >5
8	Which is your link with UNIMINUTO?	• Student • Professor • Administration staff • UAF seller • No connection
UAF and MDSM concepts and media		
9	Do you know the dates, location, and hours of the UAF and MDSM?	• Yes • No
10	What UAF and MDSM media do you consult?	• Flyers • Posters • Instagram • Facebook • Email • Word-of-mouth • Agroecological Markets Network of Bogota - Region • Radio • WhatsApp • None

11	What would you like to know about the UAF and MDSM?	• Information about the products • Dates, times, place, and activities • Information on producers • Information on the principles they promote
12	What are the principles of UAF and MDSM?	• Agroecology • Social and solidarity economy • Fair trade • Sustainable consumption • Food sovereignty • Short supply chains • Radical democracy • All • None
Characterization of consumption in UAF and MDSM		
13	What kind of buyer of the UAF and MDSM are you?	• Faithful: I visit all the UAF and shop regularly at MDSM • Occasional: I only buy if I can attend
14	Do you buy organic products in conventional markets?	• Yes • No
	In what market do you buy these products?	• Supermarkets/hypermarkets • Stores • Other
15	What products would you like to find at UAF and MDSM?	• Fast food/snacks • Drinks • Toiletries/cosmetics • Lunches • Fruits • Grains/flours/oils • Clothing • It is okay/I do not know • No response
16	Do you consider that the frequency of UAF (every month) and MDSM (Monday to Saturday) is sufficient?	• Yes • No • UAF twice a month • UAF every week • MDSM Monday to Sunday
17	What are your motivations to buy agroecological products in the UAF and MDSM?	• Products of local origin • Better organoleptic quality • For health • Contribution to a fair social and commercial system • They help to keep the environment in good condition • Curiosity
18	Why don't you buy more products from UAF and MDSM?	• I do not see the difference • Products are expensive • I have other needs • I cannot travel to the UAF and MDSM • I did not know about the fair/market • None • No response
19	How much money do you invest in each UAF or in each purchase in MDSM?	• < $19,000 • $20,000 - $49,000 • $50,000 - $99,000 • $100,000 – 199,000 • $200,000 - $299,000 • >$300,000 • None of the above • No response
20	How much are you willing to pay as a premium for the agroecological products of the UAF and MDSM?	• 0% • 1 to 10% • 11 to 20% • >20%

21	What packaging do you prefer for the products you buy from UAF and MDSM?	• None • Paper, cardboard • Plastic • Glass • Metal • Not relevant
22	How do you identify an agroecological product in the UAF and MDSM?	• Appearance (color, shape, size), smell, taste • Seal or certificate • Trust in the UAF, MDSM and its producers • Label
23	What information do you look for on UAF and MDSM product labels?	• I don't read labels • Ingredients and/or nutritional table • Place of origin or processing • Producer's data • Production and expiration date • Recommendations for conservation and use
24	What is most important when buying a product from the UAF and MDSM?	• Appearance • Price • Information on label • Certification seal • Affinity with the producer/market • Medical recommendation • Third person recommendation

Farm management succession by heritage. A Central Mexico case study

Sucesión de la gestión agrícola por herencia. Un estudio de caso del centro de México

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ABSTRACT

Farm succession involves the interaction of social, economic, and emotional factors. The process also includes the decisions of farmers and their families who consider the farm as patrimony rather than a productive asset. This article analyses the processes of succession in farms of central Mexico to understand the different stages they comprise and the problems that these processes encounter. We conducted 12 in-depth interviews with producers who went through a recent transfer process. Succession processes and their associated factors were analyzed and discussed using the multiple case study methodology; subsequently, the stages that comprise each of these processes were defined. Results show that succession is commonly managed without making decisions or taking actions that prevent future problems, and individual interests of the potential heirs prevail. Although each transfer process is different, two basic types were identified: the spouse or the descendants assume the administration of the patrimony. However, in the analyzed cases, we found that, in general, there is no planning for succession, so the successors lack training in agricultural activities. In general, succession processes are complex and generate conflicts, and their trajectory depends on family relationships and the socioeconomic conditions of the farm.

Key words: inheritance, patrimony, transfer, land.

RESUMEN

La sucesión agrícola conlleva la interacción de factores sociales, económicos y emocionales. El proceso también incluye decisiones de los agricultores y sus familias quienes consideran a la granja un patrimonio más que un activo productivo. Este artículo analiza los procesos de sucesión de granjas en el centro de México para comprender las diferentes etapas que comprenden y los problemas que cada uno de estos procesos presentan. Se aplicaron entrevistas a profundidad a 12 productores que atravesaron recientemente un proceso de transferencia. Los procesos de sucesión y sus factores asociados fueron analizados y discutidos utilizando la metodología de estudio de casos múltiples; posteriormente se definieron las etapas que los componen. Los resultados muestran que la sucesión se maneja comúnmente sin tomar decisiones o acciones que eviten problemas futuros y prevalecen los intereses individuales de los posibles herederos. Aunque cada proceso de transferencia es diferente, se identificaron dos tipos básicos: el cónyuge o los descendientes asumen la administración del patrimonio. Sin embargo, en los casos analizados, se encontró que en general no existe una planificación de la sucesión, por lo que los sucesores carecen de entrenamiento en actividades agrícolas. En general, los procesos de sucesión son complejos y generan conflictos, y su trayectoria depende de las relaciones familiares y las condiciones socioeconómicas de la granja.

Palabras clave: herencia, patrimonio, transferencia, tierra.

Introduction

Succession is essential for the development of farms since it is related to sociocultural aspects, and it is fundamental to the sustainability and productivity of global agriculture. Given the advanced age of some farmers, promoting succession is key for avoiding the rural-urban migration of younger generations and preventing losses of farms that may lead to a reduction in agricultural production, affecting food security. This is an increasingly important issue since agriculture evolves and changes, making it more difficult for farmers to maintain endogenous cycles of succession and pursue innovative activities (Fischer & Burton, 2014; Hauck & Prügl, 2015).

Although succession was not originally a big problem because farms were large enough to divide them among all descendants (Stephens, 2011), inheritance has become more important since farms are smaller, and the number of famers has increased. However, this division has led to lower individual profitability in agriculture that, combined with lower availability of services in rural areas and climatic and biological risks of farming, usually makes it an unattractive activity for successors (Barnes, 2009). Another

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problem that farm succession faces is that urbanization and non-agricultural land uses (residential and commercial) have grown considerably, increasing land prices (Schumacher *et al*., 2019).

Succession planning is essential for the success of family businesses (Barclays Wealth, 2009), as it refers to the orderly transfer of management, responsibility, ownership, and control (Stephens, 2011). It can also include the transfer of the assets and can begin when the holder is alive. Thus, succession specifies when, how, and under what circumstances the management of farms will pass from the current operator to another person (Mishra & El-Osta, 2007).

The process of succession is affected by agricultural policy regimes, opportunities for diversification on and off the farm, gender, expectations of farmers, increases in land prices, and a sense of marginalization of farmers from society (Fischer & Burton, 2014). Additionally, succession is specific to the properties and depends on their structure and composition (Leonard *et al*., 2017). Farms possess socio-emotional wealth that is relevant and must be considered, due to their nature, as family businesses. Literature about family businesses considers non-financial aspects as drivers of family business behavior and contemplates their positive and negative consequences (Berrone *et al*., 2012; Hauck & Prügl, 2015). Families should examine their environment and family relationships since agricultural succession implies walking on a tightrope requiring understanding and empathy between generations (Mann, 2007; Hauck & Prügl, 2015). Family relationships are among the fundamental factors relating to succession suggested by Camfield and Franco (2019). These authors highlight family tradition, blood relationships, family participation, conflict management, trust, and communication between siblings as relationship issues that can influence succession. Also, the intention of the successors to take over the farm is determined by their attitude, behavioral control, and perceived norms within the farm (Morais *et al*., 2018).

Thus, the processes of succession are complicated and involve the interaction of sociocultural and emotional factors of the producers and their families. For this reason, these processes often represent a conflict for families. Additionally, the discussion of succession is a topic avoided in family circles (Romero-Padilla *et al*., 2020) due to the anxiety caused by the loss of reputation when problems relating to succession and innovation are shared openly (Hauck & Prügl, 2015). There are conflicting and contradictory wishes in the older generation regarding the final transfer of a farm (Conway *et al*., 2017). These wishes can generate "symbolic violence" from the owner towards the successors when the proprietor avoids delegating responsibilities and reiterates their indispensability in the operation of the farm. Despite these problems in succession, successor effects or the "new blood effect", referring to a successful succession process when appointed successors introduce innovations on the farm, imply many rewards like farm expansion and productivity (Kerbler, 2010). However, failure in these processes can turn into significant losses, financial insecurity, and family dissatisfaction (Ahmad & Yaseen, 2018).

The interest of the heirs in heritage depends on generational involvement and the type of family in which succession takes place (Soto Maciel *et al*., 2015; Camfield & Franco, 2019). Also, interest is related to the culture and traditions of rural households and territories (Pachón-Ariza *et al*., 2019). Since the process of succession is closely related to socio-emotional factors (Arreola Bravo *et al*., 2015), family history can influence its organizational and strategic performance, as family members can impose their values, objectives, and logic (Soto Maciel *et al*., 2015).

Regarding the rural and fishing sector of Mexico, 39% of farmers are over 60 years of age (INEGI, 2018). This fact is closely linked to the migration of young people to urban areas and increased marginalization, mainly in the central and southern regions of the country (SAGARPA-FAO, 2014). Although there are several studies on agricultural succession, this issue has not been widely studied in Mexico. Thus, information on this topic is important, given the growing urbanization of central Mexico, where people easily migrate from the countryside to the city searching for better opportunities, resulting in the abandonment of agriculture because it is not attractive to new generations.

The objective of this research was to analyze land transfer on family farms, after the passing of an incumbent. The study took place within the agricultural farms located in central Mexico and includes the phases that this process encompasses. The research was carried out with the assumption that succession is a long and complex process involving the interaction of a high number of sociocultural and emotional aspects. To achieve this goal, the multiple case study method was used with producers who went through a recent succession process.

Materials and methods

The study adopted a qualitative approach using the multiple case study method. This method was chosen as a convenient tool for studying complex phenomena from unique stories (Tasci *et al*., 2020); it also enables an in-depth exploration within specific contexts (Rashid *et al*., 2019). In addition, case studies are usually useful to understand how succession is implemented in family businesses, and their wealth, depth, and closeness allow the understanding of the characteristics of each family business (De Massis & Kotlar, 2014). The qualitative approach in the study of succession processes provides a deeper understanding, and addresses beliefs, motivations, and attitudes of family members (Bertoni & Cavicchioli, 2016).

In the multiple case study methodology, the selection of cases is important. De Massis and Kotlar (2014) argue that the sample cases should be selected for theoretical reasons. In this research, the cases were selected because the participants went through a similar situation within a succession process. In this way, the producers were selected under the criterion of having gone through a family farm transfer after the incumbent's death. In all cases, the land had been transferred in the last 5 years or the process was still in progress. Additionally, the participants must have been willing to share their experiences given the sensitivity of the topic.

The producers interviewed were contacted through a "local key informant" who knew the producers in the area and understood their willingness to speak openly and honestly about their succession process. The interviews were conducted with 12 farmers in central Mexico between July and September 2019. The area of the average farm was 5 ha and the main crops cultivated were maize, bean, alfalfa, broccoli, green oats, and avocado.

Data collection was carried out through semi-structured interviews that included two main sections. The first section consisted of basic questions about the producer and the farm, while the second section included questions relating to the succession process they had gone through (Supplementary material 1). The interviews were recorded and transcribed. A content analysis was performed to find similarities and differences between the interviewed producers and to establish the categories and groups. The materials were imported to Atlas.ti and codes were established. This way, it was possible to differentiate succession pathways through the following variables: situation of the holder's spouse, number of descendants, distribution of the patrimony when the holder was alive, existence of talks about succession between the holder and their descendants, affective relationships between the producer and his spouse, involvement of descendants in agricultural work with their father, and affective relationships between the producer and his descendants.

The information on succession was then systematized and analyzed, and the factors associated with them were discussed. The sequence of events and the relatives involved in the process of succession were presented in five succession stages, adapted from the succession phases established by Belausteguigoitia Rius (2012): diagnostics, planning, training, transfer, and culmination. Finally, the importance of socioeconomic, emotional, and cultural issues in the decisions that producers make in their processes of succession was discussed.

Results and discussion

Of the twelve producers interviewed, ten were men and two women. The average age was 58 years, and only one woman had no experience in agricultural activities. Regarding the level of education, two producers completed primary school, two producers finished secondary school, five producers graduated from high school, and three had a bachelor's degree.

For the producers interviewed, the main asset of their farm was the land. Therefore, when we refer to succession and inheritance of the patrimony, we refer only to the land.

Succession pathways

Each farm underwent a specific process of succession due to the diversity of income levels, land value, family structure, agricultural systems, the emotional nexus and socioemotional characteristics in the family. Our findings agree with those of Leonard *et al*. (2017) who state that there is no specific path to succession. Nevertheless, two basic succession pathways were identified, one of them is generally a transition to the other. Thus, the final scenario leads the descendants to assume the administration of the farm (Fig. 1).

The description of these pathways and their multiple aspects are presented below.

Pathway 1: a descendant assumes the administration of the patrimony when the holder is no longer there

In this scenario, three possible situations were found. In the first one, there was a single descendant since the successor had no siblings. His mother died years before his father, so when his father died, the farm was left under his management. Although the successor had a profession and exercised it, the agricultural activity was profitable, motivating

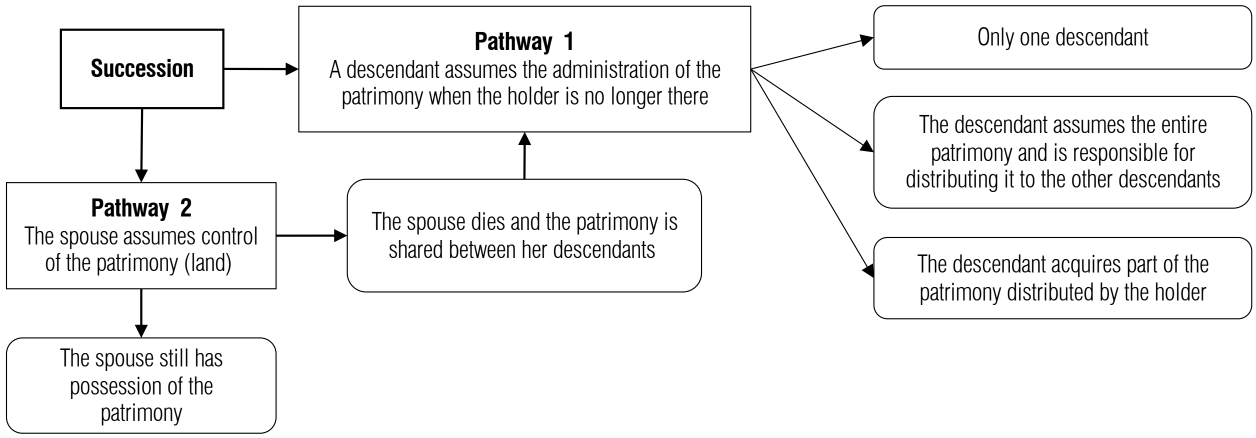

FIGURE 1. Succession pathways.

him to continue with his father's work. So, factors such as competitiveness and profitability of the farm indirectly promoted intra-family succession (Cavicchioli *et al*., 2015). An important aspect that the successor commented was that, since he was not involved in agricultural activities, after inheriting the farm many people approached him asking to buy the land. In this situation, if the heir had had a higher opportunity cost outside of agricultural activity, it is most likely that he would have sold the land, and it would have remained in agriculture, but with other owners.

The second situation occurred when one descendant received the entire property and was responsible for distributing it among the other descendants. In this situation, we found four farmers with a descendant in charge of distributing the land among his siblings according to the provisions of the holder. In these cases, the holder shared with the family the percentage of the patrimony that corresponded to each descendant. Despite this, the holder did not designate in all cases the location of the properties to be distributed; so, when there was no established location, the descendants raffled or agreed on the sites. This fomented displeasure among some heirs who did not agree with the results. In this scenario, the incumbent probably preferred to avoid problems with his descendants about the designated location of the patrimony. This constituted weak planning for the farm that brought problems between the descendants.

Finally, in the third situation, the successor acquired part of the patrimony distributed by the holder before his death. That does not mean that the inheritance was carried out in a formal way, with the existence of a testament or succession list; however, all the descendants were aware of their father's decision. The two producers in this situation received a higher percentage of the patrimony than the other heirs. Also, the holder designated a small area of land to some descendants for building their houses or to begin establishing their own farm. In the analyzed cases, the holder assigned a different proportion of land to their descendants, causing disagreements between them.

Pathway 2: the spouse assumes control of the patrimony (land)

It is common that the holder does not formally define a descendant as successor. This situation can take place due to the holder's fear of creating uncomfortable situations or losing authority, or simply due to an unforeseen death. In these cases, the administration of the patrimony invariably falls on the spouse. In this pathway, the successor, widow, and mother of the family seeks to give continuity to the prevailing situation before the death of the head of the family. Even where there are dynamic land markets, renting or selling the land are options to obtain income without engaging in agriculture. Subsequently, a new process for the designation of the land begins that is carried out by the mother towards her descendants. Although it is possible that the heirs continue working on the farm, if the land belongs to the holder's spouse, they generally do not invest, innovate, or improve the farm since it is not clear who the final successor will be.

Thus, the "symbolic violence" mentioned by Conway *et al.* (2017) is seen in this scenario when the mother refuses to transfer the land to her son, arguing for his lack of capital. Also, the barrier of the "successor effect" is seen since the son wants to take over the farm, but his mother does not allow it.

For three of the producers interviewed, their mother oversaw the designation of the available land. In these cases, when the mother died, legal procedures began for the assignment of the property to each descendant. In this scenario, disagreements were related to the final percentage that each descendant received. In the end, the descendant who cared for his mother was the one who obtained the highest percentage, causing discontent among the others.

This group included a scenario with two widowed women. One of them has already made the patrimony designation and worked together with one son, while the other has not yet appointed her successors and rents the land to other producers. Regional conditions differed between these two participants; the first person was in a large agricultural area. Additionally, she has been involved in agricultural management for years and considers this activity to be profitable. The other woman rented her land because, according to her statement, she would not know how to handle it.

In this case, the land was in a peri-urban area, where the price of land is high and agriculture becomes less important in the face of urban growth (Romero-Padilla *et al.*, 2020). The two women also acquired the responsibility of deciding whether to continue with the agricultural activity or to sell or rent the land.

Stages of the transference process

To understand the result of a succession, it is necessary to study what happens before and after the transfer of assets (Stephens, 2011). For this reason, to specify the planification of succession among the studied farmers, we analyzed the five phases of the succession process in the family businesses proposed by Belausteguigoitia Rius (2012): diagnostics, planning, training, transfer, and culmination.

Phase 1. Diagnostics

According to the producers, this phase is complicated since if they are "ejidatarios", they must choose a single successor among their family members. In México with agrarian reform, the land and water were granted by the president to a group of people named "ejido". Each person of this group is called an "ejidatario" and has the rights over a piece of land and the right to vote in the assemblies of the "ejido". New members of the "ejidos" obtain land rights, in order of importance, through a) inheritance, b) cession or direct transfer, c) purchase from another member, and d) leasing agreements (Barnes, 2009).

This implies that they would fear the disagreement among the non-chosen. In most cases, the holders did not talk to the family about their decision, and, therefore, no preparation for the successor was made. In some cases, the designation of the new successor was known only after the holder died.

For the interviewed producers, this phase involves the affective, economic, and emotional relationship that they have with their family, and it implies the condition of support that the holder expects to receive from the people identified as heirs once the transfer has been made. In turn, according to the interviewees, not only the direct successor should be considered but also his family. In this way, holders look for ways that their descendants can develop their personal work and emotional projects (López Castro, 2009).

In general, for the cases analyzed, the holders provide the diagnostics of their succession based on the economic and affective security that they would have in the future. Thus, the current economic position of the holder and his descendants affects the succession. For example, alternative sources of income, such as a retirement income, provide economic security to the holder for future years. However, this pension could motivate the transfer because the farm is not the only source of income for the farmer (Mishra & El-Osta, 2008; Grubbström & Sooväli-Sepping, 2012).

Phase 2. Planning

A formal planning phase was not found in the processes of succession studied. However, the holders talked to their family about how the transfer of the heritage would take place. In the analyzed cases, the holder sometimes started the distribution of the land so their descendants could establish their homes. In other cases, the holder designated a land area for the descendants to work but without a testament. Therefore, there was no document guaranteeing succession of the property. This at times caused frustration and disharmony among family members.

Sometimes the holder set a fraction of land apart, as an economic asset to sell in an emergency, or to cede it, at a specific time, to the descendants who would support the holder economically and emotionally in their old age. According to the anecdotes of the interviewees, the holder made changes based on the expectations and support they had from the descendants. Thus, as mentioned before, descendants did not invest in the farm because they did not know who the final heir would be.

Phase 3. Training

In the cases analyzed, no training phase was found because the successor frequently did not know whether they were the only successor. In the studied cases, sometimes it was possible to see the rungs in the succession ladder described by Errington (1998), because it was not clear who the final heir would be. This is a common problem for the farmer's boy since the holder delays his decision to leave the farm as much as possible and does not delegate sufficient management responsibility to a successor (Uchiyama *et al.*, 2008).

In some narrations, the successors stated that they had worked on the farm with the holder and had been involved in production and had also indirectly received training. In some cases, the holder commented to the successor that they would oversee the farm. Despite the above, not having an official designation kept the successor uncertain and prevented them from becoming fully involved in the production processes.

Therefore, it is important to consider that an intervention of parents in the motivation and involvement of agricultural labor could increase the intention of the successors to take over the farm, and the necessary factors could be developed to motivate interest in managing the agricultural business.

Phase 4. Transfer

This phase is inevitable, and according to the producers interviewed, it is the most complicated and the longest (Belausteguigoitia Rius, 2012). In the farms analyzed, this phase began after the holder died.

As stated before, the transfer of the farm occurs through two basic scenarios, the spouse of the holder assumes control of the patrimony, or a descendant assumes it (Fig. 1). In both cases, the holder could previously assign part of his land to his other descendants.

Phase 5. Culmination

A multiple succession was noted in the studied cases that could be caused by stress within the farm family, resulting in friction between the family members. There are a few cases where some descendants continued to work the lands inherited from the holder and buy out those who did not intend to continue in agriculture. However, this process could have some complications generated by the need to buy by other siblings and social aspects of the family unit such as the extent to which family members can work together (Burton & Walford, 2005). This intention to continue in the activity is closely related to the profitability of the farm. Thus, a successful farm links the successor to the land (Fischer & Burton, 2014).

Socio-emotional aspects and personal relationships influence the duration and complexity of the succession processes. However, the continuity of agricultural activity is largely determined by the opportunity costs of alternative uses of the land and of family work.

The culmination implies the consent of the holder's descendants after the long transfer process. Thus, in the end, those involved are satisfied or resigned to the results of the succession, and some of them continue working the farm. In this research, there are succession processes that have not reached their culmination because the transfer phase has been very long.

In some processes, the land was finally sold by the successors because the farm was not considered profitable, or the successors had higher expectations about other sources of income. Also, there were some cases where the land was rented, mainly when the farm was inherited by the spouse, and she did not know how to manage it. Another scenario was the personal use of the patrimony for non-agricultural purposes, mainly housing construction.

The typical succession process

With the comprehensive analysis of the five stages of the succession process, the typical process was designed for the cases studied (Fig. 2).

The common element in the successions that we studied is that they practically lacked the formal planning and training phases. Indeed, the first three stages were carried out unconsciously without explicit analysis or reflections. The holder avoided committing to the successors to prevent conflicts or disappointments from happening and was able to make adjustments at any time. In the end, this approach makes the next two stages, training and transfer, less effective. The emphasis was on the transfer that, according to the interviewed producers, is the most complicated and longest phase. The culmination phase usually resulted in the fragmentation of the farm and the abandonment of agriculture by most of the descendants.

Succession and continuity of agricultural activity

Succession can take place when the incumbent is still alive, and he participates in the management of the farm along with the successor, so the successor gets training. However, succession in the case studies was not planned; it rather happened because of the death of the farm holder or their inability to continue in agricultural production. At the time of the transfer of patrimony, the individual interests of the possible heirs prevailed, frequently in a framework of distrust. This results in fragmentation of the patrimony making the continuity in the agricultural activity less viable, without a route to farming that would provide an appropriate succession and efficient agriculture (Chiswell, 2016).

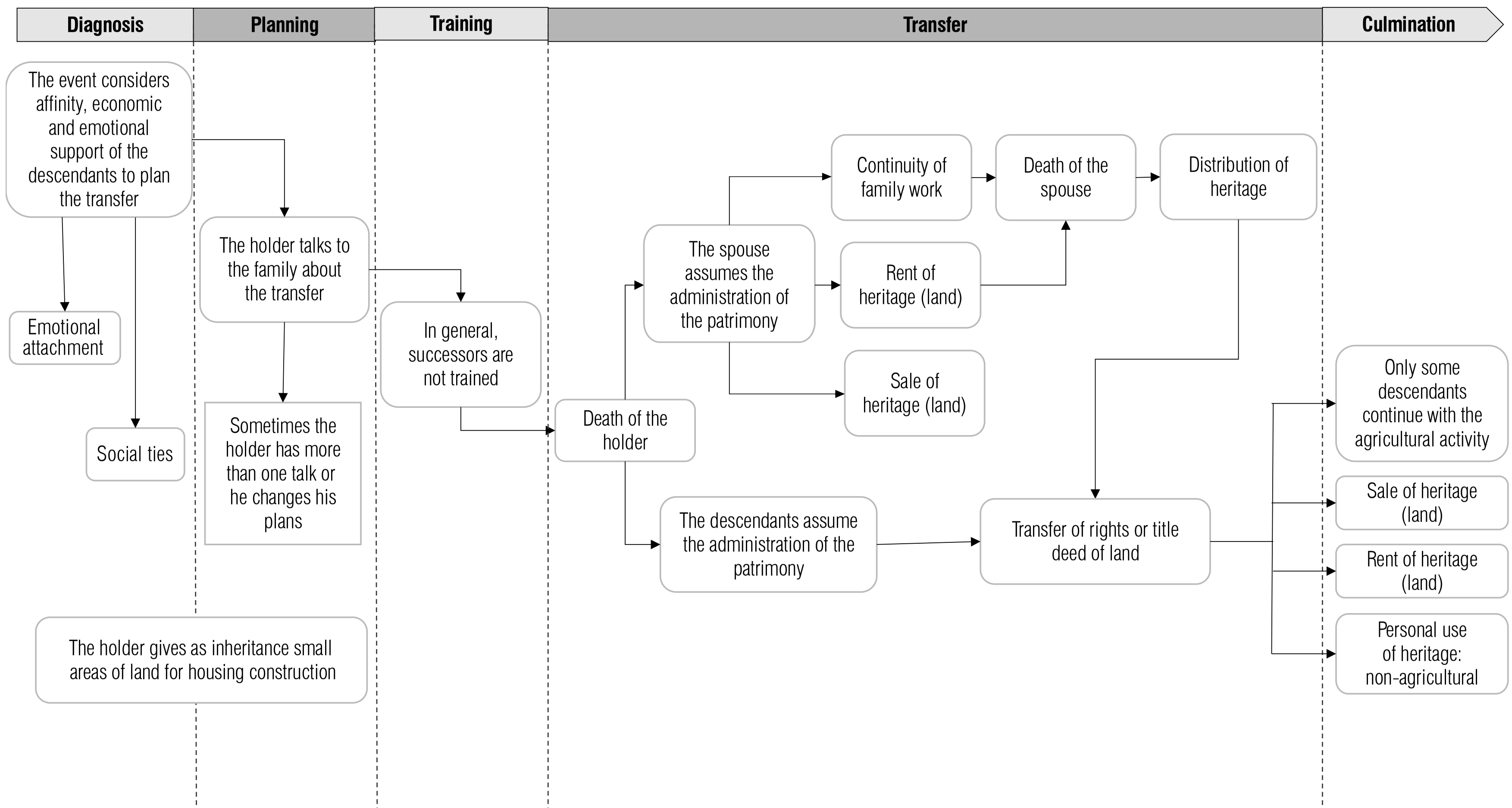

FIGURE 2. Stages of the typical process of succession in the farms studied.

Even when the decisions about management of the farm and inheritance follow an economic focus, and profitability is the main motivating factor for continuing in agricultural activities, the interviews revealed that holders and successors also have a need to satisfy personal goals. According to Gómez-Mejía *et al.* (2007), some farms continue through generations not only because they are efficient or profitable but also because they satisfy the owners and successors as family businesses, providing successful aging and social and personal identity to the older farmers (O'Callaghan & Warburton, 2017). However, in these cases studied, the greatest motivation to continue in agricultural activity was profitability that is related to the type of production system and the destination of production (Romero-Padilla *et al.*, 2020).

In the processes of successions analyzed, several factors interacted and affected the decisions made by holders and successors. For example, they were influenced by the environment in which the farm was located and the circumstances in which the producer worked. The value of the land was another important variable since proximity to cities increased the value of the land for non-agricultural uses.

Cultural and traditional aspects were also relevant in succession and have an influence on the continuity of the agricultural activities in the cases analyzed. Aspects such as gender, birth order, and age play a role in whether the successor will continue in agriculture or not. Thus, male and first-born successors are more likely to take over the farm, acquire a greater proportion of the assets, or oversee the assignment (Cavicchioli *et al.*, 2018). Although sometimes it is the wife who assumes administration of the land, the final transfer follows the same details for her descendants.

The narratives of the interviewed producers showed that the processes of succession are affected considerably by affective and emotional issues. The emotional attachment is related to the financial support the holder receives from his descendants and the economic security they will provide when the holder cannot continue the agricultural activity, and his sources of income are null or scarce. Thus, the holders maintain their authority over their descendants in an unrecognizable and silent way, exercising symbolic violence (Conway *et al.*, 2017).

Many holders preferred to assign the land to their spouse to provide security for her or simply to leave her the uncomfortable decision of choosing a successor. Finally, the main problems in the processes of succession are related to personal feelings and disagreements of the people involved. These are generated by the dissatisfaction with the area or location of the assigned patrimony.

Conclusions

The processes of transfer of farms are not planned, and decisions or actions are rarely taken to prevent future problems and difficulties. Thus, succession is long and complex and frequently has permanent consequences for family harmony and the patrimony. There are multiple aspects that depend on family relationships and the economic, social, and cultural conditions in which the farm operates.

In the most common scenarios, the continuity of the farm is unlikely due to the tendency to fragment the land and the lack of interest from family members to maintain agriculture as a source of income. Thus, at the time of transferring the land, individual interests prevail over collective ones that frequently reduce the economic viability of the farm.

In this research, the spouse has an important role in the transfer process, as she is often an executor of the main asset that is the land when the holder dies and, subsequently, the land is distributed to the descendants. This situation is important since it delays the final transfer process and the total involvement of the successors in the agricultural activities.

This vulnerability of family farming to the processes of succession represents a challenge since they alter the management of the farm and sometimes compromise continuity.

Faced with this possibility, strengthening the stages before the transfer (diagnostics, planning, and training) would help to achieve more effective and less risky succession that might prevent losses of Mexican farms. This requires those involved in the succession to be willing to seek support or advice.

In Mexico, there is no culture of succession and generally the succession process starts with the incumbent's passing. In this sense, public policies that support agrarian transference through training and advisory programs are relevant, especially for profitable farms where continuity of agricultural activity is intended and where development is limited by problems of inheritance.

Conflict of interest statement

The authors declare there is no conflict of interest regarding the publication of this article.

Author's contributions

ARP formulated the ideas and conducted the research process, performed data collection, defined the overarching research goals and aims, applied the qualitative techniques to analyze and synthesize the study data, developed the methodology, and prepared the published work specifically writing the initial draft. VHSC formulated the overarching research goals and aims, created and presented the published work, specifically writing the initial draft, developed the methodology, acquired the financial support for the project leading to this publication, and led the research activity planning and execution. SRMB formulated the overarching research goals and aims and prepared the published work. AVAG prepared the published work and performed the critical review, commentary or revision. JRAC prepared the published work, specifically data presentation and verification.

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Supplementary material 1: Semi-structured survey.

I. Farmer's information

[1] Name(s)	[2] Last name (on the father's side)	[3] Last name (on the mother's side)

	M ( ) F ( )		
[4] Age	[5] Gender	[6] Schooling (years)	[7] Time dedicated to farm work (hours per week)

[8] Importance of agricultural activity	[9] Growth expectations of the farm

II. Farm Information

[10] % income from the farm	[11] Land cost in the region	[12] Land rent in the region

[13] Crop				
[14] Cultivated area (ha)				
[15] Destination				
[16] Type of irrigation				

III. Succession

1. What do you understand as succession?
- Do you consider it important?
- Why do you think that succession is generally not planned?

2. Was there a succession plan in your case?
- Did you know it?
- Was it followed through?

3. How was the succession carried out?
- Was it what you expected?
- Was it carried out as the deceased would have liked?

4. How long was the farm in succession?
- Have there been any problems after the succession?
- What were they? Why? Could they have been avoided?

5. Do you plan to continue with the agricultural activity? Why?
- How will you carry out the succession?

Extension of umbu (*Spondias tuberosa* Arruda) postharvest life using a cassava starch-based coating

Prolongación de la vida poscosecha del umbú (*Spondias tuberosa* Arruda) mediante recubrimiento a base de almidón de yuca

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ABSTRACT

The umbu (*Spondias tuberosa* Arruda) is a fruit tree adapted to the Brazilian semiarid region and constitutes an important source of income for many families in Brazil. Due to this, sustainable methods of fruit postharvest conservation should be studied. The objective of this study was to analyze the influence of cassava starch-based biofilm coatings for the postharvest conservation of umbu. The experimental design was completely randomized in a 2x5 factorial arrangement with six replicates. The two treatments, 1% cassava starch coating and no coating, were evaluated during five periods (0, 3, 6, 9, and 12 d of storage). Fresh weight loss (WL), fruit firmness (FIR), pH, total soluble solids (TSS), total titratable acidity (TA), TSS/TA ratio, external color: luminosity (L^*), hue angle (H^*), and chromaticity (C^*) were measured. The coating of fruits with cassava starch-based biofilm improved WL, pH, and TSS. The variables TA, TSS/TA ratio, and L^* were not influenced by the use or absence of the cassava starch biofilm coating. The use of 1% cassava starch to coat the fruits ensures better postharvest quality of the umbu and extends its postharvest life.

Key words: biofilm, conservation, sustainability.

RESUMEN

El umbú (*Spondias tuberosa* Arruda) es un árbol frutal adaptado a la región semiárida brasileña y constituye una fuente de ingresos importante para muchas familias en Brasil. Debido a esto, se deben estudiar métodos sostenibles de conservación de la fruta después de la cosecha. El objetivo de este estudio fue analizar la influencia de la biopelícula de almidón de yuca para la conservación poscosecha del umbú. El diseño experimental fue completamente al azar en un arreglo factorial 2x5 con seis repeticiones. Los dos tratamientos, recubrimiento de almidón de yuca al 1% y sin recubrimiento, se evaluaron durante cinco períodos (0, 3, 6, 9 y 12 d de almacenamiento). Se midieron la pérdida de peso fresco (PPF), la firmeza de la fruta (FIR), el pH, los sólidos solubles totales (SST), la acidez total titulable (AT), la relación SST/AT, el color: la luminosidad (L^*), el ángulo de tonalidad (T^*) y la cromaticidad (C^*). El recubrimiento de los frutos con la biopelícula a base de almidón de yuca mejoró el PPF, el pH y los SST. La AT, la relación SST/AT y la L^* no fueron influenciadas por el uso o ausencia del recubrimiento. El uso de almidón de yuca al 1% asegura una mejor calidad poscosecha del umbú y prolonga su vida poscosecha.

Palabras clave: biopelícula, conservación, sostenibilidad.

Introduction

The umbu (*Spondias tuberosa* Arruda) belongs to the Anacardiaceae family and is a native, endemic species of the Brazilian semiarid region (Lima *et al.*, 2018). This species has a wide geographical distribution and shows morphologic characteristics that enable its adaptability to various edaphoclimatic conditions. For example, the plant can promote leaf abscission, has roots with structures called lignotubers with the function of storing water and nutrients (Mitchell & Daly, 2015), and efficient control of leaf transpiration (Lima Filho & Aidar, 2016).

The mechanisms of adaptation to water scarcity and irregular rain distribution make it possible to cultivate umbu in several regions of Brazil, promoting food product diversification and increasing food security. Umbu has environmental and socio-economic importance for the producing regions. Its fruits are commercialized as an extract; however, there is great capacity for expansion for

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commercial growth to meet the demand of the fresh market and the market of processed products for its pulp and peel (Ribeiro *et al.*, 2019).

The fruits are oval or oblong, and their weight can range from 5 to 100 g, according to the genotype (Oliveira *et al.*, 2014). The fruit pulp or edible fraction has bioactive compounds, namely phenolics, carotenoids, and vitamin C that confer its antioxidant potential (Ribeiro *et al.*, 2019). Besides fresh consumption, the fruit can be used in the processing industry to produce juices, beverages, and canned products (Narain *et al.*, 1992; Lima *et al.*, 2018).

Despite the importance of umbu for small and medium farmer economy, its market still faces some difficulties. Some of the problems that this crop must overcome are the lack of efficient methods to monitor fruit quality that can be used to determine the ideal harvest maturity and methodologies to quantify its quality for fresh consumption and for the industry (Marques & Freitas, 2020). Therefore, there is a great need to develop accurate and low-cost methods for assisting the conservation and postharvest quality of fresh fruits, especially those destined to be consumed *in natura*.

Different postharvest treatments have been studied to control rot and improve fruit shelf life, including the use of modified atmospheric systems (Joshi *et al.*, 2019). The modification of the atmosphere can be easily achieved using polymeric films, such as plastic bags of high or low-density polyethylene (Vieira *et al.*, 2009). Another alternative is the use of edible coatings that have recently gained prominence and are being widely used for fruit preservation (García *et al.*, 2020). These coatings are environmentally friendly and serve as carriers of food additives that facilitate safety and nutrition and enhance the sensory aspects of fruits (Khan *et al.*, 2019).

Edible coatings can be prepared from carbohydrates, proteins, and lipids, individually or in combination (Muley & Singhal, 2020). Some reports suggest that the use of cassava (yuca) starch for fruit coatings can improve their physical and chemical properties and provide greater protection against microbial agents (Aider, 2010; Pimentel *et al.*, 2018). The fruit coating based on cassava starch is of low cost compared to commercial waxes. It also preserves good flavor, is compatible with various processes, and is shiny and transparent (Assis & Britto, 2014; Sousa *et al.*, 2019).

The objective of this research was to evaluate the postharvest conservation of umbu (*Spondias tuberosa* Arruda) fruits coated with a biofilm produced from cassava starch at room temperature. Since cassava starch is a cheap and easily found product, our findings can guide the development of a new methodology to extend fruit postharvest life.

Materials and methods

Experimental design

The experiment was carried out in a completely randomized design in a 2 x 5 factorial arrangement with six replicates. Fruits with and without cassava starch coating (1%) were evaluated during five different storage periods (0, 3, 6, 9, and 12 d of storage).

Plant material

The umbu fruits were harvested on an experimental farm at the State University of Montes Claros - UNIMONTES, Janaúba, Minas Gerais, Brazil, (15°49'44'' S and 43°16'09'' W at an altitude of 544 m a.s.l.). Sixty fruits were used in the experiment. Twelve fruits (six per treatment, with and without cassava starch coating) were evaluated for each storage period (0, 3, 6, 9, and 12 d). These fruits were harvested at the breaker maturation stage and transported to the UNIMONTES Post-harvest Laboratory where they were graded for uniformity in size, shape, and green color. Fruits free from defects and blemishes were selected. The fruits had an average longitudinal length of 4.5 cm and equatorial diameter of 3.8 cm. The fruits were then washed, immersed in a sodium hypochlorite solution (100 mg L^{-1} of active chlorine), and dried at room temperature (26.7°C).

Application of cassava starch-based coating

The coating was accomplished by immersing the fruits for 3 min in the biofilm of cassava starch (BT). The solution was made with distilled water + 1% cassava starch. A control treatment (CT) was performed by immersing the umbu fruits in distilled water for 3 min. After immersion, the fruits were placed in a nylon mesh container for draining the biofilm and excess water and drying. The fruits were then stored at room temperature (26.7 ± 3°C) and 75 ± 5% humidity and evaluated at 0, 3, 6, 9, and 12 d after harvest.

Assessment of postharvest life

Weight loss

Fresh weight loss (WL) was measured using a digital scale with a precision of 0.01 g during the storage periods of 0, 3, 6, 9 and 12 d. The results were expressed as a percentage.

Firmness

Pulp firmness (FIR) was determined using a Brookfield CT3 digital texture analyzer (Brookfield Engineering, Middleboro, MA, USA) equipped with a 7.8 mm diameter stainless steel compression plate. Analyses were carried out in the equatorial region of each fruit. The results were expressed in Newton (N).

pH and total titratable acidity

A solution made from the umbu pulp (5 g) and distilled water (25 ml) was well-mixed. The solution was adjusted to a final volume of 50 ml using distilled water and filtered with Whatman 42 filter paper. The pH of the filtrate was then measured with a digital pH meter (model DM20, Digimed, São Paulo, Brazil).

The titratable acidity (TA, %) was determined by titrating the filtrate (10 ml) against 0.1 N NaOH using 1-2 drops of phenolphthalein as an indicator. The results were expressed as g of citric acid per 100 g of pulp (AOAC, 1997).

Total soluble solids and TSS/AT ratio

Total soluble solids (TSS) were quantified in an N1 bench refractometer (Atago, Tokyo, Japan) with an operating range of 0 to 32% and an accuracy of 0.2%. A few drops of umbu juice were used for the analysis. The results were expressed in °Brix. The TSS/TA ratio was calculated as the relationship between the total soluble solids and titratable acidity.

Color indices

Changes in the control and coated umbu fruit color indexes stored at 26.7°C ± 3°C were determined at 0, 3, 6, 9, and 12 d after harvest. The values of luminosity (L^*) to red or green tint (a^*) and yellow or blue tint (b^*) were measured in the ColorFlex 45/0 colorimeter, stdzMode: 45/0, HunterLab universal software system (Hunterlab, Reston, VA, USA). The chromaticity index or chroma (C^*) and hue angle or hue (H^*) were determined as previously described by Muley and Singhal (2020).

Data analysis

Data were subjected to an analysis of variance. The means for the qualitative data (with and without the biofilm) were compared using the F-test ($P<0.05$). Quantitative data (storage days) were subjected to a regression analysis using the Sisvar statistical software (Ferreira, 2014). The figures were made using the SigmaPlot version 11.0 (Systat Software, San Jose, CA, USA).

Results and discussion

The coating of fruits with cassava starch-based biofilm improves WL, pH, and TSS. TA, TSS/TA ratio, and L^* were not influenced by the use or absence of the cassava starch biofilm coating. All evaluated parameters were affected by the storage period. There was interaction for WL, FIR, H^*, and C^* (Tab. 1).

WL increased in both treatments, reaching, on average, 16% at 12 d of storage (Fig. 1). When using cassava starch biofilm, WL was reduced by 38.8% (Tab. 2). The constant loss of weight showed that even with the starch-based coating, the fruits maintained respiration, but it was slower. Biofilms have the function of contributing to the preservation of texture and nutritional value, reducing surface gas exchange and the excessive loss or gain of water (Assis & Britto, 2014).

TABLE 2. Average fresh weight loss (WL, %), pH, and total soluble solids (TSS, °Brix) of umbu fruits with and without cassava starch coating during storage.

Treatment	WL	pH	TSS
Without coating	12.1b	2.77 b	10.03 a
With cassava starch coating (1%)	7.4 a	2.85 a	9.67 b

Means followed by different letters in the columns are different by the F-test at 5% of probability.

TABLE 1. Summary of analysis of variance of fresh weight loss (WL, %), firmness (FIR, N), pH, total soluble solids (TSS, °Brix), total titratable acidity (TA, g of citric acid per 100 g of pulp), luminosity (L^*), hue angle (H^*) and chromaticity (C^*) of umbu fruits with and without cassava starch biofilm (Biof) during storage.

Source of variation	DF	Mean square								
		WL	FIR	pH	TSS	TA	TSS/TA	L^*	H^*	C^*
Biofilm	1	195.01**	598.38**	0.055**	1.26*	0.02^{ns}	1.06^{ns}	5.57^{ns}	1728.96**	243.39**
Day	4	349.36**	352.28**	0.043**	7.64**	0.008*	8.73**	11.69*	1363.44**	62.29**
Biof*day	4	18.35**	54.09**	0.005^{ns}	0.55^{ns}	0.004^{ns}	3.11^{ns}	7.71^{ns}	239.68**	33.51**
Error	30	1.46	2.76	0.002	0.23	0.005	1.96	3.46	8.35	0.89
CV (%)		9.72	10.98	1.72	4.82	8.5	11.38	3.18	2.91	2.65
Average		12.4	15.13	2.81	9.85	0.81	12.31	58.51	99.18	35.57

* and ** - significant values at 5% and 1% and ns - not significant values according to the F-test at 5% of probability. CV - coefficient of variation; DF - degrees of freedom.

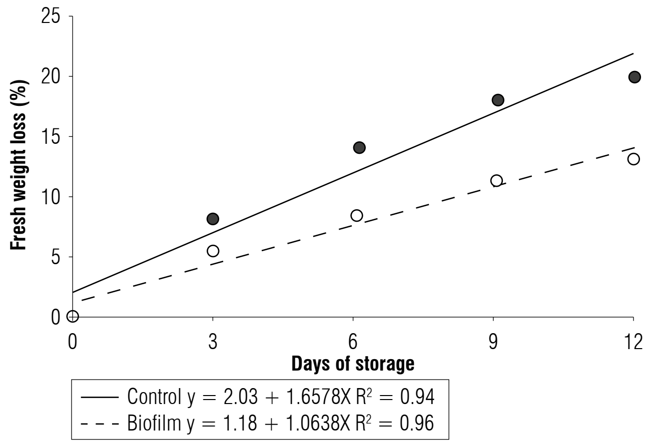

FIGURE 1. Fresh weight loss (%) of umbu fruits over storage time with and without the use of cassava starch biofilm.

FIR decreased with storage days and became stable after 9 d (Fig. 2). The FIR of the fruits treated with 1% cassava starch biofilm was 67.66% greater than the untreated fruits. Fruits not treated with biofilm obtained FIR values of approximately 5 N on the 9th d of storage that show a high degree of maturation and the beginning of deterioration. In contrast, fruits with biofilm showed FIR of approximately 15 N, with a good appearance and remaining suitable for consumption until the last day of evaluation.

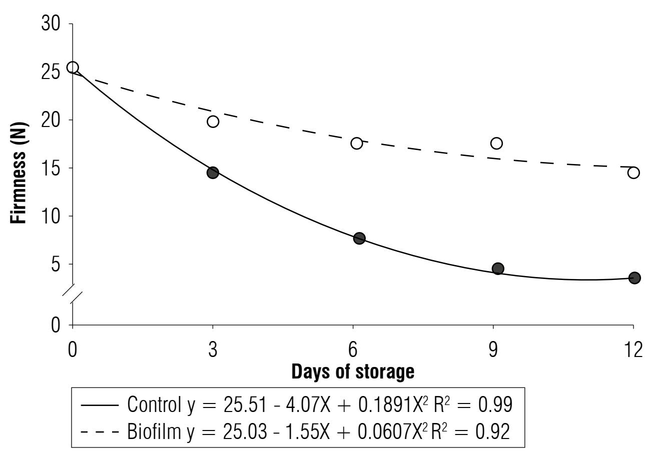

FIGURE 2. Firmness (N) of umbu fruits over storage time with and without the use of cassava starch biofilm.

The greater FIR for the fruits treated with 1% cassava starch biofilm and its reduction over the storage days is attributed to the fruit ripening process over time. The softening of the fruit during maturation is due to the activity of hydrolyses on the fruit cell wall polysaccharides leading to a modification of the cell wall composition (Yao *et al*., 2014). Thus, the application of a biofilm based on cassava starch favors the transport of fruits, avoiding mechanical damage and, consequently, increasing their shelf life.

The fruit pH increased 4.33% up to the last day of storage; the increase was more marked after the 6th d (Fig. 3). Fruits treated with cassava starch had a pH 3% lower than those not treated (Tab. 2).

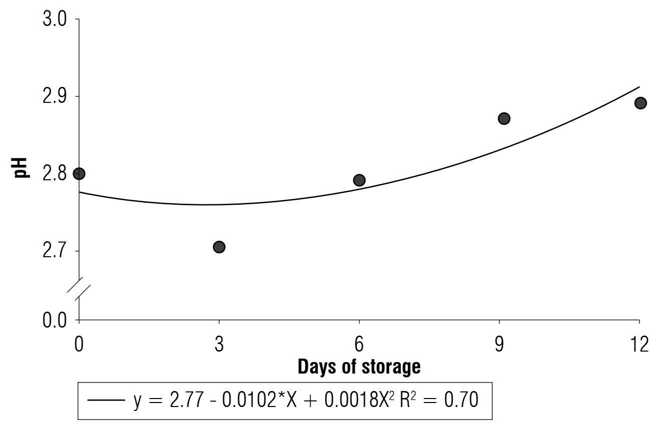

FIGURE 3. pH of umbu fruits over storage time.

The increase in fruit pH with storage is due to organic acid consumption due to breathing and converting organic acids into sugars (Chitarra & Chitarra, 2005). The higher pH in fruits not treated with 1% starch shows a higher consumption of organic acids due to faster ripening.

The TSS had a significant increase of 25.5%, from 8.4 to 10.57 °Brix during storage, decreasing after the 10th d (Fig. 4A). On the other hand, the TSS showed values 4% lower when the biofilm was used. The lower concentration of TSS is due to the delay in fruit ripening provided by the biofilm that reduces the synthesis of sugars.

The TA of fruits treated with starch was similar to that of untreated fruits, differing only according to the storage time. The highest TA occurred at 12 d of storage with increasing values from the 5th d of evaluation (Fig. 4B). Similar results have also been reported in umbu in a modified environment (Silva *et al*., 2019). The fluctuation in the TA levels with the fruit storage time can be attributed to the biochemical processes of respiratory metabolism when synthesizing and consuming organic acids (Chitarra & Chitarra, 2005).

The TSS/TA ratio increased by 36.7% over fruit storage, regardless of the use or absence of biofilm and obtained a maximum value on the 7th day of evaluation (Fig. 5). The TSS/TA ratio is related to the sugar and acid balance of fruits and is a critical flavor indicator (Freitas *et al*., 2017). The determination of this relationship is essential, as some fruits containing low levels of acids and soluble solids have high TSS/TA ratios that can lead to misinterpretations of organoleptic characteristics.

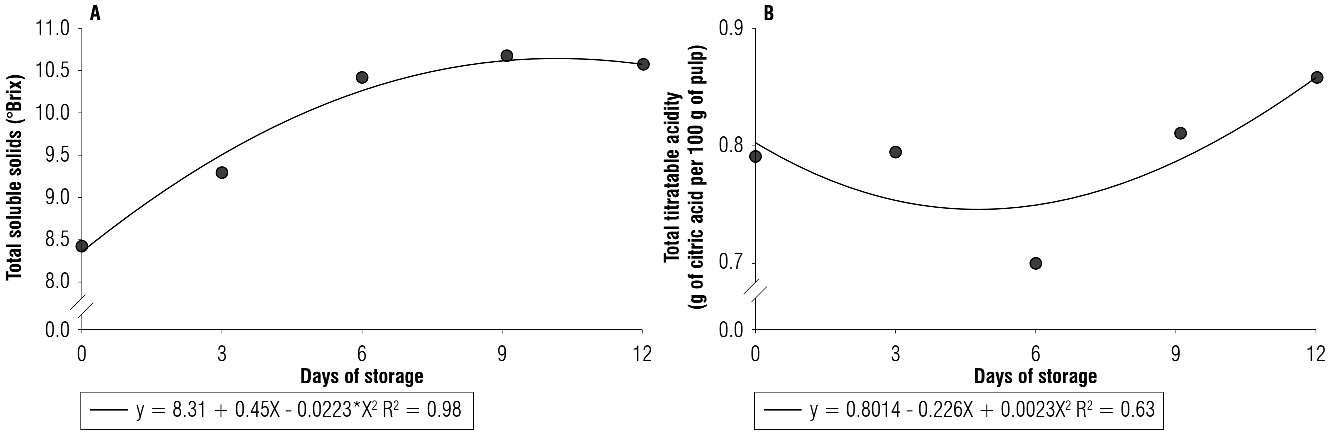

FIGURE 4. A) Total soluble solids (TSS) and B) total titratable acidity (TA) of umbu fruits over storage time.

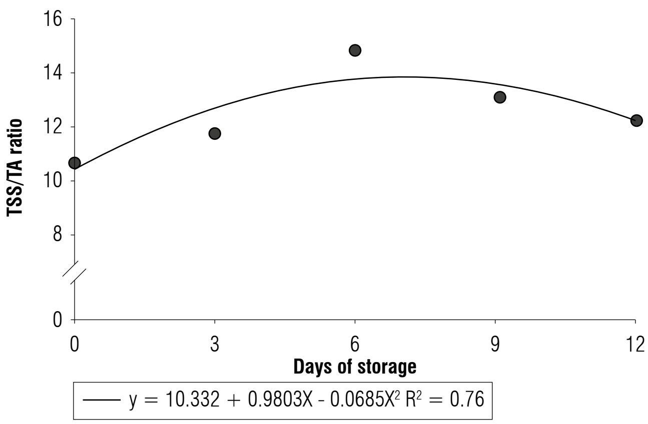

FIGURE 5. Total soluble solid/titratable acidity (TSS/TA) ratio of umbu fruits over storage time.

Fruit peel color is related to attractiveness by the consumer. The use of biofilm and the period of storage interfered in the skin color of the fruits. L^* decreased significantly until the 9th d of storage, after which it stabilized (Fig. 6A). The use or absence of starch did not interfere with the fruits L^* that maintained an average of 58.5 (Tab. 2).

H^* decreased 1.62% and 3.75% for each day of storage with and without the biofilm, respectively (Fig. 6B). For both treatments, fruits remained green until the 3rd d of storage, showing that the fruit's ripening process was delayed. From that period, lower H^* values without the biofilm were registered, indicating the change from green to yellow, together with the loss of firmness and the increase of soluble solids that was evidence of fruit ripening. However, the use of starch provided a H^* 45.8% greater in relation to untreated fruits and indicated the cassava starch-based biofilm action delayed the degradation of chlorophyll and fruit ripening. In fruits not treated with starch, chlorophyll degradation was more intense. The change in the color of the skin was characterized by a decrease in the chlorophyll content that caused the appearance of a yellow color. This color change is due to the enzymatic action on chlorophyll's structure, enabling the expression of carotenoids (Forato *et al.*, 2015).

C^* expresses the color intensity, *i.e.*, the saturation in terms of pigments of this color. The C^* in the external periderm of fruits without biofilm increased gradually up to 12 d of storage, reaching a value 28.7% higher than the treatment with biofilm (Fig. 6C). This showed that uncoated fruits had a more intense color; these fruits also showed lower H^*, exhibiting a yellow color at the end of storage. Fruits with biofilm showed lower levels of C^*, indicating lower color intensity and higher levels of H^*. These fruits showed lighter skin color, confirming the biofilm action.

In fruits and vegetables, color is the result of pigments such as chlorophyll, carotenoids in chloroplasts, chromoplasts, and phenolic pigments (proanthocyanidins, anthocyanins, and flavonols) in the vacuole that are degraded during ripening. The pigment color expression is also influenced by physical factors, such as the presence of cuticular waxes, trichomes, shapes, and orientation of cells in the epidermis and subepidermis (Lancaster *et al.*, 1997). These changes are associated with the ripening of fruits; climacteric fruits generally lose their green color during ripening. Lawson *et al.* (2019) evaluate the effect of edible coatings based on starch extracted from tropical fruits applied to mangoes and the results of that study mention that the color of the cuticle in mango shows high values for L^*, less green (increased values of a^*), and higher values of b^* as the ripening progresses. These fluctuations coincide with those reported in this research. L^* values are associated with the sugar content in the fruits. In contrast,

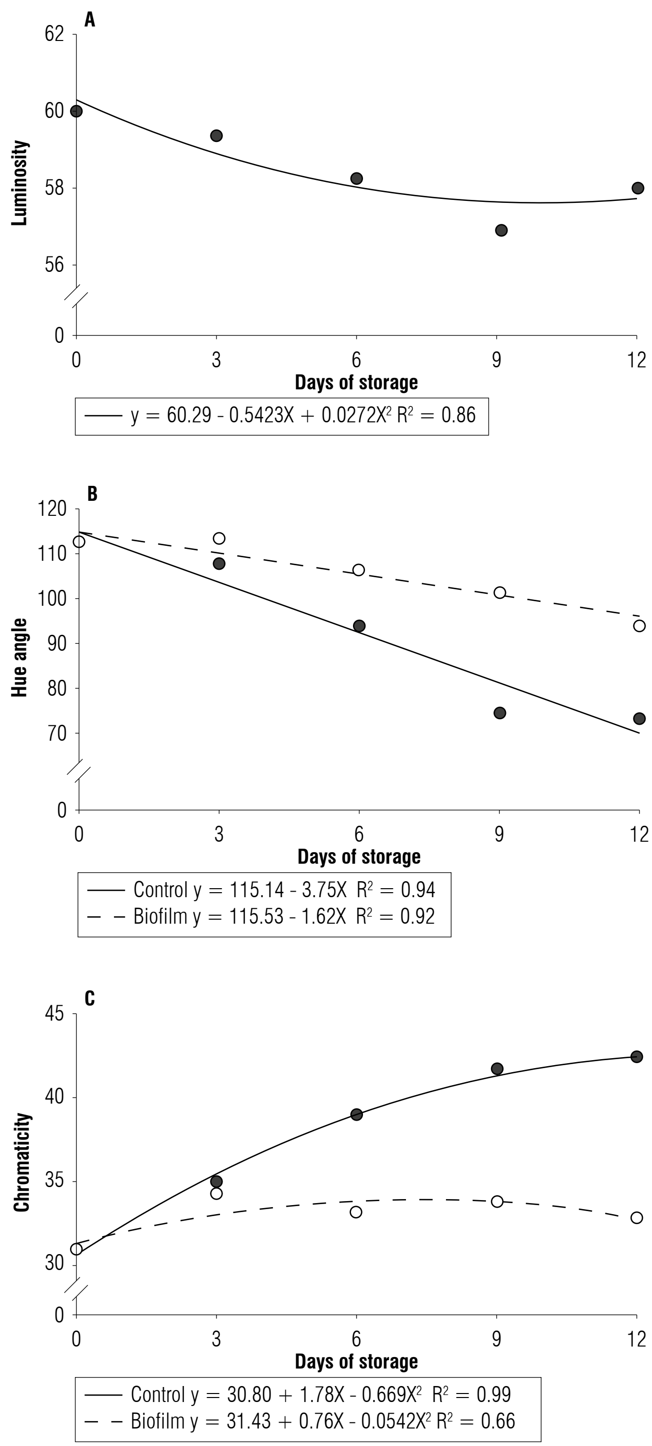

FIGURE 6. A) Luminosity, B) hue angle, and C) chromaticity of umbu fruits over storage time with and without the use of biofilm.

the values of *a** and *b** are associated with the content of chlorophyll and carotenoids, gradually increasing the total sugar, chlorophyll, and carotenoids as the fruit ripens (Corzo & Álvarez, 2014).

Conclusion

The use of cassava starch-based coating delays ripening and increases fruit postharvest life by up to 9 d at a temperature of 26.7°C ± 3°C, favoring their organoleptic characteristics. Therefore, this biofilm proved to be efficient for extending postharvest life of fruits.

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

EFS conducted the experiment, collected the data, performed the statistical analyses, and wrote this manuscript. EAP and EDP made critical revisions and contributed to the writing of this manuscript. DSM, ABD, and JCF contributed with the collected data and execution of experiment. HPP revised the manuscript.

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SCIENTIFIC NOTE

Effect of fertilization on yield and phenology of the halophyte *Sarcocornia neei* as an emerging crop

Efecto de la fertilización en el rendimiento y la fenología de la halófita *Sarcocornia neei* como cultivo emergente

Mariana Muñoz-Araya[1], Máximo Alonso[2], and Jennyfer Flórez-Méndez[1*]

ABSTRACT

Sarcocornia is a vegetable crop that can be irrigated with highly saline water. The objective of this research was to evaluate the yield and study the phenology of the halophyte *Sarcocornia neei* as an emerging crop. Trials were established in a tunnel-type greenhouse and the open field, both including the same fertilizer treatments with two doses of N (40 and 80 g N per plant). The plants were harvested three times by collecting all vegetative parts located 5 cm above the soil. Four plants per replicate were observed once a week to determine the phenological stages through the seasons in Chile (fall, spring, and summer). A two-way analysis of variance was performed for the experimental data that revealed differences between treatments and trials. The yields were significantly different between the open field and greenhouse trials in all seasons. The summer and spring harvests obtained the highest yields in treatments with 40 g N per plant. The highest yield was obtained in the greenhouse trial with 40 g N per plant at the summer harvest (45.33 g dry matter per plant). The open field trial showed no differences between the fertilizer treatments in all seasons. According to the plant phenology, the greenhouse cultivation was found to be advantageous in causing the plants to avoid the winter dormant period. The yield of *S. neei* under greenhouse conditions is the basis for further research.

Key words: greenhouse, irrigation, seawater, salinity.

RESUMEN

Sarcocornia es un cultivo vegetal que puede ser regado con agua altamente salina. El objetivo de la presente investigación fue la evaluación del rendimiento y el estudio de la fenología de la halófita *Sarcocornia neei* como un cultivo potencial. Se establecieron ensayos dentro de un invernadero y en campo abierto; ambos ensayos incluyeron los mismos tratamientos de fertilización con dos dosis de N (40 y 80 g N por planta). Las plantas se cosecharon tres veces recolectando todas las partes vegetativas ubicadas 5 cm por encima del suelo. Se evaluaron cuatro plantas por réplica una vez por semana para determinar las etapas fenológicas durante las estaciones en Chile (otoño, primavera y verano). Se realizó un análisis de varianza bifactorial para los datos que reveló diferencias entre los tratamientos y entre ensayos. Se observaron diferencias significativas en el rendimiento entre los ensayos a campo abierto y de invernadero en todas las estaciones. Las cosechas de verano y primavera obtuvieron el mayor rendimiento para los tratamientos con 40 g de N por planta. El mayor rendimiento se obtuvo en el ensayo de invernadero con 40 g de N por planta en la cosecha realizada en verano (45.33 g de masa seca por planta). El ensayo de campo abierto no mostró diferencias entre los tratamientos de fertilización en todas las estaciones. Según la fenología de la especie, el cultivo bajo invernadero presentó ventajas, observándose que las plantas evitan el período de receso invernal. El rendimiento de *S. neei* bajo condiciones de invernadero es la base para futuras investigaciones.

Palabras clave: invernadero, riego, agua de mar, salinidad.

Introduction

Climate change affects agriculture worldwide and Chile is also compromised. This context demands a higher water use efficiency through improved technologies and more efficient water management in the field (McKersie, 2015; Boisier *et al.*, 2018). There is a growing interest in irrigation with saline water, which offers a broad possibility to start intensive and commercial cultivation of various halophyte species (Basílio *et al.*, 2018; Mota *et al.*, 2021). Glassworts (genera *Salicornia* and *Sarcocornia* of the Amaranthaceae family) are halophytes widely distributed and studied as edible and non-edible crops that can be used for rehabilitation of wetlands, glass manufacture, and ruminant and human consumption. The edible species have different characteristics such as a low quantity of external inputs for their cultivation, high nutritional value, and pharmaceutical benefits, turning these crops into a sustainable food source in the context of climate change (Patel, 2016; Loconsole *et al.*, 2019). Moreover, other species of the

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Sarcocornia genus have been recently described as a good source of antioxidants (Antunes *et al.,* 2021).

Chilean coastal marshes are able to develop sustainable halophyte agriculture with low financial involvement. This provides opportunities for productive diversification and resilience to small-scale fisheries and families of coastal farmers within the integrated aquaculture systems (Buschmann *et al*., 1996). There are three species of wild glassworts in Chile, all belonging to the genus *Sarcocornia*. *Sarcocornia neei* (Lag.) M.A. Alonso & M.B. Crespo is the only species distributed along the coast. The other two species (*S. pulvinata* and *S. magellanica*) are adapted to extreme environments and could be found in the Atacama Desert and Patagonia, respectively (Alonso & Crespo, 2008). *S. neei* has the potential to be cultivated in many bays along the coastline of Chile.

S. neei is a small shrub with a height up to 80 cm, erect to decumbent stem, and wide morphological variations in its habitats (Alonso & Crespo, 2008). Its potential for human consumption has been barely studied. However, recent studies have been carried out to determine the seed germination requirements and nutritional value of the wild and cultivated plants as well as the plant's potential to serve as an indicator of heavy metal pollution in central Chile (Riquelme *et al*., 2016; Alonso *et al*., 2017; Meza *et al*., 2018). In southern Chile, the wild plant sprouts are collected in summer. Additionally, the locals let their cattle graze on the *S. neei* natural pastures in coastal marshes. These informal practices are evidence of both the potential of *S. neei* as an emerging crop and the need to develop agricultural practices for its use while avoiding the ecological depletion of these fragile ecosystems.

The cultivation of *S. neei* could be one of the first attempts to establish saline agriculture in Chile. Thus, this research aimed to study the yield and phenology of the halophyte *S. neei* as a potential novel crop in Chile. This study evaluated the effect of different fertilizer treatments on the species performance under controlled and natural conditions.

Materials and methods

Plant material and location

Whole plants were collected from the coastal marshes of Chamiza, Puerto Montt, Chile (41°29'18.9" S, 72°51'45.8" W). Trials were established in a nearby location (Lenca, 41°36'20.7" S, 72°41'20.6" W) characterized by a temperate rainy climate with coastal influence according to Köppen classification (Sarricolea *et al*., 2017). The average temperature is 10.6°C; the warmest month, January, has an average maximum temperature of 19.8°C and the coolest month, July, has an average minimum temperature of 3.5°C. The total average rainfall reaches up to 1,613 mm with 218 rainy days (World Weather Information Service, 2018).

Trial management

Two trials were established, the first one in a tunnel-type greenhouse (Fig. 1F) and the second one in the open field next to the greenhouse. The maximum temperature inside the greenhouse was 41.9ºC in January and the average air humidity was 90% in summer. Each trial consisted of nine platforms of 3.2 m long, 1.2 m wide, and 0.2 m high, with an individual area of 3.84 m^2 containing a mixture of 50% decomposed leaf litter with soil and 50% sand. Plants were collected directly from the marsh and the stems were cut 10 cm above the crown before planting. Within the platforms, the plants were arranged at a distance of 0.35 x 0.40 m from each other (16 plants per platform, Fig. 1E) to distinguish each plant at harvest. Both trials had the same fertilizer treatments: 40 g N per plant, 80 g N per plant, and a control without fertilization, with three replicates of 16 plants each. The fertilizer source was potassium nitrate (15 N - 0 P - 17 K), and 50% of the total dose was applied at plant establishment (November 2018) in each treatment. The second half of the N fertilizer was split into three applications, and each dose was applied two weeks after each harvest. All platforms received the same management and were irrigated with saline water (30 g L^{-1} NaCl) according to soil friability. The total water volume applied through the entire period was 3,046 L. Soil analysis at the end of the trial revealed a sandy soil texture, 88.4% sand, 5.4% clay, 6.2% silt, pH 6.5, and cation exchange capacity (CEC) of 45.65 cmol kg^{-1}.

Yield evaluation

All plants were cut in January 2018 to obtain uniform growth before the yield evaluation. This practice was not considered for the analysis but employed the same criterion used for the regular harvest. Thus, for yield evaluation, four plants from each platform were randomly selected; the same plants were harvested in three seasons: April (fall harvest), September (spring harvest), and February 2019 (summer harvest). Based on preliminary results, we determined the best timepoint to harvest when 50% of the shoots were larger than 10 cm. The plants were harvested by collecting the above-ground vegetative biomass, including all plant parts located 5 cm above the crown, where the roots originate. Results were expressed in grams of fresh matter per plant (g FM per plant). Then, all plant material was dried in a drying chamber (model F23, BINDER, Tuttlingen, Germany) at 70°C until constant weight to determine the water content (%) and dry matter weight per plant (g DM per plant).

Determination of phenological stages

Four plants per replicate were randomly chosen (excluding those selected for yield evaluation) and examined once a week to determine the phenological stages throughout the seasons; these were identified when 50% of the chosen plants in each trial had the same stage. Trained personnel determined the phenological stages by visual inspection, based on the available bibliography (Alonso & Crespo, 2008). Additionally, wild plants growing in Lenca coastal marsh were assessed once a week along a random 100 m transect to compare the phenological stages in both experimental trials with those recorded in wild plants. Winter dormancy period (w, plants with no vegetative growth), vegetative growth (v, plants with active growth but without flowers), flowering (f, plants at flowering), and fructification (x, plants with fruits) were registered.

Statistical analysis

A two-way analysis of variance (ANOVA) was performed for the experimental data, considering the environment (open field, greenhouse) and fertilizer treatments (0, 40, and 80 g N); the effects were considered significant with a P-value <0.05. The Tukey's test was used to compare the treatments for each analysis. A repeated measures analysis was performed to analyze the effect of the seasons. Statistical analyses were performed using the software R version 3.4.1.

Results and discussion

Yield evaluation

The yields were significantly different between the open field and greenhouse environments in all studied seasons. The highest yield was obtained in the greenhouse trial in summer, reaching 45.33 g DM per plant in the treatment with 40 g N per plant, which significantly differed from the 15.25 g DM per plant obtained in the same treatment in the open field (P<0.001). The treatment with 40 g N per plant yielded the highest biomass for both trials and at all harvests (Tab. 1). Table 1 shows the P-values of the analysis and the interaction between the environment, fertilization level, and harvest season. An experiment carried out in Brazil obtained 21.4 g FM per plant in an aquaculture system (Pinheiro *et al.*, 2020). Our results indicated that, in the 40 g N treatment, the fresh biomass reached 462.5 g per plant in summer. Therefore, the area near Lenca could be a suitable environment to establish a crop of halophytes under temperate conditions.

Greenhouse trial

The greenhouse environment showed no differences between treatments in the fall, with a mean of 12.80 g DM per plant (Tab. 1). The spring harvest obtained a significantly higher yield in the 40 g N treatment and, surprisingly, the yields from the plants fertilized with 80 g N were no different from those treated with 0 g N. Finally, the summer harvest in the 40 g N treatment showed the highest yield in the experiment, which was significantly different from the yields obtained in the 0 and 80 g N treatments (Tab. 1). The season effect was significant in this trial, with the highest yield registered in summer, followed by the fall and spring yields (P<0.001). The greenhouse-grown plants collected at the three harvests showed an average water content of 90.2% (Supplementary material 1). The treatment with 40 g N produced the highest yield at all harvests. This confirms a need for the agronomic management of *S. neei*. Also, the yields were lower in the 80 g N treatment than in the 40 g N treatment, probably because of phytotoxicity. Recently, ammonium regulation was found to be an important trait in *Salicornia europaea*, and its detoxification plays a role in the tolerance to high salinity in these halophytes (Ma *et al.*, 2020).

Open field trial

The open field trial showed no differences between the fertilizer treatments in all seasons, obtaining a mean of 3.71 g DM per plant. However, the season effect was significant, revealing the differences between the fall, spring, and summer harvests (Tab. 1). The plants from the open field trial had an average water content of 88.3% when all samples were pooled (Supplementary material 1).

Phenological stages

Figure 1 shows the four phenological stages observed in Lenca: winter dormant period (w), vegetative growth (v), flowering (f), and fructification (x).

Vegetative growth in the open field trial extended for six months, from July to December. Then, flowering occurred during January and February, and fructification was observed in March and April. The winter dormancy period was reduced in the plants from the greenhouse trial compared to wild plants, and it only lasted two months from May to June. The greenhouse conditions forced plants to avoid winter dormancy, flowering, and fructification periods during the season 2017-2018, and the vegetative growth was maintained throughout the year. According to previous

TABLE 1. Mean yield of the *Sarcocornia neii* shoots (g dry matter per plant ± standard error) in each trial, fertilization treatment, and trial*fertilization at the fall, spring, and summer harvests.

Effect	Fall harvest	Spring harvest	Summer harvest
		***P*-value**	
Trial			
Open field	1.51 ± 0.94 b	1.82 ± 0.40 b	7.81 ± 2.98 b
Greenhouse	12.80 ± 1.02 a	5.53 ± 0.43 a	24.35 ± 3.22 a
P-value	<0.0001*	<0.0001*	0.0005*
Fertilization (g N per plant)			
0	5.27 ± 1.15	2.31 ± 0.49 b	3.93 ± 3.65 c
40	8.83 ± 1.15	5.01 ± 0.49 a	30.29 ± 3.65 a
80	7.46 ± 1.29	3.35 ± 0.55 b	11.96 ± 4.09 b
P-value	0.0966	0.0012*	<0.0001*
Trial*fertilization			
Open field			
0 N per plant	1.47 ± 1.63 c	1.71 ± 0.70 c	5.17 ± 1.60 b
40 N per plant	1.45 ± 1.63 c	2.25 ± 0.70 c	15.25 ± 5.17 b
80 N per plant	1.61 ± 1.63 c	1.51 ± 0.70 c	6.59 ± 5.16 b
Greenhouse			
0 N per plant	9.07 ± 1.63 b	2.91 ± 0.70 bc	6.25 ± 5.17 b
40 N per plant	16.21 ± 1.63 a	7.76 ± 0.70 a	45.33 ± 5.17 a
80 N per plant	13.31 ± 2.00 ab	6.10 ± 0.85 ab	20.01 ± 6.3 b
P-value	0.0967	0.0078*	0.0518*

Different letters indicate significant differences between treatments or trials according to the Tukey's test ($P<0.05$). Asterisks indicate a *P*-value lower than 0.05.

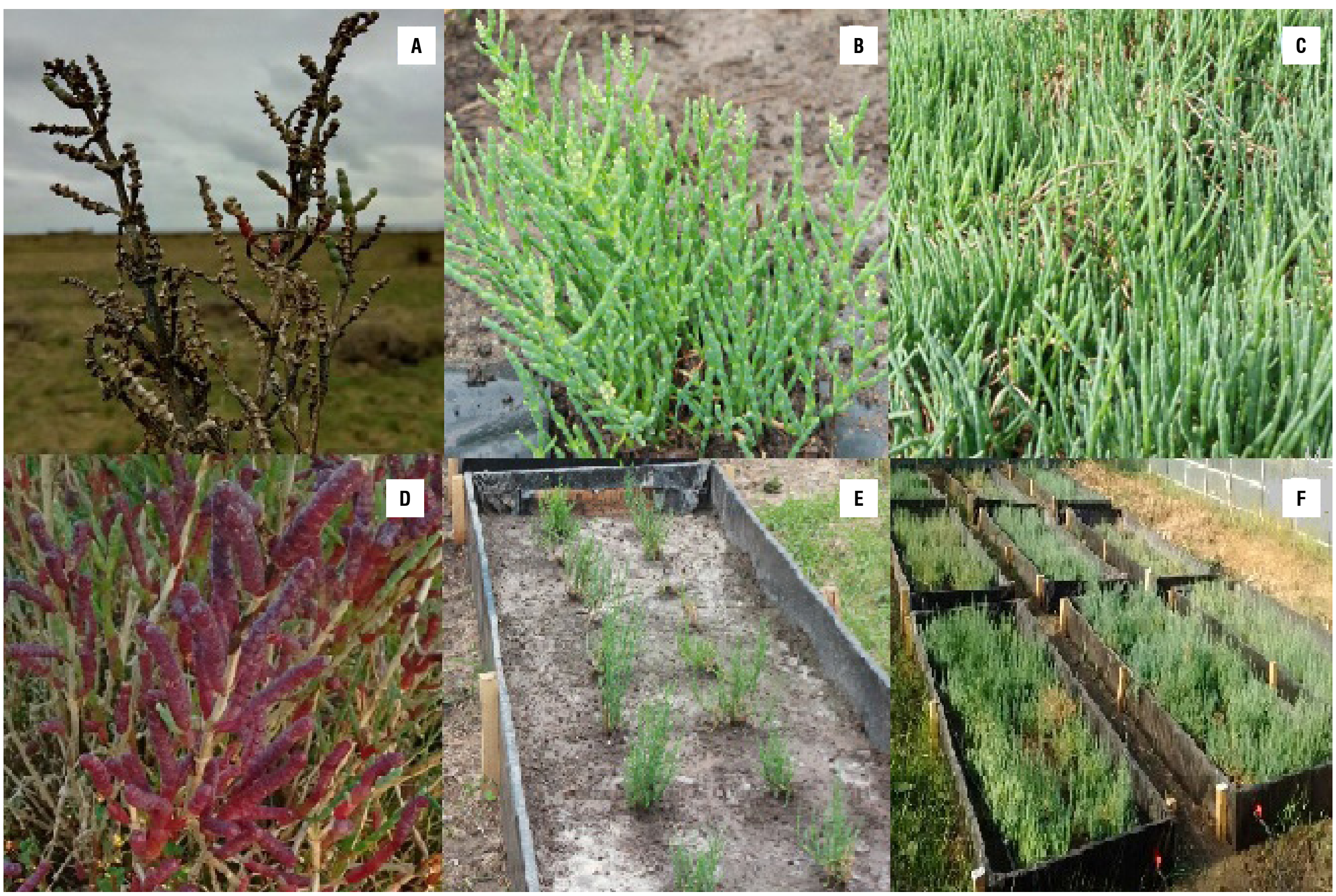

FIGURE 1. Phenological stages and trial management of *Sarcocornia neii* in the southern region of Chile: A) winter dormant period, B) vegetative growth, C) flowering, D) fructification, E) planting platforms, and F) greenhouse trial.

studies, phenological stages are conditioned by day length and repetitive harvest regime (Ventura *et al*., 2011). Thus, our results demonstrated better crop performance in the greenhouse trial since commercial standards for *S. neei* depend on its vegetative growth and a requirement of year-round cultivation.

Conclusion

The results confirm the potential of the south of Chile to diversify its commercial matrix, through the inclusion of halophyte cultivation in greenhouses in the coastal areas. Here, we introduced the general framework to establish a successful crop, including the fertilization management and better environment. However, further research is needed to fully understand the challenges of this new crop, especially related to the inputs required through the different stages of crop development and the sustainability of the production. Overall, halophytes could become new crops to support the development of small farmers and fishermen along the coast of Chile.

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

MMA and MA designed the experiments and performed the statistical analyses. MMA wrote the initial draft. MMA, MA, and JFM wrote the final version of the article.

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SUPPLEMENTARY MATERIAL 1. Mean water content of the *Sarcocornia neii* shoots (% fresh matter (g) ± standard error) in each trial, fertilizer treatment, and trial*fertilization at the fall, spring, and summer harvests.

Effect	**Fall harvest**	**Spring harvest**	**Summer harvest**
		***P*-value**	
Trial			
Open field	88.3 ± 0.973	75.2 ± 3.34	87.2 ± 0.76
Greenhouse	89.6 ± 0.966	88.1 ± 3.65	90.7 ± 0.83
P-value	0.4316	0.1244	0.0939
Fertilization (g N per plant)			
0	88.8 ± 0.732	74.5 ± 5.14	89.8 ± 1.29
40	89.3 ± 0.732	85.6 ± 5.14	88.7 ± 1.29
80	88.7 ± 0.756	85.8 ± 5.53	88.1 ± 1.42
P-value	0.5321	0.1022	0.5847
Trial*fertilization			
Open field			
0 N per plant	89.7 ± 1.06	63.3 ± 4.79	87.3 ± 1.32
40 N per plant	89.9 ± 1.06	80.1 ± 4.79	86.7 ± 1.32
80 N per plant	89.2 ± 1.06	82.2 ± 4.79	87.7 ± 1.32
Greenhouse			
0 N per plant	87.9 ± 1.06	85.7 ± 4.79	92.2 ± 1.32
40 N per plant	88.6 ± 1.06	91.0 ± 4.79	90.7 ± 1.32
80 N per plant	88.3 ± 1.06	87.3 ± 6.60	88.2 ± 1.82
P-value	0.7066	0.2923	0.3547

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The introduction must include the delimitation and current status of the problem, the theoretical or conceptual basis of the research, the literature review on the topic, and the objectives and justification of the research. Common names must be accompanied by the corresponding scientific ones, plus the abbreviation of the species author surname when mentioned for the first time.

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Besides a clear, precise and sequential description of the materials used for the research (plant or animal materials, plus agricultural or laboratory tools), this section illustrates the procedures and protocols followed, and the experimental design chosen for the statistical analysis of the data.

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A short conclusion section is useful for a long or complex discussion. It should provide readers with a brief summary of the main achievements from the results of the study. It can also contain final remarks and a brief description of future complementary studies that should be addressed.

Acknowledgments

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Agronomía Colombiana has adopted the American Psychological Association (APA) standards (https://apastyle.apa.org/about-apa-style) to elaborate the final list of references cited in the text ("Literature cited" section). This standard will be required for new manuscripts received from March 1st, 2020 onwards.

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Example: García-Arias, F., Sánchez-Betancourt, E., & Núñez, V. (2018). Fertility recovery of anther-derived haploid cape gooseberry (*Physalis peruviana* L.) plants. *Agronomía Colombiana*, *36*(3), 201–209. https://doi.org/10.15446/agron.colomb.v36n3.73108

Published dissertation or thesis references

Example: Franco, C. V. (2012). *Efecto de la colchicina sobre el número cromosómico, número de cloroplastos y características morfológicas del fruto en ecotipos de uchuva (*Physalis peruviana *L.) Colombia, Kenia y Perú* [Undergraduate thesis, Universidad Francisco de Paula Santander]. UFPS Library. http://alejandria.ufps.edu.co/descargas/tesis/1610259.pdf

Whole book

Example: Suescún, L., Sánchez, E., Gómez, M., García-Arias, F. L., & Núñez Zarantes, V. M. (2011). *Producción de plantas genéticamente puras de uchuva*. Editorial Kimpres Ltda.

Edited book chapter

Example: Ligarreto, G., Lobo, M., & Correa, A. (2005). Recursos genéticos del género *Physalis* en Colombia. In G. Fischer, D. Miranda, W. Piedrahita, & J. Romero. (Eds.), *Avances en cultivo, poscosecha y exportación de la uchuva* Physalis peruviana *L. en Colombia* (pp. 329–338). Universidad Nacional de Colombia.

For other types of references such as technical reports, conference presentations or proceedings, magazine articles or preprints see https://apastyle.apa.org/style-grammar-guidelines/references/examples. Archival documents, letters, collections and unpublished documents can be referenced using some standards users can find here: https://apastyle.apa.org/style-grammar-guidelines/references/archival. In this link (http://shorturl.at/xGIPZ) authors can find a summary of the APA style for references created by Mendeley.

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