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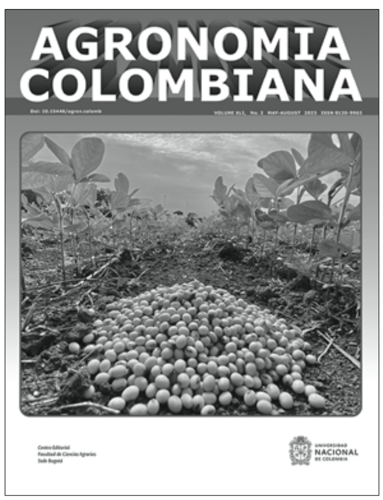

Our cover:

Effect of genotype-environment interaction on soybean (*Glycine max* (L.) Merrill) germination, vigor index, and seed yield

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Efecto de la interacción genotipo por ambiente sobre la germinación, índice de vigor y rendimiento de la semilla de soya (*Glycine max* (L.) Merrill)

Rubén Alfredo Valencia-Ramírez[1] and Yuli Stephani Tibocha-Ardila[1*]

ABSTRACT

The adverse edaphoclimatic conditions in the Orinoquía region affect the soybean crop cycle, deteriorating seed quality. The aim of this study was to investigate the effect of the genotype (G), the environment (E), and their interaction (GxE) on the yield and seed quality of six soybean varieties (Corpoica Superior 6, Orinoquía 3, Soyica P34, Corpoica Taluma 5, and Agrosavia Primavera 11 of Colombian origin and Barreira of Brazilian origin) in five environments of the Orinoquía Colombia region, two in the second half of 2020 (LIBB: La Libertad and TALB: Taluma) and three in the first half of 2021 (LIBA: La Libertad, TALA: Taluma, and LEONAS), to determine their adaptation domain. Highly significant differences (*P*<0.01) were observed between G, E, and GxE for germination (GER) and seed yield (SY). A similar situation was shown by the vigor index (VI), although without differences between environments. The GGE biplot for GER, and SY separated environments by year halves. Taluma during the first half was the most discriminating environment for the response variables, useful for genetic breeding programs with a seed quality approach. Only in La Libertad during the second half was GER above 80%. The most stable variety per environment was Soyica P34 in two response variables, and the best with specific adaptation were Corpoica Superior 6 and Orinoquía 3. These last two reached higher average values in GER (69.6%; 63.1%), VI (13.3; 13.4), and SY (1473 kg ha^{-1}; 1404 kg ha^{-1}).

Key words: environmental adaptation, seed quality, stability, water stress, oilseed crop, viability.

RESUMEN

Las condiciones edafoclimáticas adversas en la Orinoquía afectan el ciclo de cultivo de soya deteriorando la calidad de las semillas. El objetivo de este estudio fue investigar el efecto del genotipo-(G), el ambiente-(A) y su interacción-(GxA) sobre el rendimiento y la calidad de las semillas de seis variedades de soya (Corpoica Superior 6, Orinoquía 3, Soyica P34, Corpoica Taluma 5 y Agrosavia Primavera 11 de origen colombiano, y Barreira de origen brasilero) en cinco ambientes de la Orinoquía, Colombia, dos en el segundo semestre de 2020 (LIBB: La Libertad y TALB: Taluma) y tres en el primer semestre de 2021 (LIBA: La Libertad, TALA: Taluma y LEONAS), para determinar el dominio de adaptación. Se observaron diferencias altamente significativas (*P*<0.01) entre G, A y GxA para germinación (GER) y rendimiento de semillas (RS). Situación similar mostró el índice de vigor (VI), aunque sin diferencias entre ambientes. El biplot-GGA para GER y RS separó ambientes por semestre. Taluma, durante el primer semestre, fue el ambiente más discriminante para las variables respuesta, útil para programas de mejoramiento genético enfocados en calidad de semillas. Solamente en La Libertad durante el segundo semestre, GER estuvo por encima del 80%. La variedad más estable por ambiente fue Soyica P34 en dos variables respuesta, y las mejores con adaptación específica fueron Corpoica Superior 6 y Orinoquía 3. Estas dos últimas alcanzaron valores promedios más altos de GER (69.6%; 63.1%), VI (13.3; 13.4) y RS (1473 kg ha^{-1}; 1404 kg ha^{-1}).

Palabras clave: adaptación ambiental, calidad de semillas, estabilidad, estrés hídrico, cultivo oleaginoso, viabilidad.

Introduction

Food security and sovereignty in Colombia face numerous challenges due to the continuous increase in the population, climate change, international conflicts, pandemics, and the high dependence on imported food and supplies. Therefore, cost-efficient, high-quality protein that meets the needs of the human and animal populations should be pursued. Due to its high protein, unsaturated fatty acids, and carbohydrate content, soybean constitutes an excellent nutritional source for human populations and animal feed. It is also an important alternative crop for sustainable rotation due to its nitrogen fixation ability (Ritchie & Roser, 2021).

The agro-climatic conditions to produce soybean seed in Orinoquía are diverse and contrasting. In the first half from April to August, the climatic conditions include high

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rainfall that affects ripening and leads to wet grain harvest, causing seed deterioration. Seventy percent of this sowing area shows these conditions, with excesses during the entire crop cycle, particularly in the *altillanura* subregion, where improved soils facilitate surface drainage. On the contrary, in the second half from August to December, 20% is planted in the *altillanura* subregion, where alternating deficit/excess water periods occur; meanwhile, in the foothills, the remaining 10% of the total area is planted exclusively in this half. The climatic condition is highly relevant when producing seeds, particularly water excess/deficit and diseases (González *et al*., 2008). For this reason, it is essential to develop adapted varieties and their recommendation domains to produce high-quality seeds suitable to the conditions of the Orinoquía. The knowledge of the genetic variations allows classifying contrasting genotypes regarding adaptation, earliness, production, and seed quality to design effective genetic breeding strategies.

Viability and vigor are the most critical genetically controlled seed quality traits, with significant repercussions on production; their variability is due to gene pool differences. The physiological and biochemical mechanisms by which this variability is expressed are not yet fully understood, but they are significantly influenced by the genotype (G), the environment (E), management practices, and their interactions (Bellaloui *et al*., 2011). Interactions are responsible for the differential response of genotypes to environmental diversity (Allard & Bradshaw, 1964).

Biplot graphs are useful in analyzing the GxE interaction, classifying the main effects in an easy-to-interpret way, estimating magnitude and direction and allowing the visualization of the best germplasm combination per environment and a more efficient selection (León Castillo *et al*., 2005). These graphs allow investigating the response pattern of varieties in different environments. Furthermore, they increase the information available than the more standard regression methods and principal component analysis (PCA) without needing additional calculations (Kempton, 1984).

The site regression (SREG) model is frequently used for data analysis from multi-environment comparative yield trials, especially in those that are determinant in the influence of the genotype and their interaction. It includes G+GxE in the bilinear term, providing a graphical analysis of yield and stability behavior of genotypes called the GGE-biplot, identifying genotypes with the highest potential in each environment and group's genotypes and environments with similar response patterns (Ibáñez *et al*., 2006).

The adequate interpretation of the GxE interaction is critical in deciding which variety will be used and where the high-quality seed will be produced. It is not enough to have a variety with high germination power; it must also have yield potential and stability to obtain the greatest benefits in producing high-quality seeds. Vargas Hernández and Crossa (2000) describe the ease of interpreting the interaction from the SREG-GGE-biplot, where the genotypes with yields above the average are located on the right margin of the vertical reference line, and those on the left margin have lower-than-average yields. Likewise, the biplot allows visualizing the most productive environments.

Accordingly, this research aimed to evaluate the effect of the GxE interaction on germination, vigor index, and seed yield of six soybean varieties under the edaphoclimatic conditions in the Colombian Orinoquía region in order to facilitate decision-making on the adaptation domains and recommendations to produce high quality and quantity seeds.

Materials and methods

Study area and plant material

The research was carried out with six varieties, five of Colombian origin (Corpoica Superior 6 and Orinoquía 3 with days to maturity (DM) between 78 and 85 d, Soyica P34, Corpoica Taluma 5, Agrosavia Primavera 11 and the Brazilian variety Barreira with DM between 95 and 105 d. These were evaluated in five environments of the Colombian Orinoquía region, two in the second half of 2020 (LIBB: La Libertad, TALB: Taluma) planted in August, and three in the first half of 2021 (LIBA: La Libertad, TALA: Taluma, finca LEONAS) planted in April-May. These varieties show adaptation to the Colombian lower tropics (Valencia-Ramírez & Ligarreto-Moreno, 2012).

Experimental design

Six soybean varieties were planted in five environments in a complete randomized block design with four replicates; each experimental unit consisted of six rows separated by 45 cm, with plants 5 cm apart, with a useful/work plot in the four central rows. Chemical fertilization was adjusted according to the soil analysis, and the biological fertilization of nitrogen was carried out with *Bradyrhizobium japonicum* (commercial product Rhizobiol-Agrosavia at 1 L ha^{-1} according to the manufacturer recommendations).

Evaluation variables

The response variables to measure seed quality were germination (GER), vigor index (VI), and seed yield (SY), defined as follows:

- GER percentage (ISTA, 2016);
- Germination speed (number of seeds that emerge per day after sowing for 5 d);
- Viability, according to the tetrazolium test (FAO, 2019);
- Electrical conductivity, according to Marcos Filho (2015);
- Seed vigor index (VI): Composed of standardized values (mean 0, deviation 1) of seeds germinated with normal seedlings (GERe), germination speed at day 5 (V5De), viability (Ve), and electrical conductivity (CEe) according to Equation 1:

  $$VI = (GERe + V5De + Ve - CEe) \quad (1)$$

- Seed yield (SY) according to Equation 2. Seed weight per area unit (YIELD) corresponded to clean and classified seeds dried to 14% moisture, which is the recommended commercial moisture content (%).

  $$SY\ (kg\ ha^{-1}) = YIELD\ (kg\ ha^{-1}) \times GER/100 \quad (2)$$

Statistical analyses

A combined analysis of variance was performed for GER, VI, and SY using the General Linear Model procedure of the SAS statistical program. Normality and homogeneity assumptions of variances were analyzed by the Shapiro-Wilk (1965) and Bartlett tests, respectively, with this same statistical program. The GxE interaction was evaluated using the SREG-GGE-biplot methodology proposed by Crossa *et al.* (2002), Burgueño *et al.* (2002), and Yan and Kang (2003) to measure the specific adaptation and phenotypic stability (Eq. 3).

$$Y_{ge} = \mu + \beta e + \Sigma N\ \lambda n\ \gamma gn\ \delta en + \rho ge \quad (3)$$

where Y_{ge} – average performance of genotype g in environment e, μ – overall mean, β_e – deviations from environment means, N – number of Principal Components (PCs) retained in the model, λn – singular PC value, γgn – genotype vector values for each PC, δen – environment vector values for each PC, and ρge – residual.

The SAS program for the SREG-GGE fixed effects and Gollob's test (Suppl. Tab. 1), mentioned by Vargas Hernández and Crossa (2000) to determine the significance of each SREG term, were obtained from www.cimmyt.cgiar.org/biornetrics. When GxE was significant, the GGE biplot statistical tool facilitated interpreting the GxE interaction according to Yan *et al.* (2000).

Stability (*VE*) was calculated according to Purchase *et al.* (2000) to establish the phenotypic stability of each variety depending on the environment (Eq. 4).

$$VE = \sqrt{[(SS - PC1/SS - PC2) \times (PC1score)^2 + (PC2score)^2]} \quad (4)$$

where VE – GGE stability value, SS—PC1 and SS—PC2 are sum of squares of PC1 and PC2 with the highest contribution to the variance, and PC1score and PC2score are values of components 1 and 2 per genotype, respectively.

Results and discussion

The combined analysis of variance for GER, VI, and SY showed highly significant differences (P<0.01; R^2=0.87-0.92; CV=13.3-24.6%). Similarly, highly significant differences (P<0.01) were found for varieties, environments, and their interaction for germination and seed yield. Yan and Rajcan (2002) found similar results in Canada. However, no differences were observed between environments for VI. The statistical significance of the GxE interaction for the three variables implies that the average response of these cannot identify superior varieties since the phenotypic classification varies significantly across environments (Valencia & Ligarreto, 2010). When partitioning the variance components, the highest contribution in GER was by the environment, followed by GxE, for VI the highest contribution was by genotype, and for seed yield, the highest contribution was by the environment (Tab. 1).

The PCA and SREG-GGE-biplot graphs allowed interpreting the GxE interaction, defining variety performance (yield) through the environments and determining the specific or general varietal adaptation. The highest contribution of the variance was in the first two components, with an accumulated value of 89.31% for GER, 96.65% for VI, and 77.4% for SY (Tab. 2). VI was measured in the most critical environments of the first half, of greater interest since the environmental conditions due to excess water were very marked from physiological maturity to grain harvest.

The first component (SREG) represented the highest proportion of the variability, with the highest negative projection for the variety Agrosavia Primavera 11 in GER, VI, and SY; meanwhile, Corpoica Superior 6 and Orinoquía 3 had the highest positive projection (Tab. 3). Regarding the environment, the highest negative contribution for GER and SY was registered in TALB, and the highest positive projection in TALA. For VI, no statistical differences were observed in the three environments. These results indicate that TALA was the most discriminating environment for GER and SY due to its higher PC1 projection, which is highly relevant for future genetic breeding programs.

GxE interaction analysis (GGE-biplot)

The edaphoclimatic conditions contrasted greatly in precipitation, water excesses and deficits, relative humidity, temperature, and in the physicochemical properties of the soils. This singularity makes the GxE interaction study more relevant to define the best environment to produce high-quality seed and higher grain yield. However, high GxE negatively impacts heritability. Thus, the lower the heritability of a trait, the greater the difficulty in improving that trait via selection (Yan & Kang, 2003).

The main genotypic effect and the GxE interaction of a GxE data set (Yan *et al*., 2000) are shown in a GGE-biplot, where the variation sources of G and GxE constitute the most critical part of the variation when evaluating varieties (Yan & Kang 2003). This multivariate technique allowed stratifying environments and ranking varieties, clearly separating the LIBA, TALA, and LEONAS environments of the first half vs. LIBB and TALB of the second half and revealing the wide adaptation of the Soyica P34 variety,

TABLE 1. Sum of squares (SS) and contribution of the total variance by source of variation for germination (GER), vigor index (IV), and seed yield (SY) of soybean varieties in the Colombian Orinoquía region.

Source of variation	DF	GER (%)		VI		SY (kg ha^{-1})	
		SS	Participation (%)	SS	Participation (%)	SS	Participation (%)
Environment	4	7751.1**	47.8	0.100	0.04	5829197.8**	56.9
Genotype	5	2046.3**	12.6	129.6**	75.19	1239327.2**	12.1
Genotype x Environment	20	6419.9**	39.6	42.7**	24.78	3173004.9**	31.0
R^2		0.92		0.9		0.87	
CV(%)		15.3		13.3		24.6	

**Highly significant differences ($P<0.01$) according to a combined analysis of variance (ANOVA).

TABLE 2. Participation of the variance by the principal and accumulated components for germination, vigor index, and seed yield.

Principal Component	Germination		Vigor index		Seed yield	
	Part.-SS (%)	Accum. (%)	Part.-SS (%)	Accum. (%)	Part.-SS (%)	Accum. (%)
PC1	72.54**	72.54	77.62**	77.63	55.55**	55.55
PC2	16.76**	89.31	19.02**	96.65	21.85**	77.4

Part.-SS - participation in Sum of squares; Accum. - accumulated components.

**Highly significant differences ($P<0.01$) according to a principal component analysis (PCA).

TABLE 3. Means and principal components PC1 and PC2 per variety and environment.

		GER	PC1	PC2	VI	PC1	PC2	SY	PC1	PC2
Variety	Barreira	57.35	-2.24	3.71	9.25	-0.56	1.65	1,290.31	-8.25	25.06
	Orinoquía 3	69.55	4.88	1.59	13.25	1.67	-0.81	1,473.18	17.86	-2.63
	Primavera 11	46.90	**-4.36**	-1.67	6.21	**-1.91**	-1.15	929.40	**-20.65**	-13.87
	Soyica P34	58.29	-0.51	1.86	10.78	0.41	0.91	1,083.65	0.14	6.34
	Superior 6	63.06	4.84	-2.71	13.39	1.71	-0.36	1,404.6	24.06	-6.17
	Taluma 5	46.45	-2.61	-2.79	7.62	-1.32	-0.23	1,009.02	-13.16	-8.73
Environment	LEONAS 2021	56.29	3.98	-1.41	10.13	1.57	0.67	948.09	14.06	-1.64
	LIBA 2021	59.29	2.42	5.16	10.00	1.79	2.03	1,549.33	11.20	29.83
	TALA 2021	32.66	**7.16**	-0.10	10.13	2.42	-1.07	649.39	**34.59**	-8.04
	LIBB 2020	83.08	-1.49	0.50	.	.	.	1,860.6	1.51	0.15
	TALB 2020	53.34	-1.81	2.96	.	.	.	984.39	-6.62	4.99

GER – seed germination (%); VI – vigor index; SY – seed yield (kg ha^{-1}).

GxE interaction analysis (GGE-biplot).

whose coordinates were closer to zero (<GxE). The varieties or locations with negative or positive coordinates furthest from the main axis, such as Orinoquía 3 and Corpoica Superior 6, contributed the most to GxE. In general, the most favorable environment for SY was LIBB, and the most unfavorable were, in order, TALA, TALB, and LEONAS.

Germination-(GGE-biplot)

A clear separation of environments by year halves is observed (Fig. 1). The responses of the second half are found in the left margin, and those of the first half are in the right margin. This differential varietal response is attributed to a specific adaptation (Yan & Kang, 2003). Simultaneously, the biplot separated early varieties (Orinoquía 3, Corpoica Superior 6) from intermediate (Soyica P34, Barreira) and late (Corpoica Taluma 5, Agrosavia Primavera 11). TALA and LEONAS shared the same sector, with similar varietal response, so all the environments are valid for evaluating the germination performance of soybean in the first half. However, TALA, showing a greater projection with less interaction (vector closer to zero in PC2), is the most discriminating environment with the highest varietal differences in germination.

LIBA is located in a second sector with higher interaction (higher PC2) and a vectorial projection opposite Agrosavia Primavera 11 and Corpoica Taluma 5, indicating an unfavorable GER environment for these. On the left margin, TALB and LIBB were located in sectors three and four. These are similar environments to evaluate in the second half, although LIBB is a more stable and favorable environment for the varieties because it is very close to zero on both axes. Both registered the best levels of GER, but the least discriminating environment is LIBB because it projects a short vector (Yan & Kang, 2003). In LIBA, a situation with high interaction occurred; therefore, it is unrepresentative and discarded for genotype selection processes for GER.

In general, the varieties showed higher average germination in LIBB (83.1%), characterized by its higher fertility with a cation exchange capacity of 3.14 meq/100 g, organic matter contents of 2.39%, and phosphorus content of 5.94 mg kg^{-1}, with 715 mm of water availability throughout the crop cycle, and low presence of foliar and seed pathogens. The exception was Corpoica Superior 6 (71% GER) due to excess water between maturity and harvest and a greater incidence of pathogens (*Phomopsis* sp.) TALA registered the most unfavorable edaphoclimatic conditions for seed germination, with frequent and intense rainfall (478 mm) prevailing in the final phase of the cycle, and higher presence of foliar diseases such as *Cercospora sojina* and *Xanthomonas axonopodis*, and seed pathogens such as *Phomopsis* sp. and *Fusarium* sp. Pádua *et al.* (2009) demonstrated that water stress in the soybean grain-filling stage reduces seed

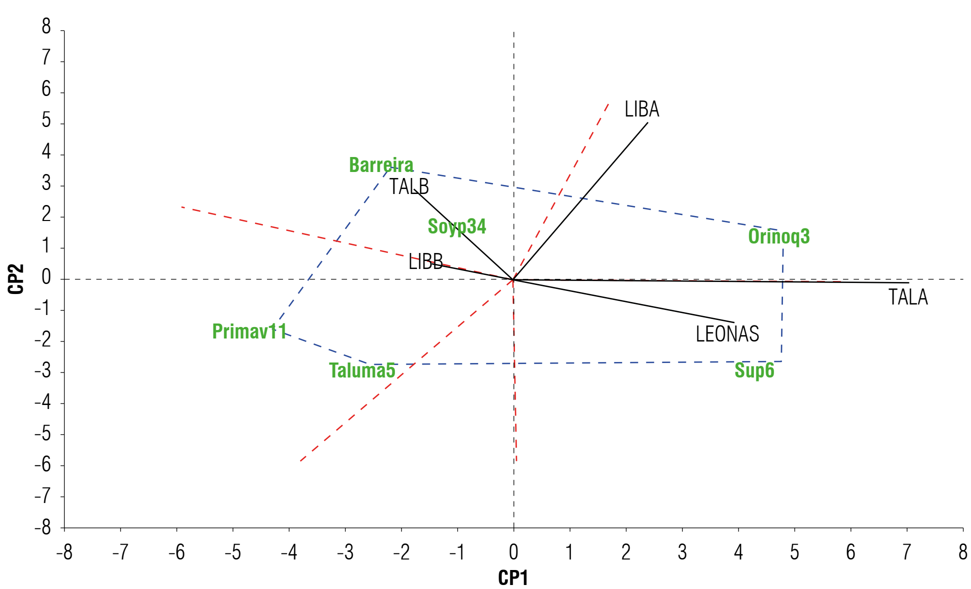

FIGURE 1. Site regression (SREG) (SREG)-model genotype-genotype x environment (GGE) biplot for seed germination of six soybean varieties (Corpoica Superior 6 (Sup6), Orinoquía 3, Soyica P34 (Soyp34), Corpoica Taluma 5, and Agrosavia Primavera 11 of Colombian origin and Barreira of Brazilian origin) in five environments of the Orinoquía region with two in the second half of 2020 (LIBB: La Libertad, TALB: Taluma) planted in August, and three in the first half of 2021 (LIBA: La Libertad, TALA: Taluma, finca LEONAS) planted in April-May.

germination, worsened by high temperatures (>28°C). In this environment, Corpoica Superior 6 and Orinoquía 3 registered the best performance of germination (68.2 and 75%, respectively) compared to the others (<26%). In the GER-biplot, Barreira, Soyica P34, Corpoica Taluma 5, and Agrosavia Primavera 11 recorded the highest GER in the second half in LIBB and TALB. Corpoica Superior 6 registered its highest germination in LEONAS (85.8%) and Orinoquia 3 in LIBA and TALA of the first half (75%). These two varieties in the first half, characterized by excess water, show relatively better behavior in GER due to their genetic attributes, such as greater earliness (>95 d after emergence) and uniform maturation without foliar retention, favoring grain quality. The most unfavorable environment for these two varieties was TALB, mainly attributed to the water deficit in the phenological stages between R5 and R7. Drought in the critical stages of seed formation and filling decreases seed size and germination capacity (Sánchez & Pinchinat, 1974).

In the GGE-biplot, PC1 represents the main effects or average behavior of the genotype, and PC2 represents the GxE effects per genotype, considered a stability measure (Yan *et al*., 2000). Based on the stability analysis proposed by Purchase *et al*. (2000), Soyica P34 was the most stable (VE=2.9), and Corpoica Superior 6 (VE=21.1) and Orinoquía 3 (VE=21.2) were the most unstable. However, the most stable variety had a lower average germination percentage than the most unstable ones, suggesting a varietal-specific adaptation to produce high-quality seeds. The GER of Soyica P34 ranged between 25.9 and 69.3% in the first half; therefore, together with Barreira, Corpoica Taluma 5, and Agrosavia Primavera 11, they are not recommended for planting in the first half due to their higher leaf retention, longer cycle and sensitivity to foliar and seed pathogens.

The contrast between the environments and the differential response of varieties is notorious. LIBB was characterized by excess water in the growth and development periods and little water in the physiological maturity to harvest period. This circumstance favored the uniform drying of the plants and the lower attack of pathogens. At the same time, TALA showed excess water at the end of the vegetative cycle, noticeably affecting intermediate and late varieties, which suffered leaf retention, green stems, and higher pathogen attack.

Sowing in the *altillanura* subregion is traditionally carried out in April, with harvest in July-August, months with high rainfall where the seed has high water content (>18%), a condition that causes its rapid deterioration. On the contrary, in the *altillanura* and foothill subregions, sowing is done in August and early September and is harvested in the dry period of December-January, where the seed reaches a favorable humidity (13-15%), showing a differential response of the varieties attributed to adaptation, earliness, uniformity at maturity and resistance to both foliar and seed pathogens. Environmental stress in soybean plant cultivation reduces germination and vigor even in healthy seeds (Egli *et al*., 2005).

Vigor index (GGE-biplot)

The VI, as a variable composed of germination, viability, and electrical conductivity, was assessed only in the first half due to the high vulnerability of the seeds to excess water that deteriorates, and favors the presence of pathogens. In the GGE-biplot, a PC1 dimension with the highest positive contribution for Corpoica Superior 6 and followed by Orinoquiaí 3. Soyica P34 was the most stable, with the vector closest to zero in both dimensions, while the others, such as Agrosavia Primavera 11, had a negative and more marked response in projection (Fig. 2).

TALA and LEONAS showed similar behavior, discriminating similarly the varietal behavior in VI. Consistent with the GER response, the biplot for VI showed the similar performance of the varieties in LEONAS and TALA, very distant from LIBA with higher positive interaction. The first two belong to the *altillanura* subregion, and the second to the foothills, suggesting that the geographical location has a high relevance in the differential response of the varieties.

The varieties with the highest VI were Corpoica Superior 6 and Orinoquía 3, with positive values. For its part, Soyica P34 was the most stable variety; the rest of the varieties showed a negative response in their order: Barreira, Corpoica Taluma 5, and Agrosavia Primavera 11 (Fig. 2). These results evidence that the varieties with higher GER showed higher VI with a highly significant correlation ($P<0.01$) of 81%. These results suggest that it is enough solely to carry out germination tests to establish a seed quality opinion.

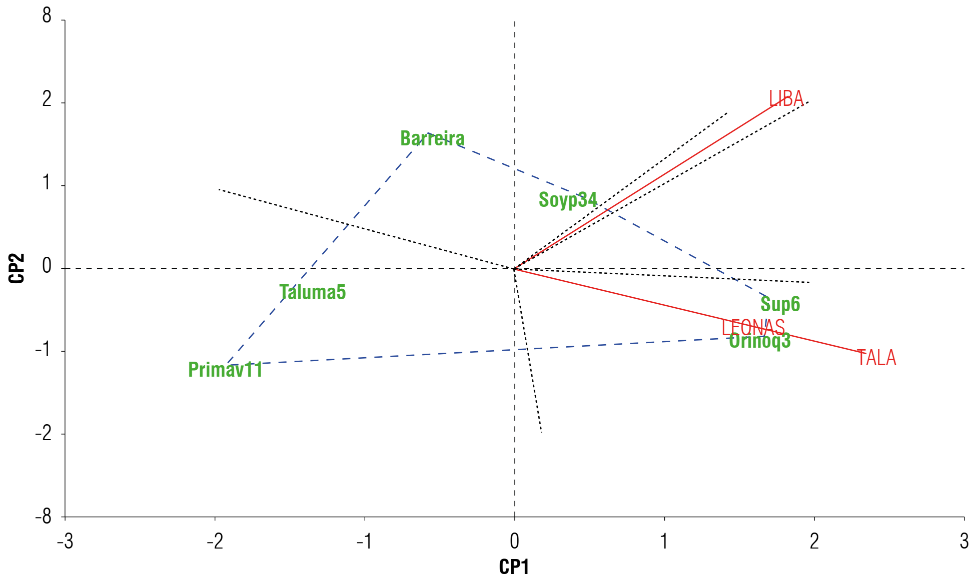

FIGURE 2. Site regression (SREG)-model genotype-genotype x environment (GGE) for seed vigor index in three environments (LIBA: La Libertad, TALA: Taluma, finca LEONAS) of the Colombian Orinoquía.

Seed yield (GGE-biplot)

In seed yield response variable composed of the SY and the GER percentage, a clear separation of environments is observed (Fig. 3), with a greater positive projection for the three environments of the first half, similar to GER. LIBB showed a positive projection for both PCs and was considered a more stable environment, and TALB exhibited a slightly negative projection for PC1 and a positive one for PC2 (Fig. 3A). An opposite response is found in LEONAS and TALA. Although they similarly discriminate varieties concerning SY, TALA has a greater projection and proximity to the abscissas. It is the most favorable environment for selecting future lines with comparative advantages in SY. According to Yan *et al.* (2000) and Yan and Rajcan (2002), sectors with higher values in PC1 and lower in PC2 receive the greatest interest because they facilitate discrimination for the trait under study. They also improve the effectiveness of the selection process for superior genotypes. The ideal evaluation environment could have a high PC1 and be more discriminating of the genotypes regarding the main genotypic effect. Furthermore, it also could have a small PC2 coefficient as the most representative of the environments (Yan & Rajcan, 2002).

In contrast, seed yield selection may not be successful in LIBA, with a low relative PC1 value and a high relative PC2 value. On the other hand, LIBB, a more stable environment, and TALB, with very low interaction, show similar varietal behavior for SY; therefore, the selection in these environments is not relevant. According to Crossa *et al.* (1990), environments with PC1 close to zero have little interaction and low genotype discrimination. Blanche and Myers (2006) state that a discriminant locality maximizes the phenotypic variation observed between genotypes for a specific trait. Genotype selection efficiency and success in genetic breeding programs are high in these locations. The discriminating ability of a locality comprises various factors, including soil type, pathogens, field drainage, temperature, precipitation, soil fertility, and management practices.

Concerning varietal behavior, Orinoquía 3 and Corpoica Superior 6 had a positive projection for PC1 and a low negative projection for PC2. Soyica P34, the most stable variety (VE=0.4), presented values close to zero. The rest of the varieties registered negative PC2 components. Agrosavia Primavera 11 recorded the highest negative projection for both components and was highly unstable (VE=56.4) (Fig. 3A). This variety has the worst SY performance in the first half, followed by Corpoica Taluma 5, demonstrating varietal differences in adaptation. This differential response of soybean genotypes in contrasting environments has previously been reported (Ngalamu *et al.*, 2013; Salmerón *et al.*, 2017).

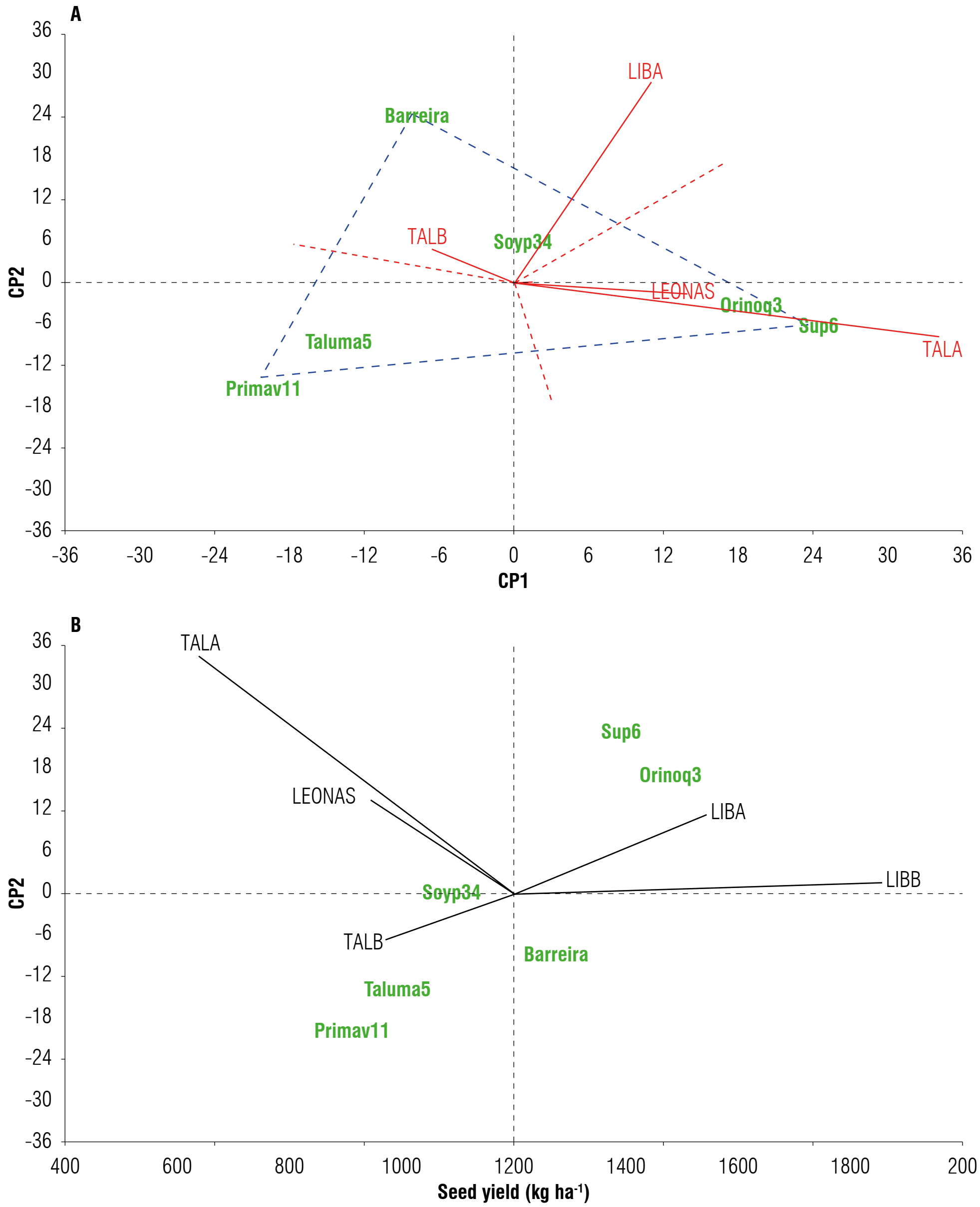

FIGURE 3. A and B) Site regression (SREG)-model genotype-genotype x environment (GGE) biplot for seed yield in five environments of the Orinoquía region, two in the second half of 2020 planted in August (LIBB: La Libertad, TALB: Taluma), and three in the first half of 2021 planted in April-May (LIBA: La Libertad, TALA: Taluma, finca LEONAS).

The highest average SY was registered in LIBB and LIBA, and varieties Orinoquía 3 and Corpoica Superior 6 stood out (1,473 and 1,404 kg ha^{-1}, respectively), while the others reached a much lower value than the average (1,210 kg ha^{-1}) (Fig. 3B). In general, all the varieties performed best in LIBB, with statistical differences in SY between Agrosavia Primavera 11 and Corpoica Taluma 5. The early varieties Corpoica Superior 6 and Orinoquía 3, as well as Barreira, significantly outperformed Agrosavia Primavera 11 and Corpoica Taluma 5 (Tab. 4). TALB showed alternating rainy and dry periods, where water deficit predominated, particularly in the phenological stages R5 and R7. Rainfall and temperature are the main factors responsible for grain yield fluctuation in much of the tropics (Carmello *et al.*, 2016).

In Taluma or LEONAS, complementary irrigation must be applied during the second half of the year, since the water deficit was the most decisive factor for seed quality and quantity in all varieties. If high seed quality and quantity need to be produced in the first half, only varieties adapted to this edaphoclimatic condition that are early or precocious and have uniform drying should be planted. Moreover, fungicides must be applied to protect the seeds from pathogenic fungi, and a seed drying unit must be used to lower seed moisture gradually without affecting the embryo at a temperature below 40°C.

Orinoquía 3 and Corpoica Superior 6 stood out for their higher average varietal response (GER, VI, and SY), with a highly favorable response for planting in the first half. In this period, high frequency and intense rainfall predominates and these early varieties ripen and dry uniformly up to harvest without leaf retention. Although Orinoquía 3 and Corpoica Superior 6 can perform well in the second half with good management practices, the same does not occur with Agrosavia Primavera 11 Barreira, Corpoica Taluma 5, and Soyica P34 in the first half. This is because they are intermediate to late varieties showing genetic leaf retention at physiological maturity and uneven maturation, which delays harvesting, facilitating pathogen proliferation. These genetic traits are vital to close the gap regarding the quality and quantity of seed produced in Orinoquía, particularly in the *altillanura* subregion, where the only adequate strategy is genetic breeding.

TABLE 4. Soybean seed yield (kg ha^{-1}) of six soybean varieties in five environments of the Colombian Orinoquía.

Genotype	Environment					
	LIBB	TALB	LIBA	TALA	LEONAS	Mean
Barreira	1871^{ab*}	1366^{a}	2180^{a}	183^{b}	852^{ab}	1290^{ab}
Orinoquía 3	2139^{ab}	1067^{ab}	1646^{ab}	1337^{a}	1178^{ab}	1473^{a}
Primavera 11	1535^{b}	1410^{a}	870^{c}	131^{b}	702^{b}	929^{c}
Soyica P34	1652^{ab}	595^{b}	1795^{a}	568^{ab}	809^{ab}	1084^{bc}
Superior 6	1701^{ab}	812^{b}	1633^{ab}	1518^{a}	1360^{a}	1405^{a}
Taluma 5	2267^{a}	657^{b}	1174^{bc}	160^{b}	787^{ab}	1009^{c}
Mean	1861^{a}	984^{c}	1549^{b}	649^{d}	948^{c}	

*Means with different letters in the same line are significantly different at $P<0.01$ according to Tukey's test.

Conclusions

The GxE interaction is highly relevant for defining the adaptation domain and recommending varieties with high germination potential and seed yield. The SREG-GGE-biplot graphic analysis identified TALA as the most discriminating and useful environment for genetic breeding processes focused on quality and seed yield.

Varieties Corpoica Superior 6 and Orinoquía 3 stood out for their better performance and seed quality due to their earliness, uniformity, and high seed yield potential. Seed germination potential and seed yield depended on the variety and the environment; GxE did not allow for generalization. The second half was the best time to produce seeds, particularly in La Libertad, with an advantage over Taluma. Moreover, La Libertad was the best environment to obtain better yields in the first half.

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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

RAVR carried out the statistical analysis of the study. RAVR and YSTA interpreted the results. RAVR and YSTA carried out the research. YSTA organized the information in databases. RAVR and YSTA wrote the manuscript. All authors revised the final version of the manuscript.

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SUPPLEMENTARY TABLE 1. Complete analysis of variance from means using Gollob's procedures (1968).

Source of variation	Gollob's degrees of freedom	Sum of squares (SS)
Genotypes	G-1	SS.(G)
Environments	A-1	SS.(E)
Interaction	(G-1)(A-1)	SS (GxE)
PC1	G+A-1-(2x1)	λ^2_1
PC2	G+A-1-(2x2)	λ^2_2
PCn	G+A-1-(2xn)	λ^2_n
Mean error	GxE(n-1)	SS (mean error)
Total	GxEr-1	SS (total)

Defoliation tolerance of soybean cultivars commercially released in different decades

Tolerancia a la defoliación de cultivares de soya liberados para su comercialización en diferentes décadas

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ABSTRACT

Breeding programs have increased the precocity and yield potential of modern soybean cultivars. Such changes may have altered the crop tolerance to defoliation due to the smaller leaf area of modern cultivars. The objective of this study was to determine the tolerance to defoliation of soybean cultivars commercialized in Brazil in different decades, their photosynthetic efficiency and the relationship between photosynthetic efficiency and tolerance to defoliation in the reproductive phase. The experiment was set in a greenhouse with controlled humidity and temperature, in the municipality of Lages, Santa Catarina State, South of Brazil, during the growing season of 2018/2019. A randomized block design was used, with treatments arranged in a 5×5 factorial scheme. The first factor was composed of five soybean cultivars released in different years: Davis (1968), Paraná (1974), BR-16 (1985), FT Abyara (1991), and Brasmax Elite IPRO (2014). The second factor consisted of five levels of defoliation applied in stage R3: 0, 16.6, 33.3, 50.0, and 66.6%. Leaf area, photosynthetic activity parameters, grain yield and its components were determined. Brasmax Elite IPRO had the lowest grain yield per plant and did not increase yield compared to older cultivars, regardless of defoliation level. There were no significant differences in photosynthetic efficiency or defoliation tolerance between the modern cultivar Brasmax Elite IPRO and the old cultivars Davis, Paraná, BR-16, and FT Abyara.

Key words: *Glycine max* L. Merrill, leaf area, grain yield, photosynthetic activity.

RESUMEN

Los programas de mejoramiento han aumentado la precocidad y el potencial de rendimiento de los cultivares de soya modernos. Dichos cambios pueden haber alterado la tolerancia del cultivo a la defoliación, siendo la pérdida de área foliar un factor agravante de la pérdida de productividad. El objetivo de este estudio fue determinar la tolerancia a la defoliación de cultivares de soya comercializados en Brasil en diferentes décadas, su eficiencia fotosintética y la relación entre la eficiencia fotosintética y la tolerancia a la defoliación en la fase reproductiva. El experimento se realizó en un invernadero con humedad y temperatura controladas, en el municipio de Lages en el estado de Santa Catarina, Sur de Brasil, en la temporada de crecimiento 2018/2019. Se utilizó un diseño de bloques al azar, con tratamientos en esquema factorial 5×5. El primer factor estuvo compuesto por cinco cultivares de soya comercializados en diferentes años: Davis (1968), Paraná (1974), BR-16 (1985), FT Abyara (1991) y Brasmax Elite IPRO (2014). El segundo factor consistió en cinco niveles de defoliación aplicados en la etapa R3: 0, 16.6, 33.3, 50.0 y 66.6%. Se determinó área foliar, parámetros de actividad fotosintética, rendimiento de grano y sus componentes. Brasmax Elite IPRO mostró el rendimiento de grano más bajo por planta y no aumentó el rendimiento en comparación con los cultivares más antiguos, independientemente del nivel de defoliación. No hubo diferencias significativas en la eficiencia fotosintética ni en la tolerancia a la defoliación entre el cultivar moderno Brasmax Elite IPRO y los antiguos cultivares Davis, Paraná, BR-16 y FT Abyara.

Palabras clave: *Glycine max* L. Merrill, área foliar, rendimiento de grano, actividad fotosintética.

Introduction

Over the past five decades, plant-breeding programs have developed soybean cultivars with desirable agronomic traits and high adaptability to different field conditions. Experimental studies are necessary to determine the best cultivar for a given production region, searching for genotypes that best fulfill a series of requirements, such as resistance to insect defoliation, suitable plant architecture to maximize light absorption, and resistance/tolerance to pathogens (Zanon *et al.*, 2018).

The leaf mesophyll is the most active photosynthetic tissue in higher plants. It is responsible for the interception of solar

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radiation and the assimilation of atmospheric CO_2, transforming light energy into chemical energy (Taiz *et al.*, 2017). Leaf area loss is one of the main factors affecting soybean yield. The maximum yield of soybean is determined by the plant's ability to intercept solar radiation and accumulate dry mass during the vegetative and reproductive stages (Heiffig *et al.*, 2006; Koester *et al.*, 2014). Although the productive potential of soybean has increased in recent years, leaf area loss is still one of the main problems studied by researchers around the world. Breeders try to develop cultivars better adapted to this condition, entomologists aim to select genotypes capable of resisting the attack of defoliating pests, and physiologists search to identify plant materials that are increasingly efficient in converting light energy into biomass (Lopes & Lima, 2015).

The reproductive phase is the most decisive time for soybean yield. This stage encompasses four periods of plant development: flowering, pod formation, grain filling, and maturation (Neumaier *et al.*, 2018). Stressful events during the reproductive phase may lead to low yields. Defoliating insects, such as *Anticarsia gemmatalis* and *Chrysodeixis includens*, can significantly reduce crop yield if defoliation surpasses 15% after the beginning of flowering (Moscardi *et al.*, 2012; Glier *et al.*, 2015).

In Brazil, soybean crop yield increased from 1,140 kg ha^{-1} in the 1970s to 3,333 kg ha^{-1} in 2017/2018 (EMBRAPA, 2018). This increase is attributed to the development of new cultivars, whose yields have increased by 34 kg ha^{-1} per year (Balbinot Junior *et al.*, 2017). Toledo *et al.* (1990) reported genetic gains of 1.8% in early-cycle cultivars and 1.3% in semi-early cultivars in the State of Paraná, Brazil. Alliprandini *et al.* (1993) observed annual yield gains of 6.62, 4.54, and 0.89% in early, semi-early, and medium-cycle cultivars, respectively. Despite these advances in crop productivity, few studies have explored how physiological characteristics have contributed to the increase in grain yield of soybean cultivars released in different decades. Furthermore, the relationships between photosynthetic efficiency, defoliation tolerance, and grain yield in old and modern soybean cultivars still need to be determined.

This study was conducted based on the following hypotheses: the modern cultivar Brasmax Elite is more productive than the old cultivars because of its higher photosynthetic efficiency; the modern cultivar Brasmax Elite is more sensitive to defoliation than the old cultivars due to its smaller leaf area and greater productive potential; and the yield gain promoted by soybean breeding is greater when leaf area is preserved.

The main objectives of the experiment were to determine the impact of leaf area loss on grain yield of soybean cultivars commercially released in different decades, compare the photosynthetic efficiency of these genotypes, and assess the relationship of photosynthetic efficiency with defoliation tolerance during the reproductive phase.

Materials and methods

A greenhouse experiment was conducted in Lages, Santa Catarina, Brazil, during the 2018/2019 growing season. The geographical coordinates of the site are 27°48'58" S; 50°19'34" W. The experimental design was a randomized block with three replicates. Treatments were arranged in a 5×5 factorial design. The experiment had 75 experimental units (5×5×3). The plants were cultivated in 5 L PVC pots, with soil-based substrate from a commercial soybean cultivation environment with the following characteristics: 405 g kg^{-1} of clay; pH (water) 5.1; 24.9 mg dm^{-3} of P, 223 mg dm^{-3} of K; 3.7 g kg^{-1} of organic matter; 4.7 cmolc dm^{-3} of Ca; 1.9 cmolc dm^{-3} of Mg; 1.0 cmolc dm^{-3} of Al and 20.9 cmolc dm^{-3} CEC, arranged over three benches (each with 25 experimental units) in an environment kept at 25±10°C and 70% relative air humidity. The first factor comprised five soybean cultivars commercially released in different decades: Davis (1968), Paraná (1974), BR-16 (1985), FT Abyara (1991), and Brasmax Elite IPRO (2014) (Tab. 1). These cultivars were selected for their relevance

TABLE 1. Characteristics of soybean cultivars used in the study.

Cultivar	Year of release	Maturity group	Growth habit	Technology
Davis	1968	7.0	Determined	Conventional
Paraná	1974	6.5	Determined	Conventional
BR-16	1985	6.5	Determined	Conventional
FT Abyara	1991	8.0	Determined	Conventional
Brasmax Elite	2014	5.5	Undetermined	IPRO

IPRO: Technology that confers resistance to the herbicide glyphosate and control and/or suppression of caterpillars.

in cultivated area during the 1960s, 1970s, 1980s, 1990s, and the current century.

The second factor comprised five defoliation levels: 0 (control), 16.6, 33.3, 50.0, and 66.6%. According to Moscardi *et al.* (2012), defoliation levels of 16.6 and 33.3% are close to the threshold of economic damage for soybean in the reproductive (15%) and vegetative (30%) phases, respectively. Defoliation treatments were applied at the R3 stage (beginning of pod formation) and assessed according to the phenological scale proposed by Ritchie *et al.* (1977). Defoliation was performed manually with scissors. The leaves were cut longitudinally until reaching the desired level of defoliation in all plants simultaneously, regardless of growth habit, as shown in Figure 1.

Disease control was performed with 1.5 ml L^{-1} of difenoconazole + cyproconazole (Cypress®), 1 g L^{-1} of azoxystrobin + benzovindiflupyr (Elatus®), 2.6 ml L^{-1} of trifloxystrobin + prothioconazole (Fox®). Fungicides were applied at the growth stages of V8, R1, and R5, respectively. Pest control was performed with 0.5 ml L^{-1} of λ-cyhalothrin + chlorantraniliprole (Ampligo®) and 1 ml L^{-1} of thiamethoxam + λ-cyhalothrin (Engeo Pleno®). Insecticides were applied on plants at the stages of V8, R1. To correct the soil used in the pots, 310 kg ha^{-1} of triple superphosphate and 155 kg ha^{-1} of potassium chloride were used.

Leaf area was determined at the R3 (beginning of pod formation) and R5 (beginning of grain filling) stages and used to calculate leaf expansion. The length and largest width of the central leaflet of each trifoliate leaf was measured in all plants. Leaf area was calculated by the following equation proposed by Ritcher *et al.* (2014):

$$LA = L \times W \times \alpha \qquad (1)$$

where LA is the leaf area (cm^2), L is the leaf length (cm), W is the leaf width (cm), and α is the angular coefficient for soybean crops (2.0185).

FIGURE 1. Representative images of soybean leaves at different defoliation levels.

Gas exchange in leaves was determined at the R5 stage. Measurements were performed between 9:00 and 11:00 h at the apex of the central leaflet of completely expanded leaves using an open-system, portable photosynthesis meter (IRGA LI-6400XT, LI-COR). Gas exchange values were used to determine net carbon assimilation rate (*A*), transpiration rate (*E*), and stomatal conductance (g_s).

Grain harvest was carried out manually on April 1, 2019, using hand pruners. The filled pods were oven-dried at 35°C for 4 d. Dried samples were used to determine the number of pods per plant, number of grains per pod, number of grains per plant, thousand-grain weight, and grain yield (adjusted to 13% moisture).

Statistical analysis

Experimental data were subjected to analysis of variance by the *F*-test. When statistical significance was detected, comparisons of mean values between cultivars (qualitative factor) were performed by Tukey's test and differences between defoliation levels (quantitative factor) were assessed by regression analysis. The level of significance was set at P<0.05. Analyses were performed using the SISVAR software (Ferreira, 2003).

Results and discussion

Leaf area at R3 (before defoliation), leaf area at R5 (after defoliation at R3), and leaf expansion (between R3 and R5) were influenced by the main effect of the cultivar (Tab. 2). Davis had the highest leaf area at the R3 stage, followed by FT Abyara and BR-16. Paraná and Brasmax Elite had the smallest leaf areas at the beginning of pod formation (Tab. 3).

TABLE 3. Leaf area at R3 (LA-R3, before defoliation), leaf area at R5 (LA-R5, after application of defoliation treatments at R3), leaf expansion (LE, from R3 to R5) in soybean cultivars subjected to different defoliation levels. Lages, Santa Catarina, Brazil, 2018/2019.

Cultivar	LA-R3 (m^2)	LA-R5 (m^2)	LE (m^2)
Davis	0.88[a]	0.93[a]	0.04[b]
Paraná	0.41[c]	0.59[c]	0.18[a]
BR-16	0.60[b]	0.66[bc]	0.06[b]
FT Abyara	0.71[b]	0.73[b]	0.03[b]
Brasmax Elite	0.39[c]	0.45[d]	0.06[b]
CV(%)	20.10	18.37	61.05

CV - coefficient of variation.

* Values are the means of five defoliation treatments. Means within columns followed by the same lowercase letters are not significantly different at $P<0.05$ according to Tukey's test.

The leaf area at R5 also differed significantly among cultivars. Similar to the results for the R3 stage, Davis and Brasmax Elite had the highest and lowest leaf areas, respectively, at R5. Paraná had the highest leaf expansion, as shown by the mean value of the five defoliation treatments. Brasmax Elite had the smallest leaf area before and after defoliation.

Brasmax Elite belongs to the maturity group 5.5, the lowest among the genotypes used in the experiment (Tab. 1), explaining why it had the smallest leaf area at R3. The lower the maturity group of a soybean cultivar, the earlier it blooms, and the smaller its leaf area at the end of flowering (Zanon *et al*., 2018). The time from emergence to R1 was 44, 49, 50, 51, and 56 d for Brasmax Elite, Paraná, BR-16, 'FT Abyara', and Davis, respectively.

Since Brasmax Elite was the only cultivar with indeterminate growth habit (Tab. 1), it was supposed to have a greater ability to expand new leaves between R3 and R5,

TABLE 2. *F*-values according to the analysis of variance for leaf area at R3 (LA-R3, before defoliation), leaf area at R5 (LA-R5, after application of defoliation treatments at R3), leaf expansion (LE, from R3 to R5), net assimilation rate (*A*), transpiration rate (*E*), and stomatal conductance (g_s) in soybean cultivars subjected to different defoliation levels. Lages, Santa Catarina, Brazil, 2018/2019.

Source of variation	df	LA-R3	LA-R5	LE	*A*	*E*	g_s
Blocks	2	2.64[ns]	2.87[ns]	0.35[ns]	1.4[ns]	0.7[ns]	1.36[ns]
Cultivars (C)	4	45.25*	30.36*	27.66*	5.7*	18.2*	3.94*
Defoliation levels (D)	4	-	0.29[ns]	0.86[ns]	2.6[ns]	1.3[ns]	0.90[ns]
C × D	16	-	0.48[ns]	0.78[ns]	1.3[ns]	0.6[ns]	0.87[ns]
Error	48						
Total	74						

df - degrees of freedom,* - significant at $P<0.05$, and ns - not significant.

which did not happen. This may be because Brasmax Elite exhibited very early maturity, reaching the end of the cycle at about 125 d, with a vegetative phase of 46 d after sowing. Compared with older cultivars, for which the vegetative stage ranged from 51 to 58 d after sowing, Brasmax Elite has a shorter cycle.

Net carbon assimilation, transpiration rate, and stomatal conductance at the beginning of grain filling were significantly influenced by the main effect of the cultivar (Tab. 2). The net carbon assimilation rate of Davis was lower than that of the other cultivars, which did not differ from each other (Tab. 4). The modern cultivar Brasmax Elite presented the highest values of transpiration rate and stomatal conductance, on the average of five defoliation levels.

TABLE 4. Net assimilation rate (*A*), transpiration rate (*E*), and stomatal conductance (g_s) of soybean cultivars at the R5 stage. Lages, Santa Catarina, Brazil, 2018/2019.

Cultivar	*A* (μmol CO_2 m^{-2} s^{-1})	*E* (mmol H_2O m^{-2} s^{-1})	g_s (μmol CO_2 m^{-2} s^{-1})
Davis	17.7^{b}	6.9^{b}	0.30^{b}
Paraná	22.2^{a}	9.7^{a}	0.34^{ab}
BR-16	22.1^{a}	6.1^{b}	0.31^{b}
FT Abyara	24.1^{a}	7.3^{b}	0.35^{ab}
Elite	22.5^{a}	9.9^{a}	0.39^{a}
CV(%)	17.8	17.3	19.41

CV - coefficient of variation, * - values are the means of five defoliation treatments. Means within columns followed by the same lowercase letters are not significantly different at $P<0.05$ according to Tukey's test.

One of the study's hypotheses was that Brasmax Elite is more productive than old cultivars because of its higher photosynthetic efficiency. The results described in Table 4 show that the modern cultivar had higher transpiration rate and stomatal conductance values. These physiological parameters are correlated with photosynthetic activity, as higher values indicate greater stomatal opening, which favors the flow of CO_2 from the atmosphere to chloroplasts (Koester *et al.*, 2014; Taiz *et al.*, 2017). However, such effects did not lead to a higher net carbon assimilation rate in the modern cultivar when compared with Paraná, BR-16, and FT Abyara.

Defoliation can reduce transpiration and photosynthesis. However, if the reduction in leaf area is not drastic, soybean plants may continue to perform photosynthesis at sufficient levels to ensure grain production (Moscardi *et al.*, 2012). In the present study, defoliation level had no significant effects on net carbon assimilation or stomatal conductance (Tab. 2). These findings suggest that defoliation of up to 66% did not compromise photosynthetic activity at the beginning of grain filling, regardless of the cultivar.

Grain yield per plant, number of pods per plant, number of grains per pod, number of grains per plant, thousand grain weight, and harvest index were influenced by the main effect of the cultivar (Tab. 5).

TABLE 5. *F*-values according to the analysis of variance for number of pods per plant (NP_{plant}), number of grains per pod (NG_{pod}), number of grains per plant (NG_{plant}), thousand grain weight (TGW), grain yield per plant (GY_{plant}), and harvest index (HI) of soybean cultivars subjected to different defoliation levels. Lages, Santa Catarina, Brazil, 2018/2019.

Source of variation	df	NP_{plant}	NG_{pod}	NG_{plant}	TGW	GY_{plant}	HI
Blocks	2	0.2^{ns}	3.8^{*}	0.5^{ns}	0.2^{ns}	0.1^{ns}	0.3^{ns}
Cultivars (C)	4	13.5^{*}	4.8^{*}	9.6^{*}	23.3^{*}	4.4^{*}	4.7^{*}
Defoliation levels (D)	4	0.2^{ns}	0.6^{ns}	0.5^{ns}	0.6^{ns}	1.6^{ns}	1.6^{ns}
C × D	16	0.6^{ns}	1.1^{ns}	0.5^{ns}	0.2^{ns}	0.6^{ns}	0.6^{ns}
Error	48						
Total	74						

df - degrees of freedom, * - significant at $P<0.05$, and ns-not significant ($P>0.05$).

FT Abyara had the highest number of pods per plant (*n*=118) and number of grains per pod (Tab. 6). As a result, it had the highest number of grains per plant. The number of grains per pod did not differ significantly among cultivars, with values ranging from 2.0 to 2.3. According to Mundstock and Thomas (2005), cropping conditions usually do not affect the number of grains per pod. The behavior of this yield component demonstrates the uniformity of genetic breeding, standardizing the parameter at approximately two grains per pod.

TABLE 6. Number of pods per plant (NP_{plant}), number of grains per pod (NG_{pod}), number of grains per plant (NG_{plant}), thousand grain weight (TGW), grain yield per plant (GY_{plant}), and harvest index (HI) of soybean cultivars subjected to different defoliation levels. Lages, Santa Catarina, Brazil, 2018/2019.

Cultivar	NP_{plant}	NG_{pod}	NG_{plant}	TGW (g)	GY_{plant} (g)	HI
Davis	96^{b}	2.0^{b}	184^{bc}	183.7^{a}	33.2^{a}	49.0^{a}
Paraná	86^{bc}	2.3^{a}	179^{bc}	180.7^{a}	32.1^{ab}	46.5^{ab}
BR-16	102^{ab}	2.2^{ab}	207^{ab}	166.0^{ab}	34.1^{a}	49.7^{a}
FT Abyara	118^{a}	2.3^{a}	238^{a}	128.7^{c}	30.6^{ab}	46.3^{ab}
Elite	79^{c}	2.2^{ab}	165^{c}	162.0^{b}	26.6^{b}	39.4^{b}
CV(%)	16.7	8.9	18.3	10.6	17.2	15.7

CV - coefficient of variation, * - values are the means of five defoliation treatments. Means within columns followed by the same lowercase letters are not significantly different at $P<0.05$ according to Tukey's test.

There was no significant effect of defoliation level on number of pods or grains per plant (Tab. 5). According to Zanon *et al.* (2018), these yield components have the highest impact on soybean yield. Souza *et al.* (2014) argued that defoliation level does not influence the total number of grains or grain yield in soybean when artificial defoliation occurs during vegetative stages. Peluzio *et al.* (2004) concluded that plants exhibit low pod numbers only when total defoliation is performed during pod formation or grain filling.

The thousand grain weight was lowest in FT Abyara, in agreement with the compensatory effect between grain number and grain weight, as reported by Mundstock and Thomas (2005) and Zanon *et al.* (2018). The cultivars with the highest thousand grain weights were Davis (183.8 g) and Paraná (180.8 g).

BR-16 and Davis had the highest grain yields and harvest indices, and Brasmax Elite the lowest (Tab. 6). Therefore, under the controlled conditions of the current study, the modern cultivar did not have a higher yield than the older cultivars. Such results disagree with those reported by Todeschini *et al.* (2019), who tested 29 soybean cultivars released between 1965 and 2011. In the referred study, modern cultivars showed higher yield, harvest index, and number of pods per plant than older cultivars. The behavior observed in the present study was contrary to the initial hypothesis that yields gains provided by genetic improvement programs are greater when leaf area is preserved. Regardless of the level of defoliation, Brasmax Elite produced fewer pods and grains per plant than cultivars released in the last century.

One factor that might have prevented Brasmax Elite from expressing its maximum productive potential is the cultivar's high nutritional requirement. Nearly 60 kg of P_2O_5, 60 kg of K_2O and 250 kg of N are required to produce 3000 kg ha^{-1} of soybean grains (Oliveira *et al.,* 2007). Contemporary soybean production is characterized by high technological levels (Balbinot Junior *et al.,* 2017). Farmers must employ various resources to meet the nutritional requirements of modern cultivars, thereby increasing yields and profit margins. All cultivars used in the experiment received the same fertilizer amount. It is possible that the fertilization rates used in the trial were not sufficient to meet the demands of Brasmax Elite. Other factors that may help to explain the results are that the experiment was conducted in a greenhouse, and each experimental unit comprised only one plant. This experimental setup may not reflect what occurs in the field, where intraspecific competition is more pronounced, particularly in older cultivars, which belong to later maturity groups and have greater leaf areas (Barros *et al*., 2002).

The reduction in leaf area did not significantly affect yield or its components in any of the cultivars used in this study. Possibly, this behavior was due to the phenological stage when the stress was imposed (R3). According to Parcianello *et al.* (2004), the most critical stage for losses in the photosynthetic area is the beginning of grain filling (R5), when yield loss is proportional to the increase in defoliation intensity. Gazzoni and Moscardi (1998) studied four levels of defoliation (0, 33, 67, and 100%) at four stages of development (V3, V8, R2, and R6) in the cultivar Paraná. Only defoliation levels above 67% affected grain production at the R6 stage. Other major agronomic characteristics, such as maturity date, lodging, and plant height, were not affected by levels. Ribeiro and Costa (2000) subjected BR 16 to different levels of defoliation (0, 17, 33, 50, 67, and 100%) at different development stages (V9, R3, R5, and R6). They found that yield decreased only in plants subjected to defoliation levels greater than 67%. Therefore, our results agree with Gazzoni and Moscardi (1998) and Ribeiro and Costa (2000), who evaluated two of the five cultivars used here.

Another factor that may explain why the defoliation level did not significantly influence the number of grains per plant was the fact that the experiment was set under greenhouse conditions, with experimental units consisting of one plant. In this environment, the amount of light received by leaves from the lower third of the stem is greater than that obtained under field conditions, where crop densities range from 200,000 to 400,000 plants ha^{-1}. Lower leaves may act as photoassimilate sinks in the field because they are shaded during the reproductive stage (Lopes & Lima, 2015). On the other hand, under greenhouse conditions, such leaves may act as sources of photoassimilates, as they receive high amounts of solar radiation. This change in source/sink ratio might have mitigated the negative effects of defoliation on grain production among cultivars.

Conclusions

Defoliation levels up to 66.6% applied at the R3 stage did not affect net carbon assimilation at R5 or the number of grains per plant of the evaluated soybean cultivars grown in a protected environment. There were no significant differences on the photosynthetic efficiency and sensibility to defoliation between the old cultivars Davis, Paraná, Br 16 and FT Abyara and the modern cultivar Elite. The cultivars with the highest mass of 1,000 grains were Davis and Paraná, with 183.8 g and 180.8 g, respectively. The modern cultivar Brasmax Elite did not show increased yield compared with older cultivars, regardless of defoliation level.

Conflict of interest statement

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

Author's contributions

TLT and LS designed the experiments; TLT, MCMJr, HFK, RK, LAT, JFM, and VLO carried out the field and laboratory experiments; TLT, MCMJr, HFK, LS, AEC and RK contributed to the data analysis; LS, TLT, MCMJr, HFK, AEC, LAT, JFM, RK, and VLO wrote the article. All authors reviewed the final version of the manuscript.

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Evaluation of the growth of maize in monoculture and when associated with peanuts and cassava in the Colombian Amazon

Evaluación del crecimiento de maíz en monocultivo y asociado con maní y yuca en la Amazonia colombiana

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ABSTRACT

Crop associations are widely recognized as a highly beneficial strategy for agriculture. By combining different crops, optimal production is achieved while minimizing the spread of pests and diseases. This practice offers numerous benefits by allowing maximum utilization of space and mutual adaptation of associated species. It is important to emphasize that crop association is oriented towards the mutual advantage of the species involved, thus guaranteeing favorable results for each of them. In this sense, the behavior of maize growth rates was evaluated in a completely randomized block design with four treatments: maize monoculture; maize and peanut association; maize and cassava association; and maize, peanut, and cassava association. The following variables were evaluated: net assimilation rate (NAR), leaf area index (LAI), relative growth rate (RGR), leaf area ratio (LAR), absolute growth rate (AGR), and leaf area duration (LAD). The association of maize and cassava obtained the highest values NAR (0.002 g cm^{-2} d^{-1}) and RGR (0.15 g g^{-1} d^{-1}) compared to the monoculture (0.001 g cm^{-2} d^{-1} and 0.08 g g^{-1} d^{-1}). This happened because maize presented higher leaf production during the vegetative growth stage indicating the physiological efficiency of maize when associated with cassava.

Key words: companion crop, efficiency, physiology, growth index.

RESUMEN

Las asociaciones de cultivos son ampliamente reconocidas como una estrategia altamente beneficiosa para la agricultura. Al combinar diferentes cultivos, se logra una producción óptima mientras se minimiza la propagación de plagas y enfermedades. Esta práctica ofrece numerosos beneficios al permitir la máxima utilización del espacio y la adaptación mutua de las especies asociadas. Es importante recalcar que la asociación de cultivos está orientada hacia el beneficio mutuo de las especies involucradas, garantizando así resultados favorables para cada una de ellas. En este sentido, se evaluó el comportamiento de los índices de crecimiento del maíz en un diseño en bloques completamente al azar con cuatro tratamientos: monocultivo de maíz; asociación de maíz y maní; asociación de maíz y yuca; y asociación de maíz, maní y yuca. Se evaluaron las siguientes variables: tasa de asimilación neta (TAN), índice de área foliar (IAF), tasa relativa de crecimiento (TRC), relación de área foliar (RAF), tasa absoluta de crecimiento (TAC) y duración de área foliar (DAF). La asociación de maíz y yuca obtuvo los valores más altos de TAN (0.002 g cm^{-2} d^{-1}) y TRC (0.15 g g^{-1} d^{-1}) en comparación con el monocultivo (0.001 g cm^{-2} d^{-1} y 0.08 g g^{-1} d^{-1}). Esto sucedió porque el maíz presentó una mayor producción de hojas durante la etapa de crecimiento vegetativo, lo que indica una mayor eficiencia fisiológica del maíz cuando se encuentra asociado con la yuca.

Palabras clave: cultivo asociado, eficiencia, fisiología, índice de crecimiento.

Introduction

Agriculture encompasses human activities and accumulated knowledge that have resulted in the domestication of animals and plants as a source of provisions for human consumption (Arenas *et al.*, 2004). Over time, agricultural methods have evolved, increasing productivity and crop diversity in different agroecosystems. It is necessary to address comprehensive food needs through sustainable agriculture to ensure future food security (Gómez-Rodríguez & Zavaleta-Mejía, 2001).

So, crop diversity is presented as a sustainable alternative for food production, since it combats hunger, promotes food security, and fosters sustainable agriculture (Gómez, 2018). It is crucial to implement more efficient planting strategies, such as multiple or diversified crops that benefit producers by improving their investments, optimizing land use,

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and reducing their production costs (Esquivel *et al*., 2019). This will allow better use of space and time, increasing crop productivity and achieving income for families that produce food on a small scale.

These diversified crops increase productivity and resource use efficiency (Castillo *et al*., 2022). They reduce the presence of weeds (Pietrobón *et al*., 2019), lead to greater integral soil fertility, recycle and retain nutrients (Bover-Felices & Suárez-Hernández, 2020); and they have a greater capacity to recover from disturbances, such as pests and diseases (López-Rivera *et al*., 2020) and extreme weather events, such as droughts and floods (Céspedes & Vargas, 2021).

In this way, the association of leguminous species, grasses, and tubers benefits the soil with the contribution of atmospheric nitrogen, light interception, and wide biomass distribution (Bedoussac *et al*., 2015). This practice is widely used in organic agriculture to promote species diversity, translating into higher total biomass and better production yields. A study by Colina *et al*. (2020) in Venezuela evaluated the yield of the association of cassava (*Manihot esculenta* Crantz), maize (*Zea mays* L.), and plantain (*Musa* AAB) and found that inter-species competition affected crop yield compared to a monoculture of each species (Jiménez, 2016).

It is, therefore, essential to consider several parameters when planting, since not all associations are beneficial. Plants have different characteristics that can affect the optimal development of associated species, so it is essential to know the favorable interactions and avoid unfavorable ones (Tamayo & Alegre, 2022).

Nowadays, significant advances have been made that demonstrate that the implementation of multiple crops leads to higher productivity (Jaramillo & Salazar, 2021; Marcía, 2021; Yanes-Simón *et al*., 2022). The present study aimed to evaluate the effect of the association of maize (*Zea mays* L.), peanut (*Arachis hypogaea* L.) and cassava (*Manihot esculenta* Crantz) crops, to analyze the growth rates of maize in various associations. In this respect, this research allows identifying the physiological efficiency of the maize crop when combined with other species.

Materials and methods

Geographic location

The study was conducted at the Centro de Investigaciones Amazónicas Macagual (CIMAZ) of the Universidad de la Amazonía, an area corresponding to the tropical rainforest life zone, located in the village of La Viciosa, in the municipality of Florencia (Caquetá), at coordinates 1°30'4.39" N, 75°39'44.8" W at an altitude of 250 m, relative humidity of 85.1%, average temperature 24°C, average annual rainfall of 3,695 mm, and sunshine of 4.6 h d^{-1} (Bonilla *et al.,* 2019; Aldana *et al*., 2021; Álvarez *et al.,* 2021).

Study design

The experimental area consisted of 33×52 m. Each plot was 10×5 m, with a separation between treatments and between replicates of 0.5 m. In each of the plots there were ridges 0.2×0.3 m. A completely randomized block design was used with four treatments and five replicates (each replicate with 528, 322 and 80 of maize, peanut and cassava plants).

The treatments corresponded to the following: maize monoculture with a planting distance of 0.4 m between plants and 0.8 m between rows; association of maize (with the planting distance of maize) and peanut (in the middle of the maize rows, with a planting distance between plants of 0.2 m); association of maize (with the planting distance of maize) and cassava (in the middle of the maize rows, with a planting distance between plants of 1 m); and association of maize (with the planting distance of maize), peanut (planting distance between plants of 0.2 m and between rows of 2.4 m) and cassava (planting distance between plants of 1 m and between rows of 2.4 m).

The ICA V-105 certified maize variety was used (Arboleda & Cassalett, 1970), and native varieties known as var. *red* (type Valencia) and var. *quindiana*, respectively, were used for peanuts and cassava (Vicaría del Sur, 2018). The species were planted on the same date; for maize and peanut, two seeds were planted per site; and for cassava, a 0.25 m long stem cutting with 3 buds was planted. At the time of planting, bokashi fertilizer was applied at a dose of 1 kg m^{-2}. Manual weed management was carried out every 30 d, and pest and disease monitoring were performed on each of the crops every 15 d. Four monitoring periods were carried out, randomly selecting 20 plants per species in a 1 m Z-shaped transept in each plot, visually verifying the presence or absence of pests and diseases on the underside and beam of leaves, stems, and the base of the stem (Velasteguí *et al*., 2010; Peralta *et al*., 2021). During the evaluation time, no diseases were recorded for any of the crops. *Spodoptera frugiperda* was found on maize leaves at stages V0 to V10 (Mora & Blanco, 2018; Abbas *et al.,* 2022; Varón de Agudelo *et al*., 2022), and garlic and chili bell pepper extract was applied at a dose of 0.1 L per m^2 with a frequency of 12 d (Ramón & Rodas, 2007; Salazar, 2010; Yaranga, 2014).

Sampling

We collected five samples starting at 21 d after planting (DAP) with a frequency of 10 d until 65 DAP. In each of the plots, ten maize plants were randomly selected for analysis.

Parameters evaluated

- Soil area: An imaginary circumference was traced around the apexes of all the leaves of the plant and was recorded in cm^2.
- Leaf area of the plant: All the leaves of each plant were placed on a white sheet at a distance of 10 cm apart; they were photographed, and the leaf area was determined with ImageJ software (Newton *et al*., 2020).
- Fresh weight: The fresh weight of all parts of the plant was recorded as follow: leaves, stem, and roots using a 0.01 g precision scale.
- Dry weight: All plant parts were placed in paper bags and into a drying oven at 80°C for 72 h; the weight was then recorded on a 0.01 g precision scale.
- Growth rates were determined from measurements of leaf area, soil area, and dry weight (Tab. 1).

Statistical analysis

Python 3.6 software with Panda, Numpy, Pingouin, and Matplotlib libraries were used. Initially, the growth index, leaf area index (LAI), and leaf area ratio (LAR) were used as growth indices. Descriptive analyses were performed using box plots to determine the distribution of block treatments and days after planting. Descriptive results were obtained to show the presence of outliers and asymmetric distributions. Subsequently, completely randomized block design tests and assumptions of normality, constant variance (equality of variances in treatments), independence (P>0.05), and interaction plots were applied. Finally, a multiple pairwise comparison analysis (*post hoc* comparison) was performed using the Tukey's HSD test.

Results and discussion

Leaf area index

The leaf area index (LAI) is a measure that evaluates physiological aspects and the total amount of photosynthetic radiation absorbed by the plants. A higher LAI is associated with an increase in biomass production (Barraza *et al.,* 2004; Zavala-Borrego *et al.,* 2022). In the maize treatment

TABLE 1. Formulas and units for growth rate indices in plants (Melgarejo, 2010).

Growth index	Symbol	Average value over a time interval (T_2-T_1)	Units
Relative growth rate	RGR	$RGR = \frac{(LnW2 - LnW1)}{(T2 - T1)}$	$g\ g^{-1}\ d^{-1}$
Net assimilation rate	NAR	$NAR = \frac{\frac{W2 - W1}{T2 - T1}}{\frac{LnLA2 - LnLA1}{LA2 - LA1}}$	$g\ cm^{-2}\ d^{-1}$
Leaf area index	LAI	$LAI = \frac{\frac{LA1 + LA2}{2}}{\frac{1}{SA}}$	Adimensional
Absolute growth rate	AGR	$AGR = \frac{\frac{1}{SA} \times (W2 - W1)}{(T2 - T1)}$	$g\ d^{-1}$
Leaf area duration	LAD	$LAD = \frac{(LAI1 + LAI2) \times (T2 - T1)}{2}$	d
Leaf area relation	LAR	$LAR = \frac{(LAI2 - LAI1)}{(W2 - W1)}$	$cm^2\ g^{-1}$

Ln: natural logarithm; *W*1 and *W*2: dry weight 1 and 2; *T*1 and *T*2: time 1 and 2; *LA*1 and *LA*2: leaf area 1 and 2; *SA*: soil area.

associated with peanut, a value of 0.3 was observed at 25 DAP; this value gradually increased until reaching a maximum value of 0.5 after 55 DAP. The association of maize and cassava did not show a significant variation, but it did show a similar behavior to the previous treatment, reaching its maximum value at 45 DAP with an LAI of 0.4 (Fig. 1).

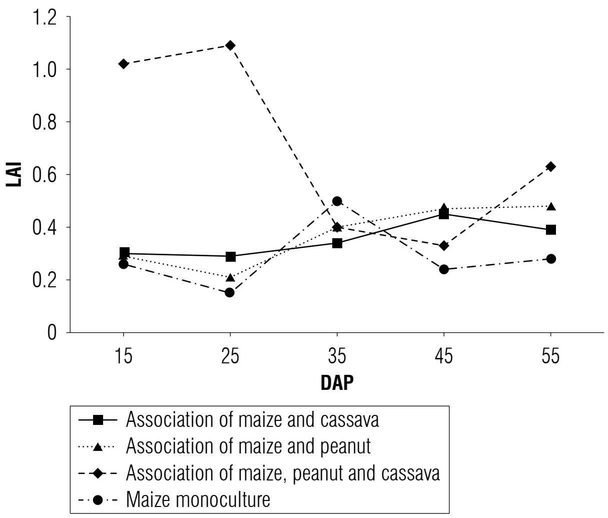

FIGURE 1. Leaf area index (LAI) behavior in relation to treatments and days after planting (DAP).

In the maize-peanut-cassava association, the LAI was higher than 1.0 during the first 25 DAP and then progressively decreased to a value of 0.3 at 45 DAP; this subsequently increased to 0.6 at 55 DAP. The variability in LAI can be attributed to temporal and spatial differences as well as to factors affecting the crops, since this association involves the presence of multiple species; and greater variability is to be expected due to the influence of diverse factors (Nafarrate, 2017).

The monoculture maize treatment obtained its maximum LAI value at 35 DAP, later reaching the lowest value at 55 DAP compared to the other treatments. In general, it can be observed that the LAI in the different associations shows maximum values after 30 DAP, indicating that there is a greater capacity of the plant to take advantage of energy after 30 DAP (Castellanos *et al.*, 2017). These findings coincide with the vegetative development of maize plants at the V10 stage that corresponds to the presence of 10 true leaves (Castellanos *et al.*, 2017), at which time the maximum uptake of solar radiation and its transformation into biomass occurs, with most of the assimilates to be used in the formation of the cob (Rincón *et al.*, 2007). However, these values for the association of maize, cassava, and peanut, and maize in monoculture decreased during this stage. According to Barrera-Violeth *et al.* (2017), this could be attributed to the effect of shading generated by the leaves within the crop itself that limits the assimilation of solar energy.

Leaf area ratio

The leaf area ratio (LAR) represents the relationship between photosynthetic activity and the cost of respiration (Hernández *et al.*, 1999). In Figure 2, the highest values of LAR were found in the initial stages of the crops, gradually decreasing as time progressed.

In the association of maize and peanut, the maximum LAR value was reached at 35 DAP, with 180 $cm^2 g^{-1}$. The other treatments showed similar behavior in terms of LAR values. In each of the treatments, the highest values were recorded at the beginning of the crop and then gradually decreased as the maize crop aged. The association of maize, peanut, and cassava showed the highest LAR during the first 25 DAP, reaching a value of 250 $cm^2 g^{-1}$. However, a progressive decrease was observed, reaching 50 $cm^2 g^{-1}$. LAR tends to be high at the beginning of a crop´s growth and decreases as developmental stages advance (Orozco-Vidal *et al.*, 2016). This is because plants allocate most of the photoassimilates to the development of their photosynthetic apparatus; and as the crop progresses, the plant deposits carbohydrates in its reproductive organs. In addition, during the initial stages of development, the plant is mainly exposed to photosynthetic activity and the generation of leaf tissues, meaning a low cost of respiration (Ayala, 2016).

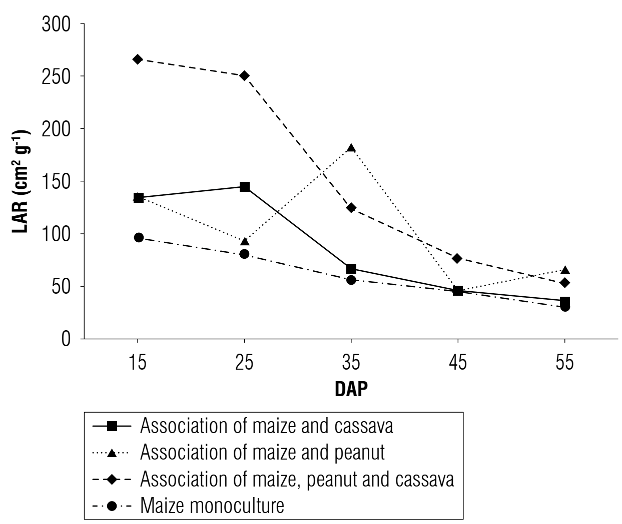

FIGURE 2. Leaf area ratio (LAR) behavior for treatments and the following days after planting (DAP).

The association of maize, peanut, and cassava showed higher LAR values during the first 30 DAP, this is because the presence of more species in this treatment favors the production of a greater number of leaves. During this initial stage, the plants are in their vegetative development phase, implying significant leaf growth and, therefore, an increase in the LAI (Hernández *et al*., 1995). With respect to the peanut and maize association, LAR increased between 25 and 35 DAP. This increase can be attributed to the growth in leaf size during this period. In general, all treatments showed a decreasing trend in LAR as time progressed. This phenomenon may be related to the fact that, as the plants grow, there is an increase in overall plant size, but a decrease in the increase of leaf area (Guevara & Guenni, 2007).

Leaf area duration

The leaf area duration (LAD) is a variable that represents the relationship between the photosynthesizing surface and the soil area occupied by the crop (Santos *et al*., 2010). So it is useful to determine the productivity and water requirements of crops. In this sense, the LAD allows for estimating the water requirements, nutritional and bioenergetic efficiency and determines the possible phytosanitary damages that the crop may have in its growth process (Mendoza-Pérez *et al.,* 2017).

Figure 3 shows that the associations of maize and cassava and maize and peanut were similar in their LAD values at 25-35 DAP, reaching an average value of 3.1 d. However, both the association of maize and peanut, as well as the association of maize, peanut, and cassava, increased at 45-55 DAP with a value of 4.8 d, followed by the association of maize and cassava that showd a value of 4.2 d. The maize monoculture was the one that showed the lowest LAD, reaching a value of 2.6 d at 45-55 DAP.

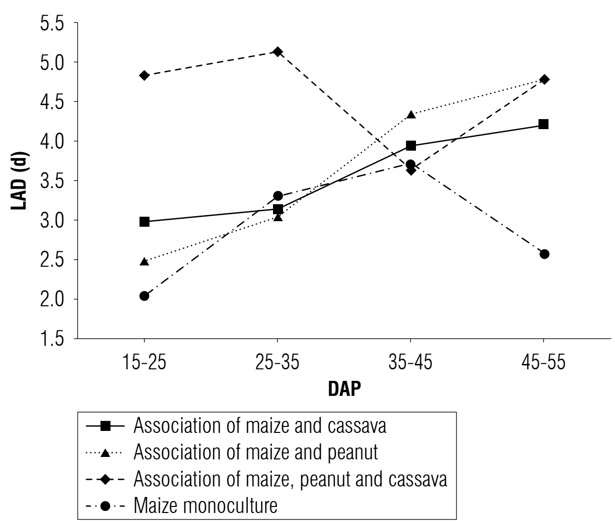

FIGURE 3. Leaf area duration (LAD) behavior in relation to treatments and days after planting (DAP).

The associations showed an increase in the LAD as time progressed. This increase was because the maize plant, after germination, went through a vegetative development stage that extends up to 60 DAP. In the associations of maize, peanut, and cassava, a higher LAD was observed compared to the maize monoculture, indicating a greater production of leaves during the vegetative growth stage of maize (Aguilar-Carpio *et al.,* 2015).

The increasing trend of all associations in general is related to the constant formation of leaf structures and biomass. This is because plants are in a constant process of capturing solar radiation over time, stimulating the growth and development of leaves (Soplín *et al*., 1993; Aguilar-García *et al*., 2005).

Absolute growth rate

The absolute growth rate (AGR) is used to measure the increase in dry mass over a given period (Barrera-Violeth *et al*., 2017). In the maize and peanut association, a significant increase was observed from 15-25 DAP to 25-35 DAP, reaching an AGR of 1.8 g d^{-1}. Then, between 45-55 DAP, this association reached its highest value with an AGR of 2.4 g d^{-1}. These results indicate that this association experienced a rapid increase in the production of dry matter compared to the other associations and the monoculture (Almanza-Merchán *et al*., 2016).

In contrast, the AGR of the maize-cassava association, as well as that of maize in monoculture, showed a similar trend during crop development, reaching values above 1.5 g d^{-1} up to 35-45 DAP. However, in the case of the association of maize and cassava, a later decrease was observed, reaching 1.0 g d^{-1} at 45-55 DAP (Fig. 4). During the vegetative stage of maize, the plants require a high amount of nutrients, and when these are insufficient, there may be a decrease in photosynthesis and in the production of assimilates (Sánchez-Olaya *et al*., 2019). This could explain the decrease in AGR observed in this association, suggesting possible nutrient deficiencies.

In the association of the three crops, a significant increase in AGR was not observed. However, it reached its maximum value of 2.3 g d^{-1} at 45-55 DAP. In comparison, the maize monoculture at 45-55 DAP had a value of 1.6 g d^{-1}. In general, the association of maize and peanut showed the highest AGR as a function of DAP (Fig. 4). An increase in AGR was observed from 35-45 DAP for all treatments, except for the maize and cassava association. Possibly at this stage the plant was in its vegetative growth phase that implied a greater photosynthetic capacity and, therefore, higher biomass production (Barrera-Violeth *et al.,* 2017).

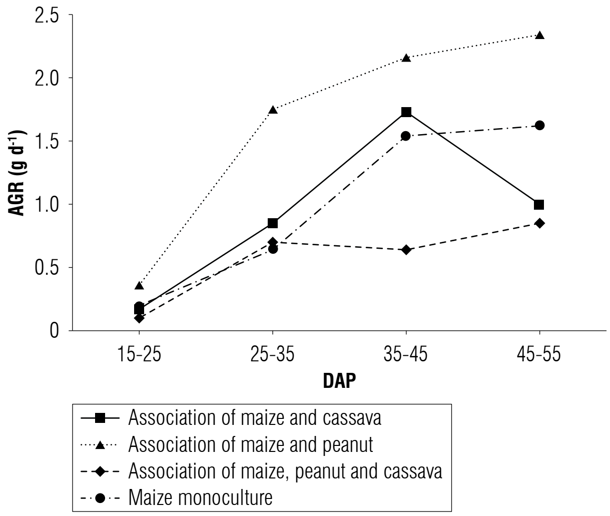

FIGURE 4. Average growth rate (AGR) behavior in relation to treatments and days after planting (DAP).

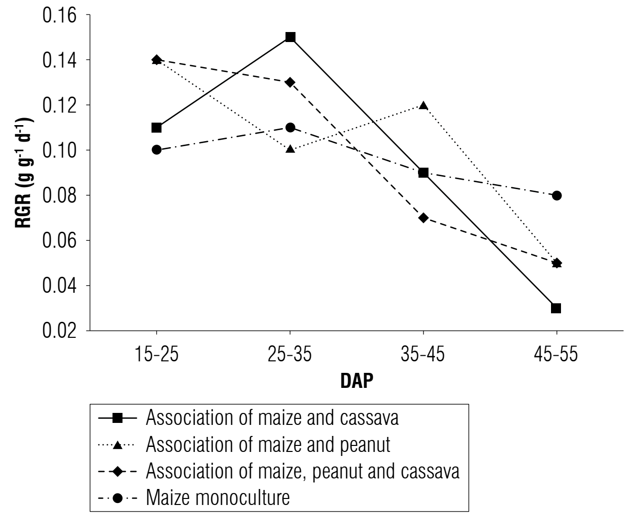

FIGURE 5. Relative growth rate (RGR) behavior in relation to treatments and days after planting (DAP).

Relative growth rate

Relative growth rate (RGR) is an important parameter for analyzing plant performance, since it represents the biomass gain at a given period of time. RGR reflects the morphological and physiological adaptations of plants in different environments and can be used as a measure of plant growth success (Gil & Miranda, 2007; Mayo-Mendoza *et al*., 2018).

The association of maize with cassava reached its highest RGR at 25-35 DAP, with a value of 0.15 g g^{-1} d^{-1}, and then it decreased considerably until 45-55 DAP with an RGR lower than 0.04 g g^{-1} d^{-1}. The association of maize and peanut showed greater variability compared to the other treatments. The maximum value was 0.12 g g^{-1} d^{-1} between 35-45 DAP, decreasing progressively until 45-55 DAP. The RGR of the association of maize, peanut, and cassava decreased after 35-45 DAP until the association reached a value of 0.05 g g^{-1} d^{-1} at 45-55 DAP. Finally, the maize monoculture showed no significant variation, remaining in a range of 0.10 to 0.08 g g^{-1} d^{-1} (Fig. 5).

In general, a considerable reduction in RGR is observed in all treatments as DAP increases. This decrease can be attributed to the change of plants from vegetative to reproductive stages and preparation for fruit/seed growth. As the crop progresses, the decrease in RGR becomes constant (Almanza-Merchán *et al*., 2016).

Differences in RGR between treatments are due to species-specific characteristics (Sánchez-Olaya *et al*., 2019) since significant differences in RGR values may result from interactions between species and mutual influences on their growth.

The RGR at the beginning of the crop shows high values and then decreases progressively at the end of the vegetative stage of the plants (Barrera-Violeth *et al*., 2017). Due to this, it is evident that the maize and cassava treatment had a higher RGR value compared to the others, reflecting the highest value for this association. However, the association of maize and peanut had a significant decrease in behavior at the beginning of the crop. The reduction could be attributed to competition between both species during the process of establishment and colonization of space in the soil; such competition may influence the behavior of the RGR (Mayo-Mendoza *et al*., 2018).

Net assimilation rate

The net assimilation rate (NAR) in crops refers to the capacity of plants to accumulate dry matter that, in turn, determines their efficiency. The NAR allows for evaluating plant efficiency of biomass accumulation (Cardona *et al*., 2021). López-Sandoval *et al*. (2018) mention that NAR is a measure that reflects the efficiency of the foliage that is the principal source of photosynthates for dry matter production.

The associations of maize and peanut and maize and cassava presented a similar trend in the NAR; both treatments reached their maximum values at 25-35 DAP and then decreased until 45-55 DAP. In the association of maize, peanut, and cassava, a variation in the NAR was observed at 25-35 DAP, with a value of 0.002 g cm^{-2} d^{-1}, followed by a decrease at 35-45 DAP. In contrast, maize in monoculture showed a different trend compared to the other treatments (Fig. 6) since it reached its highest NAR at 45-55 DAP with a value of 0.0075 g cm^{-2} d^{-1}, suggesting that maize in monoculture achieved greater efficiency in the utilization of its foliage in that period compared to the other associations.

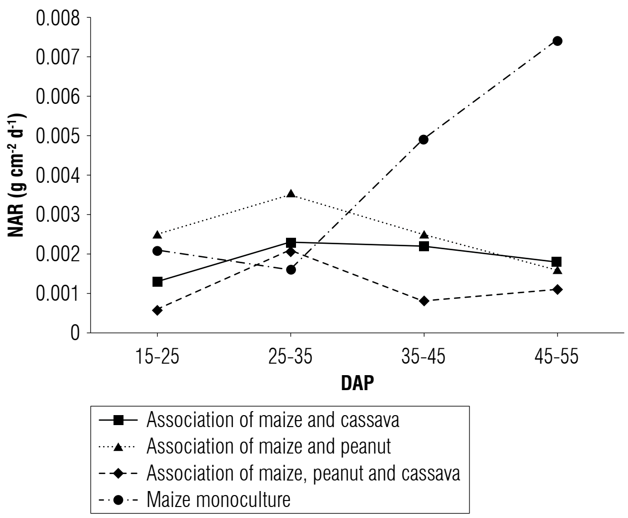

FIGURE 6. Net assimilation rate (NAR) behavior in relation to treatments and days after planting (DAP).

Shading caused by the joint growth of crops in the associations may have led to a reduction in the photosynthetic capacity of the lower leaves due to the shade cast by the upper parts of the plants. This results in a decrease in NAR due to increased biomass production by the different species in the association. In contrast, the maize monoculture, being the only species present, has a lower amount of biomass, suggesting a higher NAR (López-Sandoval *et al.*, 2018).

This indicates that the population density of the species interferes with the NAR (Aguilar-García *et al.*, 2005). In addition, the maize monoculture has higher increments compared to associations, suggesting that maize is better adapted to open spaces under monoculture conditions (Mayo-Mendoza *et al.*, 2018).

Conclusions

The growth stage of plants plays a crucial role in LAI and LAR, since in the early stages, growth focuses on the development of leaf tissues and photosynthetic apparatus, while in later stages growth allocates more resources to reproductive organs. This implies the need to consider the growth stage to optimize the relationship between photosynthetic activity and the cost of respiration.

Competition between species can affect initial growth, but certain associations, such as that of maize and cassava, can result in greater biomass gain over time as observed in the RGR. In turn, the NAR is influenced by the population density of the species and the interactions between them, since shading caused by the joint growth of the crops can reduce the photosynthetic capacity of the lower leaves and decrease the NAR.

These results highlight the importance of considering species diversity and species interactions to maximize the capture of solar radiation and to optimize biomass production in agriculture.

Conflict of interest statement

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

Author's contributions

DMSO, KTRR and ELSG designed and developed the field experiment, WSR and MFRO contributed to data analysis, KTRR and ELSG wrote and prepared the original draft, DMSO, MFRO and WSR wrote, corrected, and edited the final version of the manuscript. All authors reviewed the final version of the manuscript.

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Inoculation with mycorrhiza and *Azotobacter chroococcum* affects the quantitative and qualitative characteristics of *Eryngium caeruleum* at different planting densities

La inoculación con micorrizas y *Azotobacter chroococcum* afecta las características cuantitativas y cualitativas de *Eryngium caeruleum* a diferentes densidades de siembra

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ABSTRACT

Eryngium caeruleum is a perennial native plant that grows under diverse climatic conditions of Iran. This study aimed to investigate the effect of mycorrhizal and *Azotobacter* inoculation on the quantitative and qualitative characteristics of *E. caeruleum* at various planting densities. Factors included three levels of plant density (10×30 cm, 20×30 cm, and 30×30 cm), two levels of seed inoculation with Vesicular-Arbuscular Mycorrhiza (VAM) fungi (inoculation with *Glomus mosseae* and without inoculation), and two levels of seed inoculation with *Azotobacter chroococcum* (with and without inoculation). The application of VAM fungi and *A. chroococcum* significantly affected the measured traits. The interaction effect of plant density×mycorrhizal application and plant density×*Azotobacter* application on dry leaf weight was significant at a 1% level. The total leaf dry weight for the VAM treatments at a 30×30 cm plant density was 2.93 g. Also, the application of mycorrhizal fungus increased the essential oil percentage, the essential oil yield, and phosphorus concentration in the aerial organs by 132.68%, 100%, and 137.5%, respectively, compared to the control treatment. The simultaneous application of *A. chroococcum* and VAM improved the quantity and quality of the yield components of *E. caeruleum* by increasing the availability of mineral nutrients.

Key words: oil crop, endemic herb, free-living N_2 fixers, *Glomus mosseae*, local cuisine.

RESUMEN

Eryngium caeruleum es una planta perenne nativa que crece en diversas condiciones climáticas de Irán. Este estudio tuvo como objetivo investigar el efecto de la inoculación de micorrizas y *Azotobacter* sobre las características cuantitativas y cualitativas de *E. caeruleum* en diferentes densidades de siembra. Los factores incluyeron tres niveles de densidad de plantas (10×30 cm, 20×30 cm y 30×30 cm), dos niveles de inoculación de semillas con hongos Vesiculo-Arbuscular Micorriza (VAM) (inoculación con *Glomus mosseae* y sin inoculación) y dos niveles de inoculación de semillas con *Azotobacter chroococcum* (con y sin inoculación). La aplicación de hongos VAM y *A. chroococcum* afectó significativamente las características medidas. El efecto de interacción densidad de plantas×aplicación de micorrizas y densidad de plantas×aplicación de *Azotobacter* sobre el peso seco de la hoja fue significativo a un nivel del 1%. El peso seco total de las hojas para los tratamientos VAM a una densidad de plantas de 30×30 cm fue de 2.93 g. Además, la aplicación del hongo micorrícico incrementó el porcentaje de aceite esencial, el rendimiento de aceite esencial y la concentración de fósforo en los órganos aéreos en 132.68%, 100% y 137.5%, respectivamente, en comparación con el tratamiento control. La aplicación simultánea de *A. chroococcum* y hongos VAM mejoró la cantidad y calidad de los componentes del rendimiento en *E. caeruleum* al aumentar la disponibilidad de nutrientes minerales.

Palabras clave: cultivo oleaginoso, hierba endémica, fijadores de N_2 de vida libre, *Glomus mosseae*, gastronomía local.

Introduction

Eryngium caeruleum is one of the endemic plants of Iran with almost unknown potential that is neglected. It is an herbaceous perennial plant growing in northern Iran. *E. caeruleum* belongs to the family Apiaceae, represented by different species in the country. The plants of this family, especially of the genus *Eryngium*, could have a great potential for the future production of herbal medicines. This plant is used as a medicinal plant and as a vegetable. The leaves of this plant are used to flavor cooked vegetables in various local dishes and used in soups or consumed mixed with yogurt (Khoshbakht *et al*., 2007). Few studies have reported the content of total phenolic compounds and

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total flavonoids, and antioxidant properties of the alcoholic extract of the leaves (Dehghan *et al*., 2016). This plant is widely used in the medicine and food industry as a diuretic and appetite stimulant (Hashemabadi & Kaviani, 2011). The shoots and roots contain terpenoids, saponins, flavonoids, phenolic acids, polystyrene, and other biologically active compounds (Hashemabadi & Kaviani, 2011; Erdem *et al*., 2015). Various species of *Eryngium* contain numerous phytochemical compounds, and at least 127 compounds have been isolated and identified (Wang *et al*., 2012; Erdem *et al*., 2015). In addition, the plants of this genus contain various nutrients, including vitamins, minerals, and proteins (Paul *et al*., 2011).

Using chemical fertilizers in intensive agriculture may cause damage to plant production, soil health, and environment (Anwar *et al*., 2005). One of food security's most essential tasks is finding a suitable alternative to mineral fertilizers. The employment of these fertilizers has also increased climate pollution (Elser & Bennett, 2011; Tubiello *et al*., 2015; Bauer *et al*., 2016). Moreover, biodiversity has decreased significantly in agriculture due to habitat loss (Tilman *et al*., 2017). The microorganisms and bacteria in soil affect the cycling of mineral nutrients such as nitrogen, sulfur, and phosphorus (Kumar *et al*., 2018). Considering the beneficial microbial activities, biofertilizers have significant potential to increase the health and productivity of plants and reduce the need for synthetic fertilizers. Arbuscular mycorrhizal fungi (VAM) can form a symbiotic association with plants that benefits both partners through the acquisition and uptake of mineral nutrients, especially phosphorus, from the soil (Barea *et al*., 2011; Hoseinzade *et al*., 2016). Mycorrhiza, as a critical component in ecosystems, positively affects quantitative and qualitative characteristics of plants (Harrier & Watson, 2004; Gosling *et al*., 2006) Examples of the role of VAM in agricultural ecosystems include increasing the active surface area of the root system for better uptake of nutrients from soil, especially under phosphorus deficiency (Kapoor *et al*., 2007), increasing photosynthesis (Copetta *et al*., 2006), increasing resistance to drought, salinity, and resistance to pests and diseases (Feng *et al*., 2002; Pinior *et al*., 2005; Samarbakhsh *et al*., 2009), improving soil structure (Celik *et al*., 2004), and increasing the activity of N_2-fixing bacteria (Antunes *et al*., 2006). The variable performance of mycorrhizal fungi depends on the host plant; many studies have reported the positive effects of symbiosis of plants with mycorrhizal fungi (Jansa *et al*., 2008; Pellegrino & Bedini, 2014: Derkowska *et al*., 2015; Palencia *et al*., 2015). Koozehgar Kaleji *et al*. (2021) found that using mycorrhiza and organic fertilizers increased the quantitative and qualitative yield of *Nasturtium officinale*.

Azotobacter is a free-living N_2-fixing bacterium that produces various siderophores and can increase the absorption capacity of Zn, Fe, and Mo by plants, as well as the solubility of phosphorus from insoluble compounds; the use of these microorganisms constitutes one of the most effective ways to improve nutrient mobility and absorption (Mrkovacki *et al*., 2001). *Azotobacter* are free-living rhizobacteria that promote plant growth by producing auxins, gibberellins, and cytokinins, and making mineral nutrients, especially nitrogen, available to plants (Jnawali *et al*., 2015). Inoculation with different *Azotobacter* species increased nutrient availability and synthesis of biologically active compounds, thus, positively affecting plant growth and yield (Rojas-Tapias *et al*., 2012; Delshadi *et al*., 2017; Turan *et al*., 2017; Rodrigues *et al*., 2018).

Increasing planting density increases competition between plants for growth factors such as adequate space for branching and root growth and the acquisition of light, mineral nutrients, and moisture (Bairagi, 2014). Ahmad and Abdulla (2016) compared the effects of three planting densities (20×20 cm, 30×30 cm, and 40×40 cm) on fenugreek and observed the highest plant dry weight at the highest plant density (40×40 cm). This study evaluated the effects of the joint application of *Azotobacter chroococcum* and VAM with various plant densities on growth performance and shoot nutrient content of *E. caeruleum*.

Materials and methods

The experiment was conducted in 2016-2017 in Aboksar, a village in Mazandaran province (northern part of Iran) located at 53º62' E, 36º46' N, and an elevation of 17.3 m a.s.l. with moderate climate (Tab. 1) in a factorial randomized block design with five replicates with 685 plants per replicate and a total 3425 plants.

Factors included three levels of planting density of the *E. caeruleum* plants (10×30 cm, 20×30 cm, and 30×30 cm), two levels of seed inoculation with VAM fungi (without and with inoculation with *Glomus mosseae*, a soil-based inoculum of a local isolate, which consisted of 1200 spores/100 g of soil) and two levels of seed inoculation with *Azotobacter chroococcum* (without and with inoculation); a bacterial culture was produced by the Soil and Water Research Institute, Iran Ministry of Agriculture, using 0.5 L ha^{-1} at the time point (CFU=108). Seeds from

TABLE 1. Climate conditions of the experiment site.

Year	Month	Air temperature (°C)			Total rainfall (mm)	Relative air humidity (%)			Solar radiation (MJ m^{-2})	Total hours of sunshine
		Average	Max	Min		Average	Max	Min		
2016	September	21.6	26.7	16.5	205.9	76	95	57	448.3	184.5
	October	15.2	19.5	10.8	89.7	79	96	61	407.6	147.7
	November	10.7	15.3	6.1	133.2	79	96	62	261	144.1
	December	7.2	12.5	1.9	5.7	76	94	57	270	166.9
2017	January	5.2	11.5	1.4	25.4	79	95	60	259.4	153.4
	February	11.0	15.5	6.4	26.6	77	94	59	349.3	124.8
	March	14.2	19.8	8.6	53.3	76	97	54	407.2	217.4
	April	21.3	27.2	15.7	4.4	70	92	48	532.6	232.7
	May	25.3	30.8	20.0	136.1	72	94	50	636.2	273.6
	June	27.4	31.8	23.1	5.2	73	93	53	667.4	226.3
	July	28.1	33.8	22.5	8.8	67	90	44	615.5	309.9
	August	27.6	32.4	22.8	1.2	71	90	52	714.4	213.7

wild plants were surface sterilized with 0.5% sodium hypochlorite for 3 min and then washed thoroughly before sowing. For the VAM inoculation treatments, a thin layer of 5 g of *G. mosseae* inoculum containing 1200 spores per 100 g of soil was spread 2 cm below the soil surface; then, the seeds were sown separately in each plot. A vermicompost of 5 t ha^{-1} (animal origin) was calculated and applied on each plot. The size of each plot was 1.5×1.5 m^2, and each plot was planted with six rows. The results of the loam soil (Entisols according to the USDA soil classification) and organic manure analysis are shown in Table 2. In the first stage, the basic tests include the determination of soil texture by the hydrometric method (Bouyoucos, 1962), organic carbon by the Walkley and Black method (Nelson, 1982), equivalent calcium carbonate by back titration method, and the electrical conductivity of saturated extract and pH of saturated soil paste (Page *et al*., 1982).

Planting operations were carried out in November 2016, including irrigation, thinning, and weeding. Watering of the plants was done every 4 d. The plants were thinned to achieve adequate density at the 4-5 leaf stage (after the plants were fully established). Finally, the predefined plant density was kept constant in each plot. Vegetative organs were harvested to measure morphological characteristics and essential oil content at the end of the vegetative period (formation of the flowering stem). For this purpose, eight plants per plot were randomly selected, and leaf area and dry weight of leaves were measured and recorded separately. The relative chlorophyll content in plants was measured in leaves of the highest expansion at the pre-flowering stage using SPAD 502 PLUS. The selected plants of each plot were manually harvested and separately placed in the package and labeled. The harvested plants were dried for 10 d under natural conditions without exposure to sunlight. After crushing the leaves, 20 g of the leaf samples were mixed with distilled water in the Clevenger apparatus (Sina Glass, Tehran, Iran) to extract the essential oil. The extraction time for the essential oil was 3 h for all samples. After the oil was dehydrated with sodium sulfate, the oil content was determined as a percentage. To determine nutrient concentrations, the collected leaves were dried and pulverized with an electric mill and digested with sulfuric acid, salicylic acid,

TABLE 2. Physical and chemical properties of experiment soil and chemical properties of vermicompost.

Soil	Texture	OC (%)	Potassium (mg kg^{-1})	Nitrogen (%)	Phosphorus (mg kg^{-1})	OM (%)	Lime (%)	pH	EC (dS m^{-1})	Depth (cm)
	Loam	1.9	296	0.15	6.7	3.27	27	7.63	0.54	0-30
Vermicompost	Nitrogen	Phosphorus (%)	Potassium (%)	Copper (mg kg^{-1})	Zinc (mg kg^{-1})	Manganese (mg kg^{-1})	Iron (mg kg^{-1})	Magnesium (%)	Calcium (%)	OC (%)
	1.3	0.98	0.45	0.98	57.7	501	1069	0.67	2.85	16.8

OC – organic carbon contents; OM – soil organic matter contents; EC – electrical conductivity.

hydrogen peroxide, and selenium to prepare the extracts (Emami, 1996). The leaf area was measured using ImageJ software. The amount of essential oil was weighed with a balance of 0.0001 g and calculated as the percentage and essential oil yield in kg ha^{-1}. Nitrogen content was determined using titration after distillation in the Kjeltec Autoanalyzer (Bremner & Mulvaney, 1982). The amount of phosphorus was analyzed by the colorimetric method (yellow molybdenite vanadate) (Emami, 1996). Foliar contents of iron, copper, zinc, manganese, and potassium were measured by atomic absorption, and potassium content was analyzed using a flame emission photometer Jenway™ PFP7 (Emami, 1996). Data were statistically analyzed by analysis of variance (ANOVA) using SAS software v 9.4, and mean comparisons were estimated at a probability level of 5% using the LSD test.

Results

Leaf dry weight

Analysis of variance (Tab. 3) showed that application of biofertilizer and the interaction of plant density and biofertilizer had a significant effect on the dry weight of leaves (P<0.01). The mean comparisons showed that the highest dry weights were 2.93 g when treated with VAM at a plant density of 30×30 cm (Tab. 4).

Leaf area

According to the analysis of variance (Tab. 3), the application of *Azotobacter* and VAM had a significant effect on leaf area (P<0.01), and density had a significant effect (P<0.01). Based on the mean comparison data, all fertilizer treatments increased leaf area compared to the control group.

TABLE 3. Analysis of variance with the effects of biofertilizers on *Eryngium caeruleum* traits.

Sources	df	Leaf area	Leaf dry weight	Essential oil percentage	Essential oil yield	Chlorophyll content
Replicate	4	4538.11	0.39	22.77	6.11	0.631
Density	2	15181.54^{**}	1.01^{**}	10.33^{ns}	0.00^{ns}	2.18^{ns}
VAM fungi	1	1715692.23^{**}	5.19^{**}	702.76^{**}	0.348^{**}	59300.74^{**}
Azotobacter	1	162415.63^{**}	5.87^{**}	3484.09^{**}	1.23^{**}	107964.003^{**}
Density and VAM fungi	2	7587.73^{ns}	1.28^{**}	15.16^{ns}	0.00^{ns}	0.139^{ns}
Density and *Azotobacter*	2	798.592^{ns}	0.576^{**}	18.81^{ns}	0.00^{ns}	2.16^{ns}
Error	32	3436.91	0.04	7.77	5.73	1.98
Coefficient of variations		9.85	9.41	0.97	0.24	6.19

ns, * and **: non-significant and significant at 5% and 1% probability levels of LSD, respectively.

TABLE 4. Mean comparison effect of biofertilizers on *Eryngium caeruleum* traits.

Treatment		Leaf area (cm^2)	Leaf dry weight (g)	Chlorophyll content (SPAD units)	Essential oil percentage	Essential oil yield (kg ha^{-1})
Plant density × without biofertilizer (Control)	D_1	411.63 e	1.60 d	32.02 d	0.41 c	90.48 c
	D_2	403.47 e	2.18 b	36.04 c	0.41 c	90.62 c
	D_3	387.90 f	2.03 c	34.20 c	0.42 bc	90.78 c
Plant density × inoculation with *Azotobacter*	D_1A_1	555.07 c	2.60 a	42.66 b	0.63 b	181.56 b
	D_2A_1	569.38 c	2.00 c	44.70 b	0.63 b	179.83 b
	D_3A_1	518.22 d	2.64 a	43.94 b	0.63 b	181.43 b
Plant density × inoculation with *Glomus mosseae*	D_1V_1	846.32 b	2.04 c	54.78 a	0.82 a	210.26 a
	D_2V_1	944.19 a	2.84 a	56.80 a	0.82 a	210.53 a
	D_3V_1	845.55 b	2.93 a	55.34 a	0.81 a	210.80 a

Means in each column followed by similar letters are not significantly different at the 5% probability level- using LSD Multiple Range Test.

D_1: plant density 30×10 cm, D_2: plant density 30×20 cm, D_3: plant density 30×30 cm (without biofertilizer).

D_1A_1: plant density 30×10 cm × inoculation with *Azotobacter*, D_2A_1: plant density 30×20 cm × inoculation with *Azotobacter*, D_3A_1: plant density 30×30 cm × inoculation with *Azotobacter*.

D_1V_1: plant density 30×10 cm × inoculation with *G. mosseae*, D_2V_1: plant density 30×20 cm × inoculation with *G. mosseae*, D_3V_1: plant density 30×30 cm × inoculation with *G. mosseae*.

The highest and the lowest leaf area was recorded in the VAM treatment (944.19 cm^2) and the control group (403.47 cm^2), respectively (Fig. 1).

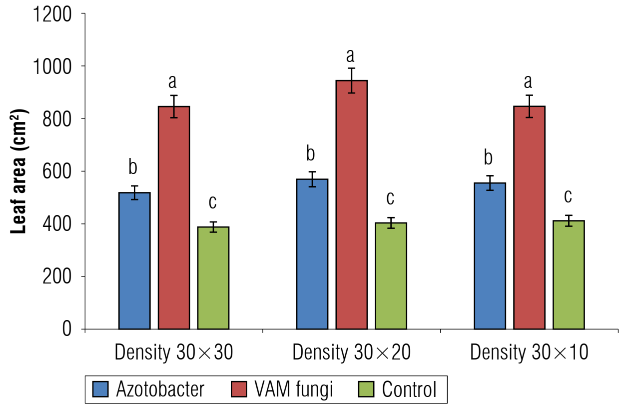

FIGURE 1. Leaf area of *Eryngium caeruleum* after applying biofertilizers at three planting densities (cm). The error bars indicate standard errors.

Essential oil content

Analysis of variance (Tab. 3) showed that the VAM, and *Azotobacter* had a significant effect on essential oil content in leaves (P<0.01). The comparison of the mean interaction showed that the highest content was found in the treatments with VAM and plant density of 10×30 cm and 20×30 cm with 0.82% and the lowest in the control treatment with 0.41% (Fig. 2).

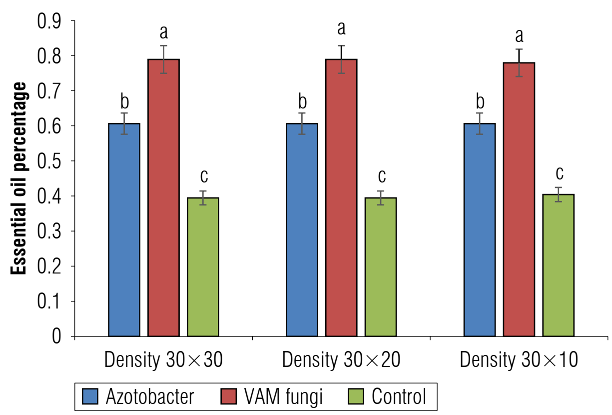

FIGURE 2. Essential oil percentage in leaves of *Eryngium caeruleum* after applying biofertilizers at three planting densities (cm). The error bars indicate standard errors.

Essential oil yield

The results of the analysis of variance (Tab. 3) showed that the different fertilizer treatments had a significant effect on the essential oil yield of *E. caeruleum* (P<0.01). A comparison of mean values showed that the VAM treatments gave the highest oil yield with an average of 210.53 kg ha^{-1}, and the control treatment gave the lowest with an average of 90.48 kg^{-1} (Fig. 3).

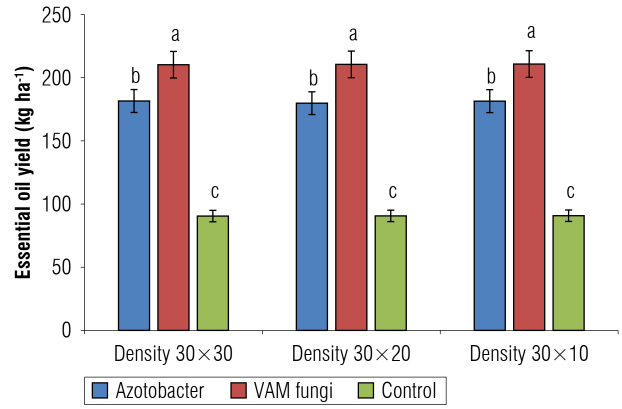

FIGURE 3. Essential oil yield in *Eryngium caeruleum* after applying biofertilizers at three planting densities (cm). The error bars indicate standard errors.

Chlorophyll content

The results of the analysis of variance (Tab. 3) showed that the application of biofertilizer had a significant effect on the chlorophyll index (P<0.01). Plant density and its interaction with biofertilizer did not affect relative chlorophyll content. The mean comparison results showed that the highest and lowest chlorophyll content were found in the VAM treatment and control treatment at 56.80 and 32.02, respectively (Fig. 4).

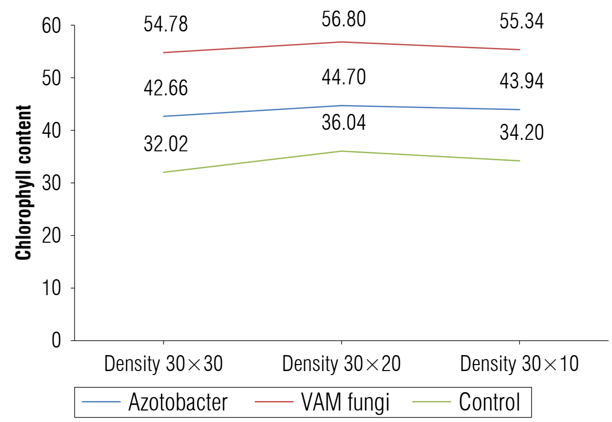

FIGURE 4. Chlorophyll content (SPAD units) in *Eryngium caeruleum* leaves after applying biofertilizers at three planting densities (cm).

Mineral nutrient uptake

Analysis of variance (Tab. 5) showed that the application of biological fertilizers significantly increased the nutrient content in *E. caeruleum* (P<0.01). The mean comparison results showed that the highest and lowest nitrogen content was found in the *Azotobacter* treatment and control treatment with 0.71% and 0.51%, respectively (Fig. 5). Similarly, the highest and lowest phosphorus contents were found in the VAM treatment at 0.19% and the control treatment at 0.08% (Fig. 6). Compared to the control treatment, inoculation with VAM and *Azotobacter* increased the concentration of iron, manganese, zinc, and copper by 1749.5, 180.9, 25.22, 14.02 mg kg^{-1}, respectively, and magnesium by 0.11%. The average comparison also showed that the VAM treatment had the highest contents of potassium (0.599%) and calcium (2.88%) (Tab. 6).

TABLE 5. Analysis of variance with the effects of biofertilizers on foliar nutrient content of *Eryngium caeruleum*.

Sources	df	Nitrogen	Phosphorus	Potassium	Magnesium	Iron	Zinc	Copper	Manganese	Calcium
Replicate	4	0.001	0.19	0.00	0.05	0.08	0.37	0.26	1.65	0.54
Density	2	1.000^{ns}	0.086^{*}	0.009^{**}	0.089^{ns}	0.81^{ns}	0.948^{ns}	0.43^{ns}	49.87^{**}	0.89^{ns}
VAM fungi	1	0.001^{ns}	1.44^{**}	0.001^{**}	0.690^{**}	1597828.40^{**}	186.00^{**}	207.50^{**}	19278.67^{**}	0.16^{ns}
Azotobacter	1	0.236^{**}	1.29^{**}	0.001^{**}	0.016^{ns}	2587438.27^{**}	103.78^{**}	1.82^{**}	74710.28^{**}	0.69^{**}
Density and VAM fungi	2	0.001^{ns}	0.52^{**}	0.001^{**}	0.27^{ns}	0.440^{*}	0.507^{**}	0.30^{**}	3.87^{**}	0.49^{ns}
Density and *Azotobacter*	2	0.001^{ns}	1.02^{**}	0.000^{ns}	0.049^{ns}	0.361^{ns}	0.22^{**}	0.284^{**}	6.82^{**}	0.02^{ns}
Error	32	0.11	0.02	0.004	0.82	0.129	0.44	0.43	0.59	0.08
Coefficient of variations		1.39	3.81	0.85	6.98	0.02	0.86	2.08	0.46	0.35

ns, * and**: non-significant and significant at 5% and 1% probability levels, respectively.

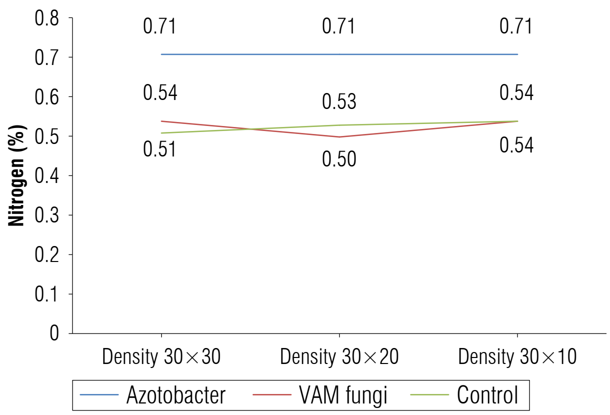

FIGURE 5. Foliar nitrogen contents of *Eryngium caeruleum* after applying biofertilizers at three planting densities (cm).

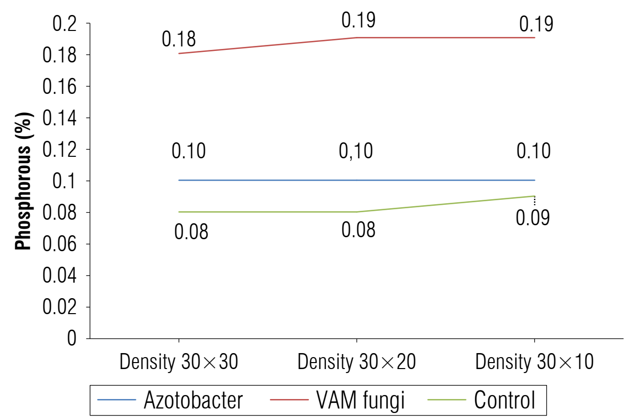

FIGURE 6. Foliar phosphorus contents of *Eryngium caeruleum* after applying biofertilizers at three planting densities (cm).

TABLE 6. Mean comparison of nutrient content in leaves of *Eryngium caeruleum* under the influence of biological fertilizer application at different planting densities.

Treatment		Nitrogen (%)	Calcium (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)	Iron (mg kg^{-1})	Zinc (mg kg^{-1})	Copper (mg kg^{-1})	Manganese (mg kg^{-1})
Plant density × without biofertilizer (Control)	D_1	0.51 c	1.77 e	0.08 c	0.564 c	0.07 b	1161.5 c	22.00 c	8.79 c	77.69 c
	D_2	0.53 b	1.80 d	0.08 c	0.608 a	0.07 b	1160.0 c	22.26 c	8.82 c	78.24 c
	D_3	0.54 b	1.80 d	0.09 c	0.566 c	0.08 b	1161.3 c	22.19 c	8.77 c	78.96 c
Plant density × inoculation with *Azotobacter*	D_1A_1	0.71 a	2.88 a	0.10 b	0.556 d	0.10 a	1748.2 a	24.48 b	9.1 b	175.84 a
	D_2A_1	0.71 a	2.88 a	0.10 b	0.558 d	0.11 a	1749.5 a	25.18 a	9.0 b	179.28 a
	D_3A_2	0.71 a	2.88 a	0.10 b	0.560 d	0.11 a	1749.3 a	25.22 a	9.2 b	180.9 a
Plant density × inoculation with *G. mosseae*	D_1V_1	0.54 b	2.42 b	0.18 a	0.571 b	0.10 a	1622.4 b	23.50 b	13.58 a	128.08 b
	D_2V_1	0.54 b	2.42 b	0.19 a	0.581 b	0.9 a	1623.2 b	24.00 a	13.74 a	128.68 b
	D_3V_1	0.54 b	2.38 c	0.19 a	0.599 a	0.10 a	1622.6 b	23.60 b	14.02 a	131.94 b

Means in each column followed by similar letters are not significantly different at the %5 probability level using LSD Multiple Range Test.

D_1: plant density 30×10 cm, D_2: plant density 30×20 cm, D_3: plant density 30×30 cm.

D_1A_1: plant density 30×10 cm × inoculation with *Azotobacter*, D_2A_1: plant density 30×20 cm × inoculation with *Azotobacter*, D_3A_2: plant density 30×30 cm × inoculation with *Azotobacter*.

D_1V_1: plant density 30×10 cm × inoculation with *G. mosseae*, D_2V_1: plant density 30×20 cm × inoculation with *G. mosseae*, D_3V_1: plant density 30×30 cm × inoculation with *G. mosseae*.

Discussion

The application of VAM and *Azotobacter* significantly affected the characteristics of *E. caeruleum*, including morphological characteristics and dry weight, leaf area, chlorophyll content, percent essential oil, essential oil yield, and nutrient uptake (Tab. 3). Vermicompost with high porosity can absorb and store considerable amounts of minerals and release them gradually. In addition, its high water-holding capacity (Arancon *et al*., 2004) affects the growth of the studied plants. Furthermore, the application of vermicompost, VAM, and *Azotobacter* increases the growth of the plants and causes a significant difference compared to the control group due to the release of macro and microelements, vitamins, enzymes, and growth-promoting hormones, as well as the increase in the number of efficient soil microorganisms. As shown in Tables 5 and 6, the application of VAM, *Azotobacter*, and vermicompost improved the morphological characteristics compared to the control group. Table 4 also shows that all measured traits improved when treated with VAM and *Azotobacter*. The results of this study are consistent with the findings of Koozehgar Kaleji and Ardakani (2018) and Koozehgar Kaleji and Ardakani (2019), who reported an increase in leaf area, essential oil percentage, essential oil yield, and dry leaf weight of *E. caeruleum* M. Bieb and *Froriepia subpinnata* with the use of organic fertilizers and mycorrhiza. Mycorrhizal symbiosis of fungi with plants can increase nutrient uptake by the plants and yield (Chen *et al*., 2017). Other experimental results on tomato plants induced by VAM inoculation showed that leaf area and nitrogen, potassium, calcium, and phosphorus content increased (Balliu *et al*., 2015). Another study observed that the application of *Azotobacter* and mycorrhiza increased the chlorophyll content of the plant *Prosopis chilensis* ([Molina] C.E. Hughes & G.P. Lewis) compared to the control (Faramawy, 2014). Determining the optimum plant density could be critical for plant growth and yield. Optimal plant density prevents inter-plant competition and increases product and final dry matter by increasing light, nutrient, and water uptake efficiency and reducing evapotranspiration. The increase can be attributed to the uptake of nutrients, especially phosphorus (Larimi *et al*., 2014). Other studies have shown that *G. mosseae* increases the essential oil content of *Origanum majorana* (L.) (Khaosaad *et al*., 2006). Also, two species of mycorrhizal fungi (*Glomus fasciculatum* [*Rhizoglomus fasciculatum* (Thaxter) Sieverding, G.A. Silva & Oehl], *Glomus macrocarpum* Tul. & C. Tul.) increased the growth and essential oil concentration of fennel (Kapoor *et al*., 2004) and coriander (Kapoor *et al*., 2002). Using biofertilizers increased the yield and essential oil content of *Ocimum basilicum* L. (Al-Mansour *et al*., 2018). Since the essential oil yield is related to the percentage of essential oil and the dry weight of the plant, any increase in these two parameters may result in a higher yield of oil. Rueda *et al*. (2016) showed that inoculation of *Azospirillum* and *Azotobacter* with *Fragaria vesca* L. increased plant height, dry weight of roots and shoots, leaf area, chlorophyll content, nutrient content, and plant yield. Tang *et al*. (2009) found that maize inoculated with *G. mosseae* had higher chlorophyll synthesis, significantly improving plant photosynthesis. This improvement was attributed to increased nitrogen uptake by inoculated plants. Inoculation of *A. chroococcum*, *Pseudomonas putida* (Trevisan), *Bacillus polymyxa* or *Paenibacillus polymyxa* (Prazmowski), and VAM significantly increased root and shoot biomass, chlorophyll, and NPK content in plants (Vafadar *et al*., 2014). The results on phosphorus, copper, zinc, and manganese agree with the results of Koozehgar Kaleji and Ardakani (2018). Another study showed that the effect of mycorrhizal inoculation on the concentrations of iron, manganese, and copper was significant (P<0.01); inoculation with VAM fungi increased the concentrations of nutrients such as iron, manganese, zinc, and copper by 142, 67.6, 21, and 12 units, respectively, compared to the control treatment (Ortas & Bykova, 2018). The results of this study are consistent with the findings of Zhang *et al*. (2016), who found that inoculation of mycorrhiza increased phosphorus contents in plants. Root colonization by VAM and bacteria can increase root length and surface area, thus, increasing water and nutrient uptake, especially under drought conditions (Marulanda *et al*., 2006; Marulanda *et al*., 2009). Other research results showed that inoculation with mycorrhizal fungi increased the absorption of nitrogen, phosphorus, potassium, zinc, iron, and copper in sesame plants compared to the control (Askari *et al*., 2018).

Conclusions

Application of VAM and *Azotobacter* treatments at different plant densities increased the dry weight of leaves, essential oil yield, leaf area, and oil content. In addition, increasing plant density increased yield components. Increases in yield and yield components of *E. caeruleum* due to the use of a biological fertilizer can be attributed to the increased absorption of nutrients, such as nitrogen, phosphorus, potassium, and a microbial population in the rhizosphere in the soil. Inoculating the seed with growth-promoting bacteria apparently increased the growth of *E. caeruleum* by creating a cycle that provided nutrients through the production of fungal mycelia, which increased nutrient uptake by the roots and indicated enhanced microbial activity in the soil. Additional tests showed that mycorrhizal symbiosis, vermicompost and *Azotobacter* applications increased plant growth and yield. The study show that the combination of low-input systems, free-living nitrogen-fixing bacteria, and VAM could be a suitable alternative to chemical fertilizers and complete fertilizers, especially in low-input systems or organic farming.

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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

MK and MRA designed the experiments, MK carried out field and laboratory experiments, SK contributed to the data analysis, and MK, MRA, and SK wrote the article. All authors reviewed the final version of the manuscript.

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Impact of waterlogging on fruit crops in the era of climate change, with emphasis on tropical and subtropical species: A review

Impacto del anegamiento sobre los frutales en la era del cambio climático, con énfasis en especies tropicales y subtropicales: una revisión

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ABSTRACT

Incidents of flooding in tropical and subtropical fruit trees have increased as a result of climate change. Because of flooding, the anaerobic conditions of the rhizosphere increase the conditions for phytotoxicity and infection by pathogenic fungi and bacteria. Due to oxygen depletion in waterlogged soils, growth, functions of the roots and of the entire plant are impaired. The decrease in the photosynthetic rate is considerable because of the reduced functional leaf area because of chlorosis, necrosis, leaf drop and stomatal closure, as well as chlorophyll degradation. Plants have developed different morphological, physiological, and biochemical adaptations to survive hypoxic stress. Some fruit trees form an aerenchyma in roots for the diffusion of oxygen from the aerial parts, create aerenchyma-containing adventitious roots, rapidly elongate stems into deeply flooded soils; or they form hypertrophied lenticels, like some mango varieties. Measures for better adaptations and tolerance of tropical fruit trees to climatic impact include the following: adaptations of the cultivated terrain, selection of varieties, rootstocks more tolerant to hypoxic stress, pruning to reestablish the balance of the aerial part/roots, and foliar applications (*e.g.*, of glycine betaine or hydrogen peroxide (H_2O_2)). Mycorrhizal colonization of roots can increase tolerance to waterlogging, while the application of fertilizers, such as CaO or MgO, can improve the redox potential of flooded soils. We present results of studies on this problem for the following fruits: yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) and purple passion fruit (*P. edulis* f. *edulis*), cape gooseberry (*Physalis peruviana*), lulo or naranjilla (*Solanum quitoense*), tree tomato (*Solanum betaceum*), citrus (*Citrus* spp.), guava (*Psidium guajava*), papaya (*Carica papaya*), and mango (*Mangifera indica*).

Key words: flooding, hypoxia, aerenchyma, photosynthesis, adaptation, tolerance.

RESUMEN

Los incidentes por inundaciones en los frutales tropicales y subtropicales han aumentado como resultado del cambio climático. En consecuencia, las condiciones anaeróbicas de la rizosfera aumentan las condiciones de fitotoxicidad y contagio por hongos y bacterias patógenos. Debido al agotamiento del oxígeno en suelos anegados, el crecimiento, las funciones de las raíces y finalmente de toda la planta resultan perjudicados. Se presenta disminución de la tasa fotosintética, debido a la reducida área foliar efectiva como consecuencia de la clorosis, necrosis y caída foliar, además del cierre estomático y la degradación de la clorofila. Las plantas han desarrollado diferentes adaptaciones de tipo morfológico, fisiológico y bioquímico para sobrevivir al estrés por hipoxia. Algunos frutales forman un aerénquima en raíces para facilitar el transporte del oxígeno desde las partes aéreas, inducen raíces adventicias que contienen aerénquima, alargan rápidamente los tallos hacia suelos inundados más profundos o forman lenticelas hipertrofiadas, como en las variedades de mango. Dentro de las medidas para una mejor adaptación y tolerancia de los frutales tropicales a esta adversidad climática se recomiendan una adecuada preparación del suelo, la selección de variedades y patrones más tolerantes al estrés por hipoxia, podas, para reestablecer el equilibrio de la relación parte aérea/raíz en los árboles, aplicaciones foliares como por ejemplo de glicina betaína o peróxido de hidrogeno (H_2O_2). La colonización micorrícica en las raíces puede aumentar la tolerancia al anegamiento y el potencial redox en suelos inundados puede mejorarse con la aplicación de enmiendas como CaO o MgO. Se presentan resultados de estudios sobre esta adversidad en maracuyá (*Passiflora edulis* f. *flavicarpa*), gulupa (*P. edulis* f. *edulis*), uchuva (*Physalis peruviana*), lulo o naranjilla (*Solanum quitoense*), tomate de árbol (*Solanum betaceum*), cítricos (*Citrus* spp.), guayaba (*Psidium guajava*), papaya (*Carica papaya*) y mango (*Mangifera indica*).

Palabras clave: inundación, hipoxia, aerénquima, fotosíntesis, adaptación, tolerancia.

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Introduction

In many cases, climate defines the geographical distribution of a species, the types of vegetation, and the appropriate management of the crops, demonstrating climate´s great influence on the growth of plants (Dey *et al*., 2016). Negative influences on food security include climate variability (CV), climate change (CC) manifested through global warming, the change in rainfall patterns, and the increased incidence of extreme weather events (IPCC, 2019). In addition, the effects on crops are more intense in low latitude countries, with higher initial temperatures (Fischer *et al*., 2022; Fischer, Orduz-Rodríguez *et al*., 2022), and even more so in marginal or already degraded soils and in underdeveloped regions with insufficient potential for adaptation to these changes (Yohannes, 2016). Extreme events due to flooding of agricultural land will continue to increase, including in regions where previously it was not expected (Fischer, 2021). Contradictorily, in northern areas CC can have effects, mostly positive, manifested in an increase in productivity and diversity of cultivated varieties, unlike southern areas where the inconveniences of CC prevail with lower usable yields, greater instability in plant yield of cultivated plants, and decreases in areas suitable for traditional crops (Potopová *et al*., 2015).

In these scenarios, not only at a local level but also globally, the intensity and frequency of abiotic stress in plants constantly increases (Dubey *et al*., 2021). Recent studies reveal that the multifactorial stresses generated by CC and global warming cause severe decreases in plant growth and survival and biodiversity of the microbiome (Zandalinas *et al*., 2021).

Sthapit *et al*. (2012) affirm that global warming will have a significant impact on small farmers who depend on rainfed agriculture. Likewise, increases in the frequency of coastal flooding induced by CC can significantly reduce the area available for agriculture (Sthapit *et al*., 2012). In general, adaptations of fruit species to CC require time and long-term investments (Chmielewski *et al*., 2008).

A shortage of precipitation because of recent CC is expected. However, most fruit growing regions experience almost constant precipitation (Drogoudi *et al*., 2020) with a slight shift of more rain during certain periods than in other periods of the year (Bisbis *et al*., 2018). A similar situation occurs in the Colombian Andes with an increase in rainfall in the highlands (Hirabayashi *et al*., 2013). Long-term (60 year) weather records show a change from moderate rainfall to an increase in the incidence of heavy rainfall (> 20 mm) (Kunz & Blanke, 2022) that sometimes leads to flooding.

Fruit production can increase in some areas due to increased rainfall, but in other areas, production can fall seriously due to decreased rainfall (Sthapit *et al*., 2012). In general, it is estimated that fruit trees will be less affected by CC compared to semi-annual or annual crops (*e.g*., cereals) (Sthapit *et al*., 2012); however, fruit species in temperate zones will move to new areas closer to the poles (Jones *et al*., 2005), while tropical fruit trees may extend over their latitudinal strip and/or to higher altitudes (Fischer & Melgarejo, 2020).

In recent years, because of CC and especially global warming (Hirabayashi *et al*., 2013), waterlogging and flooding have increased in frequency and are unpredictable all over the world (Zhang *et al*., 2021), especially due to erratic and non-seasonal rainfall (Dubey *et al*., 2021). Hirabayashi *et al*. (2013) model flood frequencies through global warming predictions and report a 42% increase in frequencies, compared to an 18% reduction in flooding globally. Flooding will increase across large areas of South and Southeast Asia, Northeast Eurasia, East and low-latitude Africa, and into South America, where flooding will increase especially in the northern part of the Andes, while decreasing in southern South America (Hirabayashi *et al*., 2013).

In Latin America, Wood *et al*. (2000) estimate that 13.3% of arable land suffers from poor drainage due to the physiography that favors flooding as well as high groundwater levels or stagnant surface water. The rainy seasons in soils with poor drainage produce anaerobic conditions that are harmful to the roots (Moreno & Fischer, 2014). In addition, Kaur *et al*. (2020) report other causes of waterlogging or flooding that include rainfall after irrigation, excessive irrigation, poor surface and internal drainage in heavy clay soil types as well as in coarse-textured topsoil over compacted clay subsoil. Plantations in the vicinity of large and small rivers are also at risk, as these often overflow; Larcher (2003) classifies these as phenomena that do not depend solely on CC.

Waterlogging reduces the quality of soils and the productivity of many crops (Singh *et al*., 2010), affecting large areas of the world (Martínez-Alcántara *et al*., 2012). And Kaur *et al*. (2020) characterize soil flooding as the most harmful abiotic stress that affects crops apart from drought.

Waterlogging in the rhizosphere of plants is influenced by several factors, but especially climate that affects the amount of water incorporated into the soil, the volume of water that passes through or over the soil surface, and the amount of soil water used by plants and other organisms (Kaur *et al*., 2020).

For the survival of many plants and microorganisms in the soil, oxygen (O_2) is a crucial element; losses caused by this type of abiotic stress can affect up to 40% of the total production (Moreno & Fischer, 2014). The availability of O_2 is much lower in stagnant water and generates much stronger stress conditions compared to moving water, since turbulence facilitates the solubilization of O_2 in flooded water (Kreuzwieser & Rennenberg, 2014).

The availability of O_2 for flooded roots decreases because water contains fewer gases than the atmosphere since the diffusion of these gases dissolved in water is 10,000 times slower than in air (Parent *et al*., 2008). This generates an energy crisis in the tissues subjected to hypoxia that can cause the death of a plant (Moreno & Fischer, 2014).

The objective of this review was to characterize the effect of waterlogging and flooding on fruit crops in the context of global warming and the probability of very abundant rains that can greatly harm the growth and development of crops. We emphasize tropical and subtropical species in order to facilitate decision-making for future research and to enhance fruit production better adapted to these new conditions.

Influence on the soil

Kreuzwieser and Rennenberg (2014) and Kaur *et al.* (2020) define flooding as water above the soil level, while waterlogging refers to water that has completely saturated the soil, leading to O_2 deprivation since excess inflowing water displaces soil air from the soil pores (Revelo, 2020). This means that O_2 concentration at the root surface decreases drastically since the O_2 level of the water is markedly lower than that of the air, and the air in the atmosphere contains about 20% O_2 (200 kg m^{-3}) compared to <0.01 kg m^{-3} dissolved O_2 in a flooded soil (Taiz *et al*., 2017).

Schaffer (2006) differentiates between normoxia (soil with sufficient O_2 content), hypoxia (low O_2 content in the soil), and anoxia (trace O_2 content in the soil, like the case of prolonged waterlogging). The remaining O_2 in the soil is consumed by microbial activity and plant roots (Kreuzwieser & Rennenberg, 2014). Thus, in waterlogged soils, anoxia is always preceded by hypoxia (Drew, 1997).

In flooded soils, the redox potential decreases (Sanclemente *et al*., 2014), indicating that the low level of O_2 affects the availability of nutrients for the plant (Unger, Kennedy *et al*., 2009; Unger, Motavalli *et al*., 2009). Flooding hinders the activity of enzymes such as β-D-glucosidase and phosphatase, necessary in the carbon, nitrogen (N), sulfur (S) and phosphorus (P) cycles (Wang & Lu, 2006); and ethylene concentration increases (Malik *et al*., 2003). These conditions in the soil negatively affect the growth and development of plants (Jiménez *et al*., 2012).

During waterlogging, O_2 deficiency changes the physicochemical properties of soils considerably (Kreuzwieser & Rennenberg, 2014). On the one hand, the lack of O_2 harms the microbial communities in the soil (Unger, Kennedy *et al*., 2009); and it reduces numerous oxidized nutrients as follow: (nitrate [NO^{3-}], iron(III) [Fe^{3+}], sulfate [SO_4^{2-}]) and generates high levels of reduced compounds (iron (II) [Fe^{2+}], manganese (II) [Mn^{2+}], ammonium [NH_4^+], hydrogen sulfide [H_2S]) and organic compounds (acids, carbonyls, alkanes, etc.). In many cases, these low oxygen changes are toxic to plants (Jackson & Colmer, 2005; Kreuzwieser & Rennenberg, 2014). The physicochemical properties change in their speed and magnitude according to the type of soil, duration of waterlogging or flooding and the prevailing environmental conditions, mainly temperature (Drew, 1997; Kozlowski & Pallardy, 1997).

The porosity of the soil is very important for the impact of waterlogging and is a determining factor in the availability of O_2 for the roots (Moreno & Fischer, 2014). Waterlogged pores immediately generate O_2 deficiency, and the remaining O_2 is consumed very quickly by roots and microorganisms (Pallardy, 2008). Clay soils with their fine pores are particularly susceptible to O_2 deficiency, compared to sandy soils that remain aerated, even within flowing water (Larcher, 2003). Waterlogging leads to O_2 depletion in the soil, since the O_2 diffusion rate in waterlogged soils is 10,000 times lower than in a well-drained one (Hossain & Uddin, 2011).

Flooded soils greatly limit the operation by machinery that can affect soil preparation, plant management, and crop harvesting, leading to lower yields (Kaur *et al*., 2020).

Influence on the plants

Plants are aerobic organisms that require O_2 (Pucciariello & Perata, 2012) for the absorption of nutrients. Excessive moisture around the roots can cause lethal conditions (Lacona *et al*., 2012) in most terrestrial plants (Jiménez *et al*., 2012). Schaffer *et al.* (2009) report that the soil O_2 level can be reduced from 20 to <5% within 1 to 2 d of waterlogging. To respond to O_2 deprivation, plants have developed different morphological, anatomical, and metabolic adaptations to prevent cell damage in such hostile environments (Bailey-Serres & Voesenek, 2008; Xie *et al.*, 2021). These are listed below.

Growth

Kozlowski and Pallardy (1997) mention that the most deleterious effects of waterlogging on woody plants are reduced root and stem growth, changes in nutrient uptake, and carbohydrate translocation that increases senescence and plant mortality. Crane *et al.* (2020) point out that the symptoms of waterlogging are very different in trees, reducing growth and yield, not only in non-tolerant but also in tolerant fruit species. Symptoms of waterlogging or excessively wet soils on fruit trees, gradually progress as follow: (1) wilting and scorching leaves; (2) fruit drop, leaf chlorosis and drop; (3) regressive death of the stem and limb dieback; (4) death of the tree (Crane *et al.,* 2020).

The root zone is the region of the plant that is first and directly affected by an excess of water and O_2 deficiency of the soils (Moreno & Fischer, 2014); root growth stops or the apices are injured or die (Larcher, 2003). This starts with the fine and fibrous roots (Fischer & Orduz-Rodríguez, 2012), because thicker roots are less susceptible to radial loss of O_2 (Pedersen *et al.*, 2020). Due to the anaerobic conditions in the rhizosphere, phytotoxic conditions increase from the accumulation of reduced ions and the products of anaerobic organisms that limit soil aerobic microorganisms and increase the presence of fungal pathogens such as *Pythium, Phytophthor*a, and *Fusarium* (Fischer & Orduz-Rodríguez, 2012). In general, for fruit trees a water table of 1.5 m is the most recommended (Fischer & Orduz-Rodríguez, 2012).

Waterlogging affects stem growth according to the degree of adaptation of the plants. It can be reduced or accelerated (Blom & Voesenek, 1996) to position their flowers and leaves above the water level (Schopfer & Brennicke, 2010). However, in plants susceptible to waterlogging such as papaya (*Carica papaya*) (Crane, 2020), waterlogging for only 48 h seriously affects the growth in diameter and length of the stem and of the root (Khondaker & Ozawa, 2007).

Leaves react to waterlogging like plants exposed to water stress; stomatal conductance is reduced as well as the rate of elongation of the leaves (Lambers & Oliveira, 2019). As the time of exposure to hypoxic stress of the roots increases, the leaves yellow and necrosis increases, followed by their abscission. These symptoms are caused by the lack of uptake and transport of water and nutrients by the roots (Kozlowski & Pallardy, 1997; Aldana *et al.*, 2014; Fischer *et al.*, 2016). Interestingly, when apple (*Malus domestica*) trees are in full fructification, leaf-fall is less than that compared to trees without fruits (Lenz, 2009).

However, the growth and reproductive development of fruit trees that are not tolerant of waterlogging because of the inhibition of root respiration and the accumulation of toxic substances (Pan *et al.*, 2021) is highly impaired. Processes as energetic as the formation of flower buds, flowering, and set and growth of fruits is inhibited, and the premature fall of these organs due to the suppression of photosynthesis and hormonal imbalance induced under anoxic conditions (Kozlowski & Pallardy, 1997) occurs. For example, peach (*Prunus persica*) fruits were smaller after waterlogging for 12 h per day for 8 weeks that produces ethylene and softening of the pulp earlier in postharvest than do fruits from non-waterlogged trees (Insausti & Gorjón, 2013).

Exposure to short-term hypoxia in the roots reduces the redox potential of the soil and can promote the availability of micronutrients in basic-reactive soils, by reducing Fe^{3+} to Fe^{2+} that can be metabolized by plants. This stimulates flowering and fruit production in carambola (*Averrhoa carambola*) (Schaffer *et al.,* 2006).

Physiological considerations

Basic processes of development, physiology, and metabolism, such as cell division, respiration, growth, nutrient and water intake, and transpiration are O_2-dependent processes; so O_2 deprivation results in reduced growth (Sathi *et al.*, 2022).

The anaerobic stress of the root suppresses its respiration, leading to energy wastage (Lambers & Oliveira, 2019), an increase in fermentation, acidification of the cytosol (cytoplasmic acidosis) and toxic effects due to the accumulation of ethanol, lactic acid, and acetaldehyde (Bailey-Serres & Voesenek, 2008; Xie *et al.*, 2021) that can lead to root cell death (Drew, 1997). This energetic deterioration affects many metabolic processes, such as protein synthesis (Taiz *et al.*, 2017). The amount of adenosine triphosphate (ATP) generated during fermentation is two ATP molecules per glucose molecule, produced by substrate phosphorylation (Taiz & Zeiger, 2010). Thirty-two ATP molecules are produced during oxidative phosphorylation (Jiménez *et al.*, 2012) corresponding to a 16-times lower energy production through fermentation. Especially if photosynthesis is also decreased, an energy shortage is assumed under flooding conditions (Menezes-Silva *et al.*, 2019). In general, plants suffering because of anaerobic conditions can switch their metabolism from aerobic respiration to the fermentation pathway, as an adaptive mechanism to oxygen deprivation through waterlogging conditions (Peña-Fronteras *et al*., 2008; Jiménez *et al*., 2012).

The result of this stress can be cell death in a few hours or days depending on the adaptation of the species to low O_2 levels. In addition, if the stressed plant returns to normal O_2 levels the recovery process can be endangered (Mielke & Schaffer, 2010; Cardona *et al*., 2016; Taiz *et al*., 2017). Roots under anaerobic stress do not suffer from the formation of reactive oxygen species (ROS) due to the absence of O_2; instead, if the O_2 concentration in the soil increases rapidly, a part is used to form ROS that generates oxidative damage to the root cells (Taiz *et al*., 2017).

The effect of waterlogging on decreases of the photosynthetic rate is great, especially in plants that are intolerant to this stress (Parent *et al*., 2008); it is primarily related to stomatal as well to nonstomatal limitations (Kreuzwieser & Rennenberg, 2014). Early decrease is associated with stomatal closure and consequently with the reduction of the photosynthetic process, also because of the reduction of leaf area (Pallardy, 2008). Pallardy (2008) characterizes the decrease in leaf area due to suppression in the formation and extension of leaves, as well as by leaf lesions and abscission. The reduction of the chlorophyll content and premature senescence of leaves contributes to an inhibition of photosynthesis in a later stage of waterlogging (Parent *et al*., 2008; Aldana *et al*., 2014).

Stomatal closure due to hypoxic stress of the root system has been observed in many species, *e.g*., lemon (Moreno & Fischer, 2014) and strawberry (Blanke & Cooke, 2004), known to remedy water loss (Taiz & Zeiger, 2010). In addition, flood-sensitive species appear to lack a mechanism to reopen stomata that have been closed by hypoxic soil conditions (Pallardy, 2008). Interestingly, waterlogging could not only increase stomatal resistance but also restrict water intake through reductions in root hydraulic conductivity (Else *et al*., 1995; Menezes-Silva *et al*., 2019), leading to an internal water deficit in the plant (Parent *et al*., 2008; Sanclemente *et al*., 2014).

Water transport within a plant is driven by a combination of three mechanisms: a) root pressure, b) cohesion, and c) suction forces generated by evaporation. The latter relies on the presence and functioning of open stomata and water vapor pressure deficit (VPD) between the plant and the ambient atmosphere (Taiz *et al*., 2017). Water channels are described as key regulatory proteins in cellular apoplastic water transport. They are also responsible for the response to changes in xylem water potential and are required for the transport of water (Lambers & Oliveira, 2019).

Studies of flooded strawberry plants have shown that leaf water potentials remain unchanged after flooding, without changes in water channel activity (Blanke & Cooke, 2004). In this case, turgor may be preserved by maintaining root pressure, an electrochemical and ion gradient, and xylem differentiation, assuming water channels remain open. This contrasts with drought-stressed strawberry plants, where water channel activity is reduced. In any case, the effect of flooding on water relations of strawberry stolons and leaves is less pronounced than that of drought (Blanke & Cooke, 2004). The stomatal closure under drought could be attributed to increased delivery of abscisic acid (ABA) from roots to the leaves (Lambers & Oliveira, 2019). However, Blanke and Cooke (2004) observe stomatal closure in strawberry leaves to be more rapid under flooding conditions than under drought; and they suggest this reaction is due to the release of stress ethylene because the ABA transfer from the roots to the leaves is severely depressed in the xylem.

By reducing stomatal conductance, plants can prevent water loss; however, this reaction also affects net photosynthesis under waterlogging stress (Davies & Flore, 1986). Taiz and Zeiger (2010) and Kozlowski and Pallardy (1997) characterize the decrease in photosynthetic activity due to waterlogged conditions by the following: 1) low water potential, 2) reduced stomatal conductance, 3) decreased activity of photosynthetic enzymes, 4) lower content of chlorophylls, and 5) irregular transport of photoassimilates that can alone or in combination with these mentioned reasons decrease photosynthesis. Additionally, Pan *et al*. (2018) and the other cited authors by them explain that during the waterlogging of the plants the leaf stomata close; and the degradation of chlorophyll causes yellowing and senescence of the leaves so that the capacity of the leaves to take advantage of light is reduced, generating a decrease in the photosynthetic rate.

The distributional pattern of photoassimilates can change under these conditions in the Chandler and Camarosa strawberry (*Fragaria* × *ananassa*) varieties, with an increase of 15.9% and 18.4% of the dry mass of the roots in waterlogged plants (Casierra-Posada & Vargas, 2007).

Anaerobic root conditions also affect hormone metabolism; root ethylene concentrations increase because this gas moves slower in flooded soil than in well-aerated soil, and because of greater production of this hormone under stress conditions (Lambers & Oliveira, 2019). Ethylene is a recognized signaling molecule that is involved in the acclimation of plants to hypoxia (Xie *et al*., 2021). Similarly, the synthesis of abscisic acid in flooded roots is a responsible signal in the leaves for their stomatal closure (Else *et al*., 1995) and is also involved in the development of aerenchyma in roots (Pan *et al*., 2021).

Absorption of mineral nutrients

The severe energy deficit of roots due to O_2 deficiency induces an irregular functioning of these organs, resulting in deficient absorption and transport and an insufficient supply of the plant with nutrients and water (Zeng *et al*., 2014; Lambers & Oliveira, 2019). Suppression of the root colonization by mycorrhizal fungi in waterlogged soils contributes to reduced nutrient uptake (Kozlowski & Pallardy, 1997), but in peach seedlings an arbuscular mycorrhizal (AM) infection provides limited tolerance to waterlogging and flooding, possibly due to increased plant nutrition (Rutto *et al*., 2002). However, waterlogging favors the risk of nutrients leaching to deeper layers of the soil (Friedrich & Fischer, 2000).

Waterlogging impairs the absorption of ions due to its impact on soil conditions as well as the response of plants to nutrient uptake that is less affected in species tolerant to waterlogging (Pallardy, 2008). Especially for species intolerant to waterlogging, the uptake of N, P, and K is decreased by the decrease in energy due to anaerobiosis, while the absorption of Ca and Mg is less affected, and that of Fe and Mn increases due to its reduction to Fe^{2+} and Mn^{2+}, respectively (Kozlowski & Pallardy, 1997). Regardless of the increase in the levels of Fe and Mn in the rhizosphere, their uptake by the roots is reduced due to slower growth of the flooded plants (Pallardy, 2008). In contrast, 'Peach' mango (*Mangifera indica*) trees increase their photosynthetic rate by increasing Fe and Mn levels in calcareous soil, when the 10 to 20 d of waterlogging was pushed to higher levels than in the previous stage of waterlogging (Schaffer *et al.,* 2009).

Waterlogging increases soil N loss due to denitrification, nitrate leaching, and runoff, reducing the N mineralization rate in the soil (Kaur *et al.,* 2020). Foliar applications of N could reduce the detrimental effect of waterlogging in lulo (naranjilla) (*Solanum quitoense*) plants (Flórez-Velasco *et al*., 2015). The foliar application of nutrients can compensate for the inhibited absorption of mineral nutrients by the roots. In addition, the foliar application of phytohormones can compensate their reduced synthesis due to the limited function of the roots, as is the case of cytokinins and gibberellins (Moreno & Fischer, 2014).

Interaction with other climatic factors

In flooded pitanga (*Eugenia uniflora*), a pre-acclimatization to high solar radiation (44 mol m^{-2} d^{-1}), for 55 d, decreased the photosynthetic rate and the accumulation of dry weight compared to those only exposed to a radiation of 12 mol m^{-2} d^{-1} (Mielke and Schaffer, 2010). Additionally, lulo (naranjilla) plants under shade (56%) tolerated waterlogging better than those without shade (Sánchez-Reinoso *et al*., 2019).

However, low solar radiation arising during periods of intense and prolonged rains that flood the plantations can reduce photosynthesis and further affect plant growth (Pucciariello & Perata, 2012). For the chonto tomato (*Lycopersicon esculentum*), 56% shading combined with 12 d waterlogging reduced biomass accumulation in the plant more than shade or waterlogging alone (Baracaldo *et al*., 2014). Two or more combined abiotic stresses can affect physiology and productivity of plants that are different from effects of individual stress (Taiz *et al*., 2017).

High temperatures aggravate the effects of waterlogging and increase plant mortality due to decreased solubility of O_2 and CO_2 in warm flooded water (Panda & Barik, 2021). Citrus roots soaked for 3 d at 30-35°C die, however, at temperatures below 15°C can survive for several months (Orduz-Rodríguez, 2012). The abscission of leaves, flowers, and fruits due to waterlogging is more accentuated when the temperature of the soil and water increase above tolerance limits (Fischer *et al*., 2016).

Adaptation and tolerance mechanisms

Tolerance to waterlogging depends on several factors; for example, in fruit species, the previous stress of the plant (*e.g*., in too cold or dry climates), the fruit load, the air temperature (warmer ones being more harmful), the type of soil, and the depth of the flood and its duration influence this tolerance (Crane *et al*., 2020).

Plants have developed different morphological, physiological, and biochemical adaptations to survive O_2 deficiency stress, including metabolic acclimation and signaling networks, so that plants can resist or escape low-O_2 environments that disturb their metabolism and growth (Xie *et al*., 2021).

Parent *et al.* (2008) consider that species tolerant to waterlogging are generally those that are able to continue their energetic state through fermentation. As a short-term response, there is an increase in the antioxidant defense system as a reaction to the increase in ROS; and this increases the level of antioxidant enzymes serving as a parameter for tolerance to waterlogging stress (Jiménez *et al.*, 2012). Thus, in apples the activity of superoxide dismutase (SOD), peroxidase (POD), and glutathione reductase (GR) increases during hypoxic conditions (Bai *et al.*, 2010).

After the metabolic adjustment, the plant generates morphological changes, such as formation of aerenchyma in roots of some species to survive long periods of flooding (Jiménez *et al.*, 2012). The formation of an aerenchyma, induced by waterlogging, consists of interconnected intercellular spaces caused by the programmed death of cortical cells that can promote gas exchange with the aerial part of the plant and improve adaptation to hypoxic conditions (Rankenberg *et al.*, 2021) due to the significant increase in the energy status of the plants (Jiménez *et al.*, 2012). In the submerged soils, ethylene induces the formation of aerenchyma in the mature parts of the root (Pedersen *et al.*, 2020) within its fully developed cortex (Rankenberg *et al.*, 2021). Ethylene prevents the elongation of the roots and leads to the formation of an aerenchyma (Lambers & Oliveira, 2019).

Above all, in trees with deep roots that grow in humid soils the O_2 supply is greatly limited. This is why some species survive through anaerobic metabolism (by fermentation) or through the formation of pneumatophores that allow the movement of O_2 to the roots (Taiz *et al.*, 2017). In addition, plants that show a certain tolerance to waterlogging can induce formation of adventitious roots that contain aerenchyma for the diffusion of O_2 from the aerial parts of the plant (Jackson & Colmer, 2005) and in waterlogged chonto tomato (Baracaldo *et al.*, 2014). Generally, endogenous auxin is responsible for the formation of adventitious roots (Lambers & Oliveira, 2019). These new roots can compensate for the loss of the ability to absorb water and nutrients from the decayed root system (Kozlowski & Pallardy, 1997). In many cases, the adventitious roots are formed near the base of the stem or in the area of numerous lenticels (Parent *et al.*, 2008).

Apart from adventitious root formation, a rapid elongation of the stem is one of the most important morphological adaptations in highly flooded plants (Xie *et al.*, 2021). Also, in short-term flooding (2-6 d) in tree tomato (*Solanum betaceum*), the plants react with greater stem elongation; however, they are accompanied by a loss of plant biomass (Betancourt-Osorio *et al.*, 2016).

Several mango genotypes react to waterlogging with the formation of hypertrophied lenticels that facilitate O_2 diffusion (Schaffer *et al.*, 2009) and the release of toxic substances produced by anaerobic metabolism (Parent *et al.*, 2008). Because of that, Zeng *et al.* (2014) highlight the importance of the supply of internal O_2 from the tree trunk that is crucial for the functioning of the roots in an anoxic environment.

In waterlogged plants, the induction of epinasty can be observed that has the effect of decreasing foliar transpiration, generating protection against direct sun and heat to the leaves (Schaffer *et al.*, 2006).

Some measures to relieve stress from waterlogging

In case of fruit trees that need to be grafted, the selection of more tolerant rootstocks reduces hypoxia stress to a minimum (Schwarz *et al.*, 2010); the use of rootstocks from other species, such as plum (*Prunus domestica*) and for some peach and apricot (*Prunus armeniaca)* varieties has led to greater resistance to this problem (Orazem *et al.*, 2011; Reig *et al.*, 2018). Likewise, in tropical and subtropical fruit trees, such as citrus, the *Citrus macrophylla* rootstock shows greater tolerance to waterlogging than 'Cleopatra' (*C. reshni*) (Pérez-Jiménez & Pérez-Tornero, 2021), just as the 'DusaTM' rootstock is more resistant than the 'Duke 7' avocado (*Persea americana*) (Reeksting *et al.*, 2014). The type of rootstock plays an important role in the adaptation to waterlogging; guava plants propagated by seedlings are more tolerant than those propagated by shoot layering (Kongsri *et al.,* 2020).

When waterlogging ends, pruning the branches can restore the balance of the shoot/root ratio in the tree, especially if there are damaged roots; thus, pruned avocado trees recover more quickly from hypoxic stress, compared to those not pruned (Sanclemente *et al.*, 2014). In addition, the reduced leaf area limits transpiration, which is difficult in plants with damaged roots.

The redox potential of flooded soil can be improved with the application of solid fertilizer such as CaO or MgO. For papaya (*Carica papaya*), adding 5 g of magnesium peroxide (MgO) to the potting medium prior flooding increases the leaf area and the total dry weight of the flooded plants compared to the non-flooded ones because of the oxygenation of the soil (Thani *et al.,* 2016). This improves the redox potential, an indirect indication of O_2 content (Liu & Porterfield, 2014).

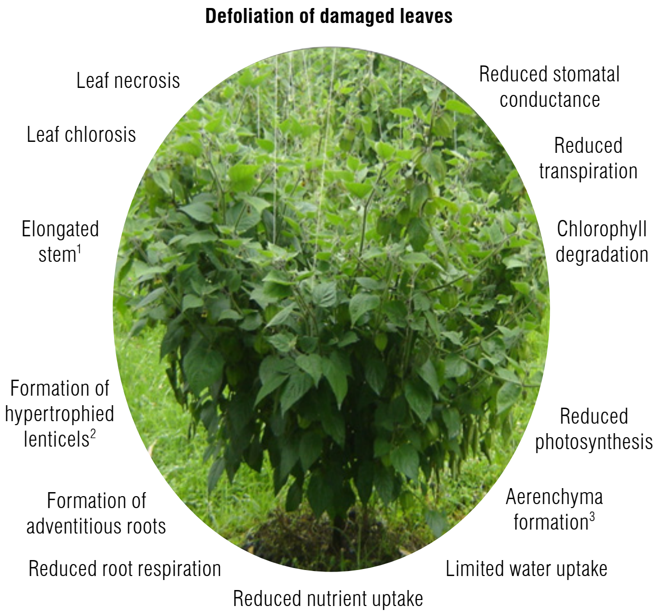

FIGURE 1. Schematic diagram of the effect of waterlogging stress on important processes in tropical and subtropical fruit crops, including adaptive responses. Modified from Sathi, K. S., Masud, A. A. C., Anee, T. I., Rahman, K., Ahmed, N., & Hasanuzzaman, M. (2022). Soybean plants under waterlogging stress: responses and adaptation mechanisms. In M. Hasanuzzaman, G. J. Ahammed, & K. Nahar (Eds.), *Managing plant production under changing environment* (pp. 103–134). (With permission from Springer Nature Singapore).

[1] Under flooded conditions; [2] in some mango varieties; [3] in some species.

Similarly, mycorrhizal colonization of roots can increase tolerance to waterlogging because they promote growth and biomass of plants by improving nutritional conditions and potential adjustment (Tuheteru & Wu, 2017). Flooded purple passionfruit (*Passiflora edulis* f. *edulis*) plants inoculated with a mixture of mycorrhizae (*Glomus caledonium*, *G. etunicatum*, *Gigaspora margarita,* and *Scutellospora* sp.) showed an increased retention of leaves and increased proline and chlorophyll content in leaves and longer maintenance of the foliar content of N and P (Chebet *et al.,* 2020). These authors supposed that the increasing dry and fresh root weights contributed to a higher root health of mycorrhized plants favoring the uptake of mineral nutrients.

Foliar applications of glycine betaine (GB) or hydrogen peroxide (H_2O_2) provides plants with a greater tolerance to waterlogged conditions, favoring their acclimatization. Cape gooseberry (*Physalis peruviana*) flooded for 6 d with applications of 100 mM GB or H_2O_2 show better responses in terms of growth and physiology (stomatal conductance, maximum photochemical efficiency of PSII [F_v/F_m], leaf water potential, relative water content, chlorophyll content, and net photosynthesis) (Castro-Duque *et al.,* 2020). In general, chlorophyll fluorescence is a fast and easy technique to measure the effect of waterlogging stress (Flórez-Velasco *et al*., 2015; Sánchez-Reinoso *et al*., 2019; León-Burgos *et al*., 2022).

Fruit trees adapt better to conditions of abundant rainfall when they are planted in places where floods have not historically occurred, such as on sloping land (Fig. 2). Other measures include avoiding planting crops near bodies of water (lakes, rivers, etc.), avoiding unevenness in planting lots, planting trees in raised beds (Fig. 3) with deep subsoil, and installing drainage (Moreno & Fischer, 2014; Kaur *et al*., 2020).

FIGURE 2. Flooding of Chicamocha river near Duitama (Colombia), with 'Anna' apple (*Malus domestica*) plantation on slopes. Photo: G. Fischer.

FIGURE 3. Raised beds of papaya (*Carica papaya*) plantation prevent waterlogging effects on the roots, La Union (Valle, Colombia). Photo: G. Fischer.

In soils that experience waterlogging and flooding, the long-term use of cover plants improves the soil structure and reduces its compaction by increasing the water infiltration rate (Blanco-Canqui *et al.*, 2015).

Waterlogging and plant diseases

Waterlogged soil, especially in the case of standing water, provides an ideal culture medium for many fungal and bacterial pathogens (Friedrich & Fischer, 2000). This potent combined stress weakens plants, especially the root system that becomes more susceptible to diseases, particularly in avocado, papaya, lychee (*Litchi chinensis*), and pineapple (*Ananas comosus*) (Paull &Duarte, 2012).

Stagnant water leads to root rot in avocado by *Phytophthora cinnamomi* (Reeksting *et al.*, 2014), in pineapple by *Phytophthora* spp. (Fischer, 2012) (Fig. 4), in papaya because of *Pythium aphanidermatum* (Koul *et al.*, 2022), in banana by *Fusarium oxysporum* f. sp. *cubense* (Bolaños, 2019), and in cape gooseberry by *F. oxysporum* (Villareal-Navarrete *et al.*, 2017; Fischer & Melgarejo, 2020). Cape gooseberry infected with *F. oxysporu*m f. sp. *physali*s shows low acclimatization for periods that exceed 6 d of flooding (Arias *et al.*, 2019). One measure to alleviate disease stress in waterlogged soils is the use of rootstocks that are more resistant to these pathogens.

FIGURE 4. Pineapple (*Ananas comosus*) plantation affected by waterlogging due to *Phytophthora* spp. (La Tebaida, Colombia). Photo: G. Fischer.

Some examples of studies on fruit trees

Crane *et al.* (2020) defined three flood tolerance groups of fruit trees: 1) Tolerant – trees can survive high water table and flooded conditions up to a few weeks, but these conditions can reduce growth and production. In this group these authors include: guava (*Psidium guajava*), sapodilla (*Manilkara sapota*), caimito (*Pouteria caimito*), coconut (*Cocos nucifera*) and grafted citrus (*Citrus* spp.); 2) Moderately tolerant trees can survive several days of waterlogged or flooded soil conditions, but these conditions can reduce growth and production (*e.g.* lychee (*Litchi chinensis*), longan (*Dimocarpus longan*), 'Tahiti' lime (*Citrus* × *latifolia*), canistel (*Pouteria campechiana*), mango (*Mangifera indica*), carambola (*Averrhoa carambola*) and banana (*Musa* × *paradisiaca*); 3) Intolerant trees do not tolerate waterlogged or flooded soil conditions that after one or a few days of these conditions can heavily damage or kill the trees (*e.g.* avocado (*Persea ame*ricana), papaya (*Carica papaya*), mamey sapote (*Pouteria sapota*), sugar apple (*Annona squamosa*), atemoya (*Annona* × *atemoya*), passion fruits (*Passiflora* spp.) and jackfruit (*Artocarpus heterophyllus*). Crane *et al.* (2020) underline that the first and second group of fruit crops mentioned above can be damaged or killed by root diseases.

Peach and avocado do not tolerate waterlogging and need about 15% O_2 in the soil solution for survival compared to pear (*Pyrus communis*) and apple that resist up to a 5% O_2 in the soil solution (Fischer & Orduz-Rodríguez, 2012). Passion fruit species, in particular yellow passion fruit (*P. edulis* f. *flavicarpa*) and purple passion fruit (Tab. 1), can tolerate waterlogging conditions for a few days (Fischer & Miranda, 2021), although these findings are probably due to the distinct experimental methods used by the researchers.

Paull and Duarte (2012) also include pineapple as a very sensitive crop to waterlogging. Saavedra *et al.* (2012) attribute the intolerance of avocado to waterlogging because of a lack of absorbing root hairs. Moreno and Fischer (2014) classify species of the genus *Prunus* such as cherry (*P. avium*) and plum (*P. domestica* and *P. salicina*) as intermediate tolerant to waterlogging, as well as citrus (*Citrus* spp.). In the case of bananas, Bolaños (2019) distinguishes between flooding with circulating water that the plants can tolerate up to 72 h, while under non-circulating flooding they only tolerate 24-48 h (Robinson & Galán-Sauco, 2010).

For several fruit trees of tropical and subtropical origin (Tab. 1), waterlogging reduces stomatal conductance, thus affecting the photosynthetic rate, leading to lower leaf area and biomass production in the plant organs (Sanclemente *et al.*, 2014). Increase in chlorosis and necrosis of the leaves leads to a decrease in the chlorophyll content: cape gooseberry shows a 51% reduction in the chlorophyll index for 8 d of waterlogging, observed after 50 d (Aldana *et al.*, 2014). This premature leaf senescence is attributed to the reallocation of mobile elements in the phloem as N, P, and K to younger leaves because of the hypoxic root conditions (Kläring & Zude, 2009).

Measurements of chlorophyll fluorescence in lulo (naranjilla) shows damage at the level of photosystem II from 3 d of waterlogging; the drastic decrease in the maximum efficiency of photosystem II (PSII) (F_v/F_m) is especially evident in plants waterloged for 9 d (Sánchez-Reinoso *et al.*, 2019). In papaya, a decrease in the F_v/F_m values occurs after only 2 d of flooding (Rodríguez *et al.,* 2014). Due to its practical use, Moreno *et al.* (2019) recommended the use of the maximum photosynthetic efficiency of PSII and the stability of the cell membranes as markers for tolerance under waterlogging conditions in trees. The increase in chlorosis and necrosis in the leaves leads to a decrease in the chlorophyll content, probably, caused by the deficient absorption and translocation of mineral nutrients and water by the roots (Fischer *et al.*, 2016).

In general, the results presented in Table 1 show that the tolerance of tropical and subtropical fruit trees to flooding conditions depends on the species, with Solanaceae being among the most affected. These include cape gooseberry (Aldana *et al.*, 2014) (Fig. 5) or naranjilla (Sánchez-Reinoso *et al.*, 2019), which tolerate less than 6 d of flooding. Guava is a very waterlogging-tolerant species (Crane *et al.*, 2020); its seedlings tolerate waterlogging for 8 weeks without dying, and they react with the formation of adventitious roots after 5 weeks (Kongsri *et al.*, 2020).

Basso *et al.* (2019) classify the yellow passion fruit as a species that does not tolerate more than 4 d of waterlogging due to the irreversible effects on its growth and physiology. Govêa *et al.* (2018) find that the waterlogged yellow

TABLE 1. Effect of waterlogging on the growth and physiology of some tropical and subtropical fruit trees.

Species	Waterlogging duration	Decrease	Increase	Authors
Yellow passion fruit (*Passiflora edulis* f. *flavicarpa*)	7 d	Photosynthetic rate; stomatal conductance; intracellular CO_2 concentration; instant carboxylation efficiency.	Root diameter; epidermal thickness; cortex thickness; endodermis thickness; aerenchyma formation.	Govêa *et al.* (2018)
Purple passion fruit (*Passiflora edulis* f. *edulis*)	28 d	Leaf number; foliar N and P; total chlorophyll content.	Carotenoids; proline; soluble sugar.	Chebet *et al.* (2020)
Cape gooseberry (*Physalis peruviana*)	2, 4, 6, and 8 d	DW of the plant after 4 d WL; leaf area, basal stem diameter, plant height and number of reproductive organs after 6 d WL; chlorophyll content after 8 d WL.	Chlorosis, necrosis, epinasty, and leaf abscission, mostly after 8 d WL.	Aldana *et al.* (2014)
Lulo (*Solanum quitoense*)	0, 3, 6, and 9 d	Chlorophyll content; photosystem II damaged after 3 d WL; RWC after 6 d WL; root growth (diameter, length, volume and DW) after 6 d WL.	Electrolyte leakage most on 9 d WL; aerial part/root biomass ratio.	Sánchez-Reinoso *et al.* (2019); Cardona *et al.* (2016)
Tree tomato (*Solanum betaceum*)	2 d (between 35 and 37 DAT); 4 d (51-55 DAT); 6 d (64-70 DAT)	Leaf area; stem diameter; DW of leaves, stem, roots and total plant; F_v/Fm ratio; nitrogen use efficiency.	Stem length.	Betancourt-Osorio *et al.* (2016)
Citrus 'Cleopatra' *Citrus reshni* Hort. Ex Tanaka	7 d	RWC; photosynthetic rate; stomatal conductance; carbon gain (*Ci*/Ca); total chlorophyll content.	WUE (*A*/E); no effect on the concentration of ions in the plant.	Pérez-Jiménez and Pérez-Tornero, (2021)
Guava (*Psidium guajava*)	8 weeks	Photosynthesis; transpiration; SPAD units; plant height; DW of leaves, stem and roots.	Wilting; chlorosis and leaf drop; broken stem bark; formation of adventitious roots.	Kongsri *et al.* (2020)
Avocado (*Persea americana*)	(1) 0, 3 d; (2) 0, 6 d	Photosynthetic rate; stomatal conductance; transpiration; WUE; xylem sap flow; DW of roots, stem, leaves and total plant.	Leaf abscission.	Sanclemente *et al.* (2014)
Papaya (*Carica papaya*)	0, 1, 2, 3, 4, 5 d	Photosynthetic rate; stomatal conductance; transpiration; F_v/F_m; chlorophyll index.	Leaf senescence and death.	Rodríguez *et al.* (2014)
Mango (*Mangifera indica*)	10-20 d	Maximum photosynthesis, stomatal conductance and transpiration at 2-3 d WL.	Absorption of Fe and Mn in calcareous soils.	Schaffer *et al.* (2009)

DW: dry weight; WL: waterlogging; RWC: relative water content; WUE (*A/E*): water use efficiency; F_v/F_m: maximum photochemical efficiency of photosystem II; *Ci*/Ca: carbon gain; DAT: days after transplanting.

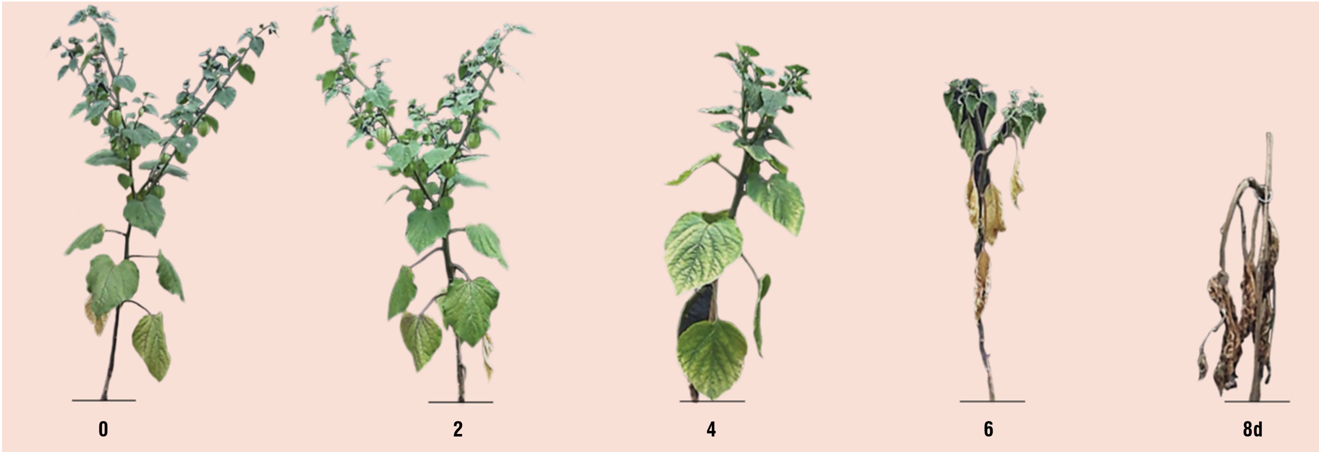

FIGURE 5. Symptomatology of cape gooseberry plants through 0, 2, 4, 6, and 8 d of waterlogging, at 50 d after the beginning of waterlogging (modified from Aldana *et al*. (2014), with permission of *Revista de la Academia Colombiana de Ciencias Básicas Exactas, Físicas y Naturales*).

passion fruit plants had a certain tolerance due to an increase in the diameter of the roots, epidermis, cortex, and endodermis, and the formation of aerenchyma compared to non-waterlogged plants. Faria *et al.* (2020) classify passion fruit plants as moderately susceptible to excess water in their root system.

No studies have been carried out on the effect of waterlogging on fruit trees from the tropical and subtropical zone growing in the highlands; these will be greatly affected by climate change. This situation is mentioned by Fischer and Parra-Coronado (2020) and Fischer *et al.* (2020) for feijoa (*Acca sellowiana*), Fischer *et al.* (2021) for sweet cucumber (*S. muricatum*), and by Fischer and Miranda (2021) for banana passion fruit (*P. tripartita* var. *mollissima*).

Conclusions

Because of the effects of climate change and especially global warming, waterlogging and flooding incidents in tropical and subtropical fruit crops have increased in frequency and are unpredictable. As a result of the anaerobic conditions in the rhizosphere, phytotoxic conditions increase from the accumulation of reduced ions and products of anaerobic organisms, offering an ideal culture medium for many fungal and bacterial pathogens.

Waterlogging leads to depletion of O_2 in the soil with detrimental impacts on root growth and functions and ultimately on the entire plant and its physiology. A decrease in the photosynthetic rate is especially considerable, due to reduced intact leaf areas as a result of chlorosis, necrosis and leaf drop, stomatal closure, and chlorophyll degradation.

Plants have developed different morphological, physiological, and biochemical adaptations to survive stress due to O_2 deficiency in soil. Some fruit trees form an aerenchyma for the diffusion of O_2 from the aerial parts of the plant in order to survive long periods of flooding, in addition to the induction of aerenchyma-containing adventitious roots. A rapid elongation of the stem is one of the most important morphological adaptations of some flooded plants, likewise the formation of hypertrophied lenticels in mango varieties.

Measures for better adaptation of tropical fruit crops to this climatic impact include adaptation of the area of cultivation, selection of varieties and rootstocks more tolerant to hypoxia stress, pruning to reestablish the balance of the aerial part/root ratio in trees, and foliar applications of glycine betaine or H_2O_2. Mycorrhizal colonization in the roots can increase tolerance to waterlogging and the application of fertilizers such as CaO or MgO can improve the redox potential of flooded soil.

Results of studies on the effect of waterlogging on the growth and physiology of tropical and subtropical fruit trees show that guava and coconut are the most tolerant species resisting hypoxia stress during some weeks.

Conflict of interest statement

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

Author's contributions

GF reviewed the bibliography; GF, FCP, and MB wrote the article. All authors reviewed the final version of the manuscript.

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Contribution of community-based companies to sovereignty and food security in the Central Highlands of Ecuador

Contribución de las empresas comunitarias a la soberanía y seguridad alimentaria en la Sierra Central del Ecuador

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ABSTRACT

Popular and solidarity economy (PSE) organizations number around 5,280 throughout Ecuador, with the participation of 153,390 registered members. However, only 1.02% of these companies are community-based and operate in the food production and processing sector. This research aimed to determine the contribution of community-based companies (CBCs) to food sovereignty in the province of Tungurahua, using a mixed methodology including the use of interviews and surveys. The qualitative analysis integrated data on the features of this sector to observe their relationship with social, environmental, and economic dimensions using the RQDA package in R Studio. The results show that CBCs contribute to food sovereignty by adding value to locally produced products relevant to local traditions and culture. The social and environmental contributions of CBCs play a part in rural development. Thus, the promotion of CBCs in food production represents a mechanism through which to strengthen food sovereignty and preserve food production relevant to the local culture in these areas.

Key words: community participation, sustainable development, RQDA, environment and territory.

RESUMEN

Las organizaciones de la economía popular y solidaria suman un total de 5,280 en todo el Ecuador, con una participación de 153,390 miembros que las conforman. Sin embargo, sólo el 1.02% es comunitario para el sector de la producción y transformación de alimentos. El propósito de este trabajo se centró en establecer la contribución a la soberanía alimentaria de las empresas de base comunitaria (EBC). Esta investigación siguió una metodología mixta y utilizó la entrevista y la encuesta como instrumentos de investigación para un grupo de EBC, relacionados con la producción de alimentos en la provincia de Tungurahua. El análisis incluyó variables que describen este sector, además de variables para observar su relación con las dimensiones social, ambiental y económica a través del paquete RQDA de R Studio. Los resultados mostraron que las EBC contribuyen a la soberanía alimentaria ya que se enfocan en dar valor agregado a productos que se producen localmente y que están relacionados con las tradiciones y la cultura de las comunidades. Así, también se incorporan componentes de contribución social y ambiental, que forman parte del desarrollo rural. Esto concluye que la promoción de las EBC para la producción de alimentos representa un mecanismo para fortalecer la soberanía alimentaria y preservar la producción de alimentos apegados a la cultura de los territorios.

Palabras clave: participación comunitaria, desarrollo sostenible, RQDA, medio ambiente y territorio.

Introduction

Ecuador's favorable agroclimatic conditions have supported its agriculture production. A major part of this production is based on primary products. The supply, however, has not reduced unequal access and, in the period after the COVID-19 pandemic, has increased food insecurity by 12% from moderate to severe. The observed increase in poverty affects more than 41% of the rural population (INEC, 2021) and reached 60.1% in 2021 (Latin American Center for Rural Development, 2021). The average income of rural households was about USD 559 per month in 2021, while urban households made USD 896 per month (Banco Central del Ecuador, 2022). An analysis of urban-rural differences in the highlands of Ecuador has indicated a need for public policies that promote productive activities in the rural sector, focusing on strengthening organizational and community aspects at the regional level (North & Cameron, 2008).

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The popular and social economy is an alternative to the traditional company production model. Furthermore, it is a practice where small producers constitute a company as partners and participate with the same contribution of inputs. Also, the political reform in Ecuador of 2008 introduced the framework for boosting a popular and solidarity economy (PSE). Under this reform, organizations had the institutional support to include public policies to the local socio-economic development that contribute to sovereignty and the food security of consumers. Among these are the elimination of limitations associated with access to resources such as information, credit, and technological inputs, which hamper their competitiveness within the global marketplace. The formulation and implementation of such policies can serve to bolster the resilience and competitiveness of these systems, making them more appealing and lucrative for all stakeholders involved. Additionally, associative practices - the strategic fostering of relationships among diverse entities such as public and private institutions, commercial sectors, and communities - have been identified as powerful catalysts for economic growth and regional social development (Ranaboldo & Arosio, 2016).

Locally, food sovereignty is recognized as a cultural nexus with the production and consumption of food, alongside the principles of food security, recovering the collective right to natural resources. Some Andean crops contribute to food sovereignty, in which origin and traditions promote their production. Potatoes or maize represent a base of family food and are part of a model of household farming production characterized by diversification and mixed crops. Food security, in this study, analyzes the availability of nutritional and healthy food in a local area. Significantly, maintaining connections to traditional agricultural practices contributes to the preservation of the environment and resilience in the face of multidimensional changes in the neoliberal economy (Resico, 2011).

Part of the new structure developed since the popular social economy is a Community-based Company (CBC) which is defined as a group of people who have a background in community practices. Likewise, associative promotions with an economic purpose are called community enterprises, social enterprises, or popular and or solidarity economy entities (PSE) (Camacho *et al*., 2005). Community-based companies (CBCs) participate in food security and sovereignty to promote the local production of crops associated with the cultural heritage of the Andean zone.

Moreover, CBC practices respond to the problem of declining incomes for small farmers and the gradual loss of cultivated areas with products related to the culture and customs of rural territories (Mota Botello *et al*., 2018). The self-organization of producers into networks that generate added value represents an opportunity to maintain traditions while bringing forth positive social, environmental, and economic impacts.

The common thread in these definitions is the involvement of multiple actors in the market economy to further local development (Giovannini, 2016). The main distinctive features of CBCs are their emphasis on solidarity, reciprocity, and cooperation (Superintendencia de Economía Popular y Solidaria, 2018).

According to Evert-Jan (2017), CBCs encompass employment, inclusion and entrepreneurship, with the participation of the community of family members of rural territories. This creates an opportunity for the development of rural areas, in that CBCs emerge from a local context, through groups or communities that implement business ideas that generate added value. Thus, the sustainable use of local resources is harnessed in support of social innovation, finding solutions to environmental problems, job creation, and support for knowledge, skills, and the supply of goods and services (Marín Pérez, 2012).

According to the United Nations Environment Program (UNEP), the adoption and application of sustainable food value chain systems can serve as effective mechanisms for breaking the cycle of poverty by offering a comprehensive approach to socioeconomic development. Nonetheless, their sustainability and effectiveness are often encumbered by an array of challenges and complexities. The success of these value chain systems is inextricably tied to the cooperation between various stakeholders involved in the chain. This cooperative effort must span the entire process, from primary production through to final consumption. It is critical, therefore, that all actors within the value chain collaborate effectively, ensuring the system's robustness and resilience. Moreover, there is an acute need for well-designed public policy interventions aimed at enhancing collective bargaining power and competency. By promoting interconnectivity and cooperation, these practices enable the sharing of resources and knowledge, leading to the evolution of more efficient and sustainable food value chain systems (Quiroz *et al*., 2021).

Food sovereignty involves communities applying their policies regarding the sustainable production and accessibility of nutritious food products (Medina Rey *et al*., 2021). In this context, food is a basic need that displays a relationship with the cultural identity of communities, as reflected in the origins of products and the people who provide them. The promotion of food sovereignty practices incorporates direct forms of producer-consumer relationships to strengthen rural development. Thus, there is an emphasis on short marketing circuits that focus on geographical proximity and enhance the social capital of communities (Ranaboldo & Arosio, 2016). These mechanisms play a role in food security and sovereignty, a vital tool for physical, social, and economic access to safe and nutritious food at all times (FAO, 2011).

Food CBCs operate through relationships that contribute to food sovereignty, promoting regional development, increasing the employment of both men and women, and absorbing local production, with positive consequences for the economy and environment. They are involved in the entire food production supply chain: production, transformation, distribution, marketing, and consumption, and are, thus, a key factor in supporting food sovereignty and security (Nusantoro, 2018). They also contribute to health, nutrition, socioeconomic development, equity, and the protection of the environment, since they are directly involved in the implementation of sustainable food systems and provide tangible benefits for the population (Soares *et al.*, 2020). This contrasts with the food industry's focus on increasing production yields, extending the shelf life of food, and incorporating food additives for the transformation and production of processed and ultra-processed food products (Floros *et al.*, 2010).

Finally, a fundamental divergence exists between industrial models and community-based food production. Firstly, CBCs operate through an integrated approach, actively participating in all aspects of the food production supply chain. This ranges from the initial stages of production and transformation to distribution, marketing, and ultimately consumption (Nusantoro, 2018). This analysis determines that a key characteristic of these enterprises is their commitment to food sovereignty, which they achieve by absorbing local production and fostering regional development. In addition, they also enhance employment opportunities for both men and women, leading to positive economic consequences.

The multifaceted involvement of CBCs in the food supply chain also allows them to actively contribute to health, nutrition, socioeconomic development, equity, and environmental protection. This is primarily achieved by implementing sustainable food systems, which inherently yield tangible benefits for the population at large (Soares *et al.*, 2020). In essence, the focus of CBCs is not only on food production but also on the holistic betterment of the community, ensuring food security and making a significant contribution to overall societal well-being.

On the other hand, the industrial model of food production has a distinct focus. This model emphasizes increasing production yields and extending the shelf life of food, often through the use of food additives (Floros *et al.*, 2010). These processes typically result in the production of processed and ultra-processed food products, which can potentially have adverse health impacts eventually. Consequently, while the industrial model may contribute to food availability, it may not necessarily enhance food sovereignty or security, and its impact on overall societal well-being may be less balanced.

In the context of the Central Highlands of Ecuador, this study examines the contributions of CBCs to food sovereignty, using data on food-producing CBCs and local impact indicators. The contrasting approaches between CBCs and industrial food production underscore the need for more sustainable and community-oriented methods of food production that directly contribute to societal well-being.

Materials and methods

Data collection

This study was conducted in December 2021 in the province of Tungurahua, in the Central Highlands of Ecuador. A non-experimental design with a mixed methodological approach using both descriptive and correlational analyses was used to understand the role of community enterprises in terms of their characteristics, contributions, and processes, with the primary data collected through an interview and a questionnaire.

The interview, which aimed to gather information on the contexts of operation and the characteristics of CBCs, was conducted at the beginning of the study and was used to inform the design of the questionnaire, which consisted of open-ended questions to gather information on the following aspects of operation: type of raw materials used, number of workers, target market, date of incorporation, and location (Tab. 1). The interview was carried out with the manager of one company.

Surveys were done on 21 CBCs using the database from the National Census from the Superintendencia de Economía Popular y Solidaria from Ecuador. The questionnaire had 24 semi-structured open and closed questions. A section regarding the classification of food products and their contribution to food security was designed using PAHO, World Health Organization (WHO), and FAO documents focusing on food classification and its health implications (OMS & OPS, 2015; Coronel Carbo & Marzo Páez, 2017). Hence, to analyze the contribution of CBCs to food sovereignty, the questionnaire was based on "The Six Pillars of Food Sovereignty" by Food Secure Canada (Food Secure Canada, 2020). The validation of questions was undertaken by experts, who contributed to their final formulation. The collection of information was done online, due to the restrictions caused by the COVID-19 pandemic.

Population and sample

For the determination of the population, a database was obtained from the Superintendent of Popular and Solidarity Economy in 2021, from which we extracted 275 popular and solidarity economy organizations focusing on agricultural, artisanal, industrial, and food production (Superintendencia de Economía Popular y Solidaria, 2021). Our sample was selected from this population of organizations using non-probabilistic convenience selection.

More specifically, only those community companies engaged in the production of value-added food products were selected. Twenty-one surveys were applied, with one interview at the beginning of the study to formulate and validate the questionnaire. Table 1 lists the twenty-one community-based enterprises in our sample.

Table 1 provides a dataset that serves as a comprehensive registry of industrial and agricultural organizations, primarily located within the Tungurahua province of Ecuador. Each entry in the dataset corresponds to an organization and provides a multi-faceted characterization of the entity in question.

TABLE 1. Community companies producing value-added food analyzed in this study.

Name	Location	Years of functioning	Economic capital (USD)	Member	Number of employees	Type of production
Asociación de Producción Industrial de Migrantes y Familiares de Tungurahua	Tisaleo	3	400	Family members	13	Minimally processed/raw, semi-processed
Asociación de Producción Agrícola Cadena Provincial de la Mora	Tisaleo	11	5500	Community members	18	Semi-processed
Asociación de Producción Alimenticia Zare	Pelileo	1	400	Family members	4	Minimally processed/raw, semi-processed
Asociación de Producción Alimenticia Vergel Green	Quero	5	300	Family members	13	Semi-processed
Asociacion Agropecuaria Mulanleo	Ambato	40	0	Community members	2	Processed
Asociación de Productores y Comercializadores de Leche del Cantón Quero	Quero	15	600	Community members	4	Minimally processed/raw, processed
Asociación de Producción Alimenticia Industrial Chiquipulp	Pelileo	12	100	Community members	7	Processed
Asociación Artesanal la Chocolatera Ambateña	Ambato	15	1900	Friends	15	Processed
Asociación de Productores Agropecuarios Las Viñas Pachanlica	Pelileo	10	1000	Community members	30	Minimally processed/raw
Asociación de Producción Pecuaria Tamboloma	Ambato	8	400	Community members	2	Minimally processed/raw
Asociación de Productores Agropecuarios San Luis de Tisaleo	Tisaleo	15	50	Community members	15	Processed
Asociación Unión Tisaleña	Tisaleo	4	0	Community members	20	Semi-processed
Asociación de Ganaderos y Productores de Leche Fe por la Leche Yatchil	Píllaro	1	100	Community members	37	Minimally processed/raw
Asociación de Producción Ganadera Mirador del Condor	Píllaro	2	500	Community members	3	Minimally processed/raw
Asociación Serafín Montesdeoca	Ambato	21	5000	Community members	1	Processed
Asociación de Productores Alternativo la Dolorosa	Tisaleo	17	200	Women	18	Minimally processed/raw

Continued

Name	Location	Years of functioning	Economic capital (USD)	Member	Number of employees	Type of production
Asociación Agro Artesanal de Productos Lácteos el Lindero	Ambato	20	800	Family members	2	Processed
Asociación de Producción Agropecuaria Benicultores	Pelileo	5	0	Community members	12	Semi-processed, processed
Asociación de Producción Agroecológica Sabiduría Pillareña	Píllaro	4	200	Community members	1	Minimally processed/raw, semi-processed
Asociación Artesanal de Producción de Bienes Agrícolas y Pecuarios del cantón Píllaro	Píllaro	15	1000	Community members	20	Minimally processed/raw, semi-processed, processed
Cooperativa de Producción, Acopio, Industrialización y Comercialización de Cuy Tungurahua	Ambato	6	1360	Friends	1	Minimally processed/raw
Asociación de Alimentación Orquideans (Orquídeas)	Píllaro	4	20	Community members	15	Semi-processed

The first aspect of characterization is the organization name, a vital identifier that often also offers a glimpse into the organization's operations or objectives.

The next aspect is location, which, given the rural nature of the regions in the dataset (Tisaleo, Pelileo, Quero, Ambato, and Píllaro), could have implications for the type of agricultural or industrial activities the organizations are engaged in, or the markets they have access to.

The third aspect is the number of years since the organization constitution. This information serves as a proxy for the organization stability, its ability to withstand market dynamics, and its accumulated experience within its field of operation.

The dataset also provides insights into the financial backbone of the organizations as indicated by their economic capital expressed in USD. This refers to the total financial assets, net worth, or operational budget of each entity, which would, in turn, influence their ability to invest and expand.

The demographic or relational constitution of the membership of each organization is also included, with categories such as "Family Members," "Community Members," "Friends", or "Women." These affiliations could significantly influence the organization governance structure, decision-making process, and the alignment of its objectives.

Also included is the number of employees, providing an estimate of each organization size, the scope of its operations, and its contribution to local employment.

Lastly, the type of production undertaken by each organization is mentioned, segmented into minimally processed or raw goods, semi-processed goods, and fully processed goods. This classification not only sheds light on the kind of products each organization deals with but also points to the complexity and sophistication of the organization operations, its potential markets, and likely sources of revenue.

Data analysis

The data from surveys that provided qualitative information was processed using a Likert scale. Each response was given a numerical value and then quantified. This operation is frequently used in other similar studies (Gutiérrez-Pérez *et al*., 2013; Akhtar *et al*., 2018) . Hence, information was analyzed with descriptive statistics. In the second stage, the database was administered using Excel. To understand the connection between a CBCs business and the contribution to food sovereignty, a qualitative analysis was applied and correlation analyses were conducted using R and R Studio (Mellado *et al*., 2020) (Tab. 2). Text analysis was completed using the RQDA package (Dușa, 2019).

TABLE 2. Components analyzed with qualitative methods.

Components	Variables
Administration	• Parameters and enterprise development • Gender equality
Production	• Value added production. • Food processing contribution • Food security • Food sovereignty • Environment protection
Marketing	• Popular and solidarity economy • Family and collective well-being contribution

In Table 2, the variables analyzed are presented. The social structure, information, and raw material are highlighted as characteristics of CBCs. The definition of these variables came from the interview where the experts developed the importance of the role of CBCs and the consumption of local raw material, the origin of a social organization, and the problems for obtaining technology and equipment.

Results

The results obtained in this research show that community companies in Tungurahua participate in food production through sustainable food system economic factors as well as financial development of small groups. The results highlight the added-value to local production, a direct and indirect benefit to local farmers. Additionally, consumers obtain healthy and nutritious food, thus contributing to food security and sovereignty.

Figure 1 shows the reasons these organizations were formed, which mainly involve the expansion of entrepreneurship (50%) and the promotion of added value to local products (45%). Another main reason was the better commercial price received for products and, thus, generating a fair and direct income for producers, whose resources represent a benefit for organizations and the community (20%). Caring for the environment is also an important purpose of the CBCs in our sample, in addition to obtaining aid from public institutions and creating opportunities to access credit (10%).

We found community companies operating in five of the nine cantons of the province of Tungurahua. Most of the community enterprises are located in the cantons of Ambato, Tisaleo, and Píllaro, and to a lesser extent in the cantons of Pelileo and Quero. Organizations are mostly made up of community residents (70% of the CBCs), reflecting the empowerment of communities through such companies. The participation of family members, including women (30% of the CBCs) as managers, reflects the family structure. There appears to be no concentration of CBCs in areas of high population density, contrary to the distribution of SMEs and food industries (Zapatta & Isc, 2010).

Analysis of administration, production, and marketing processes

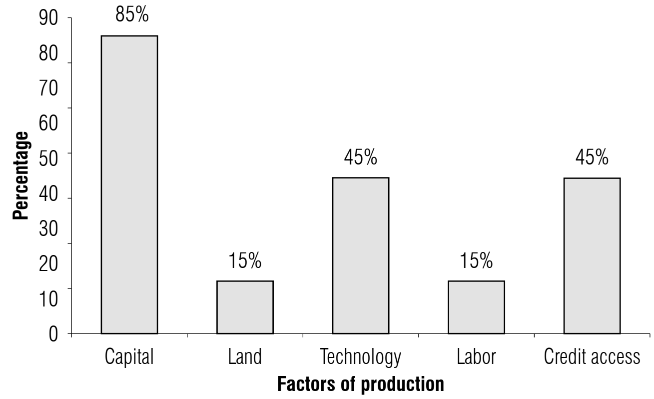

FIGURE 2. Factors of production in community enterprises.

Figure 2 shows trends regarding factors of production, with 85% of community enterprises emphasizing that capital is one of the essential components for the acquisition of all types of assets needed to conduct productive activities.

Forty-five percent of CBCs companies stress the importance of technology for driving innovation, improving product quality, and lowering production costs. Likewise, 45% consider access to credit as a key resource to continue operations, while 15% consider land to be an indispensable resource in terms of food production from agricultural activities. Depending on the type of production and demand for the food products produced, the CBCs in our sample are made up of between 1 and 37 workers. In summary, capital was most commonly mentioned as being vital, compared to the other production factors.

Table 3 shows various difficulties faced by community-based companies in the processes of administration,

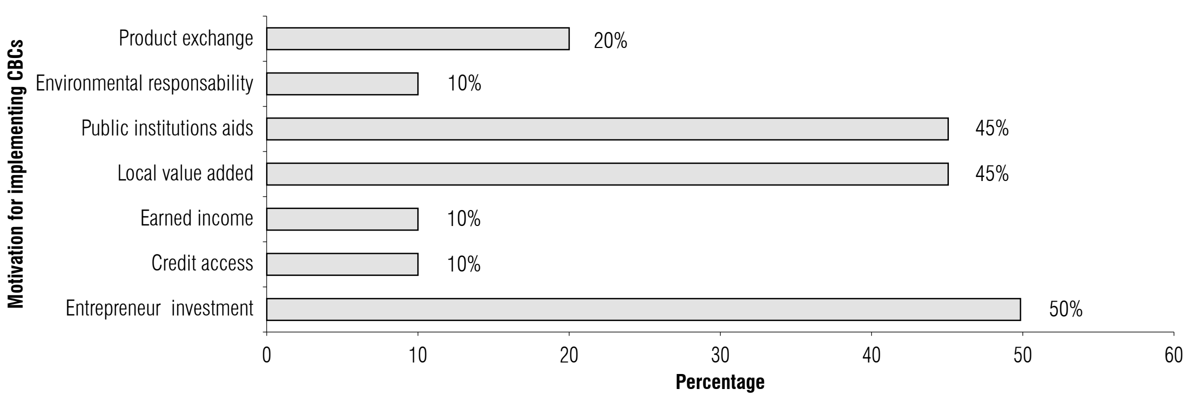

FIGURE 1. Motivations of founding members to form community enterprises.

production, and marketing. In terms of management, 36% of companies report facing obstacles related to leadership, organization, and knowledge. Meanwhile, production or processing presents difficulties for 20% of the CBCs in our sample. These processes are affected by a lack of resources to obtain raw materials and equipment, among others, as well as the situation caused by the COVID-19 pandemic, which caused production to become unstable and even paralyzed.

TABLE 3. Challenges faced by community-based companies.

Components	Variables	Reporting frequency	Reporting percent
Administration	• Lack of leadership • Lack of knowledge • Lack of organization	10	36
Production	• Unstable • Lack of raw materials • Lack of equipment and materials • Lack of knowledge	5	20
Marketing	• More competitors • Lack of sales planning • Unbranded products	11	44

Marketing appears to present greater difficulties, with 44% of companies reporting issues in this area. The main problems faced are a large supply of related products, a lack of planning or strategies, and products without any identification or brand. Social and economic inclusion is a large way to successful due to the capital constraint. This limitation has been solved the support of national NGOs involved in the territory (*i.e.*, Maquita or Heifer) and international cooperation.

Table 4 details the variety of food products produced by the CBCs in our sample and the raw materials used to produce them. The provenance of raw materials used by the organizations in our sample reflects their focus on the local economy. More specifically, 68% of community companies use raw materials from their local area, taking advantage of local crops for the welfare of the inhabitants of the communities. Hence, a 20% include the use of raw materials from a different province and 8% from a different territory. It should be noted that some of the community companies use the agroecology food production mode, bringing benefits to the population in terms of health and food sovereignty. Thus, the contribution of CBCs to food security and sovereignty is evident.

Table 4 shows the raw materials and products with a relationship to food sovereignty. The information shows that vegetables and animals are the most common primary product used for CBCs companies. In general, most of these raw materials are grown in the communities where community enterprises are located. The whole province of Tungurahua is characterized by high levels of agricultural activity, particularly the production of domestic species, and products such as milk, fruits, and vegetables that have high nutritional value for human consumption. Products of marine origin come from coastal provinces. All the raw materials indicated are processed to produce food products such as dairy, meat, cereals, preserves, condiments, oil, snacks, and beverages. Certainly, most products contribute favorably to nutrition, since their raw materials are produced ecologically and do not contain components that affect the health of consumers, such as substances from chemical products.

This dataset underscores the significant role popular and social economic companies play in strengthening food sovereignty in Ecuador by fostering local supply chains and economic stability (FAO, 2014). By processing a diverse range of primary products into market-ready goods, these entities boost local self-sufficiency, insulating communities from external market volatilities (Altieri, 2009). The data indicates a strong emphasis on food safety and demonstrates a clear commitment to food sovereignty, reflecting the alignment with sustainable development goals. Moreover, the economic capital and employment data point towards these organization potentials to contribute to rural livelihoods, further embedding the principles of food sovereignty within the local economies (Holt-Giménez *et al.*, 2011).

The findings in this study align with the principles of effective food handling and safety protocols widely acknowledged in scientific literature. Effective food safety management involves rigorous controls, personnel hygiene, prevention of contamination in production areas, adequate cleaning and disinfection practices, and appropriate use of protective equipment (World Health Organization, 2006). Indeed, a study by Park *et al.* (2010) found that compliance with these protocols played a pivotal role in enhancing food safety and thereby contributing to food security.

The data presented also correlate food safety practices to food sovereignty, underscoring the importance of safe and responsible food production at the local level for self-sufficiency (Windfuhr & Jonsén, 2005). This view is reflected in the works of Claeys (2015), who argues that the ability to exercise control over local food systems and practices - a core principle of food sovereignty - is inherently tied to ensuring food safety standards.

TABLE 4. Contribution to food security and sovereignty of community enterprises.

Primary product	Processed products	Food safety	Food sovereignty
Seawater	Salt	No	Yes
Mango and salt	Canned mango	Yes	Yes
Blackberry	Nectar	Yes	Yes
	Wine	Yes	
	Juice	Yes	
	Jam	Yes	
Fruits and vegetables	Frozen foods	Yes	Yes
Quinoa, oats, flaxseed, stevia, and honey	Healthy snacks	Yes	Yes
Milk	Butter	No	Yes
	Fresh cheese	Yes	
Milk	Dairy products	Yes	Yes
Fruits	Fruit pulp	Yes	Yes
Fine aroma cocoa	Chocolate bars	No	Yes
Avocado	Oil	No	Yes
Fruits	Jam	Yes	Yes
Milk	Fresh cheese	Yes	Yes
Blackberry	Wine	Yes	Yes
Blackberry	Wine	Yes	Yes
Milk	Yogurt	Yes	Yes
Milk	Cheese	Yes	Yes
Milk	Cheese	Yes	Yes
	Yogurt	Yes	
Blackberry	Wine	Yes	Yes
Milk	Fresh cheese	Yes	Yes
Barley	Barley flour	No	Yes
Peas, Corn, broad beans	Wheat flour	No	Yes
Potato	Wine	Yes	Yes
Blackberry	Jam	Yes	Yes
	Wine	Yes	
Milk	Creams	Yes	Yes
Rabbit meat	Sausages	Yes	Yes
Fruits	Jams	Yes	Yes
Milk	Yogurt	Yes	Yes
	Fresh cheese	Yes	
Guinea pig, rabbit, chicken	Slaughtered animals	Yes	Yes
Guinea pig	Slaughtered guinea pigs	Yes	Yes
Blackberry	Wine	Yes	Yes
	Jams	Yes	
Native potatoes	Potato chips	Yes	Yes

TABLE 5. Classification of food products and contribution to food security and sovereignty.

Classification	%	Food safety (%)	Food sovereignty (%)
Minimally processed	5.5	83.3	100
Culinary ingredients	16.7		
Processed foods	77.8		
Ultra-processed foods	0		

Table 5 shows the level of production of processed food products in our sample. Added value is generated through processing for 77.8% of processed foods, followed by 16.7% of culinary ingredients and 5.5% of minimally processed foods. Our sample contained no community company focused on the production of ultra-processed foods, a benefit to consumers since consumption of these foods leads to chronic diseases. It was found that 83.3% of the food products produced by community companies contribute to food security, especially in terms of nutritional quality. Similarly, 100% of companies contribute to food sovereignty through practices that are beneficial in terms of environmental protection, local production, and food accessibility.

TABLE 6. Marketing channels and target market of community enterprises.

Marketing channels	Target market
Short marketing circuits	
Neighborhood shops	Children
Squares and markets	Young people
Supermarkets	Adults
Entrepreneurship fairs	Self-consumption (family)
Requests	Pregnant/lactating women
Baskets at home	Vegans
Business services	Health food consumers
Electronic media/websites	

Table 6 presents the primary marketing strategies employed by CBCs. These strategies exhibit considerable heterogeneity and diversification, reflecting the dynamic nature of CBC practices. A key strategy employed by CBCs involves direct sales, which is the transfer of goods directly from producers to consumers. This mode of distribution facilitates personal engagement, supports local economies, and boosts food sovereignty by connecting consumers directly to the food production process.

Moreover, the CBCs actively participate in local entrepreneurship fairs, squares, markets, and other short marketing circuits, strengthening the local food system and enhancing the community's access to locally produced goods. These channels not only promote the fair trade of products but also generate valuable resources that directly benefit businesses and communities. By eliminating intermediaries, CBCs ensure a larger portion of the economic benefit stays within the local economy, boosting food sovereignty and self-sufficiency.

Likewise, marketing promotes better social relations between producers and consumers, who share information and have the desire to know details regarding the food products on offer, their benefits, and the production processes undergone by both the raw materials and the product itself. This then provides benefits for the producer through the generation of more income and for the consumer through the supply of healthy food.

Half of the companies in our sample (50%) also contribute to feeding the families of their own CBC members. Nevertheless, a large portion of the food products produced is destined for trade, reaching target markets of people of all ages.

The contribution of community enterprises to community development

Community enterprises generate positive impacts in terms of development in their communities, as detailed in Table 7. This is favorable for the inhabitants and therefore for the communities because rural areas are the most marginalized and tend to lack development opportunities.

TABLE 7. Impacts generated by community enterprises.

Dimension	Impact	%
Social	Gender equality	50
	Avoidance of emigration	50
	Support for vulnerable people	45
Economic	Trading opportunities	60
	Employment opportunities	50
	Creating wealth for social purposes	25
Environmental	Ecosystem protection	45
	Community *mingas**	45
	Production for self-consumption	70

* Term used in the Quechua language for community labor.

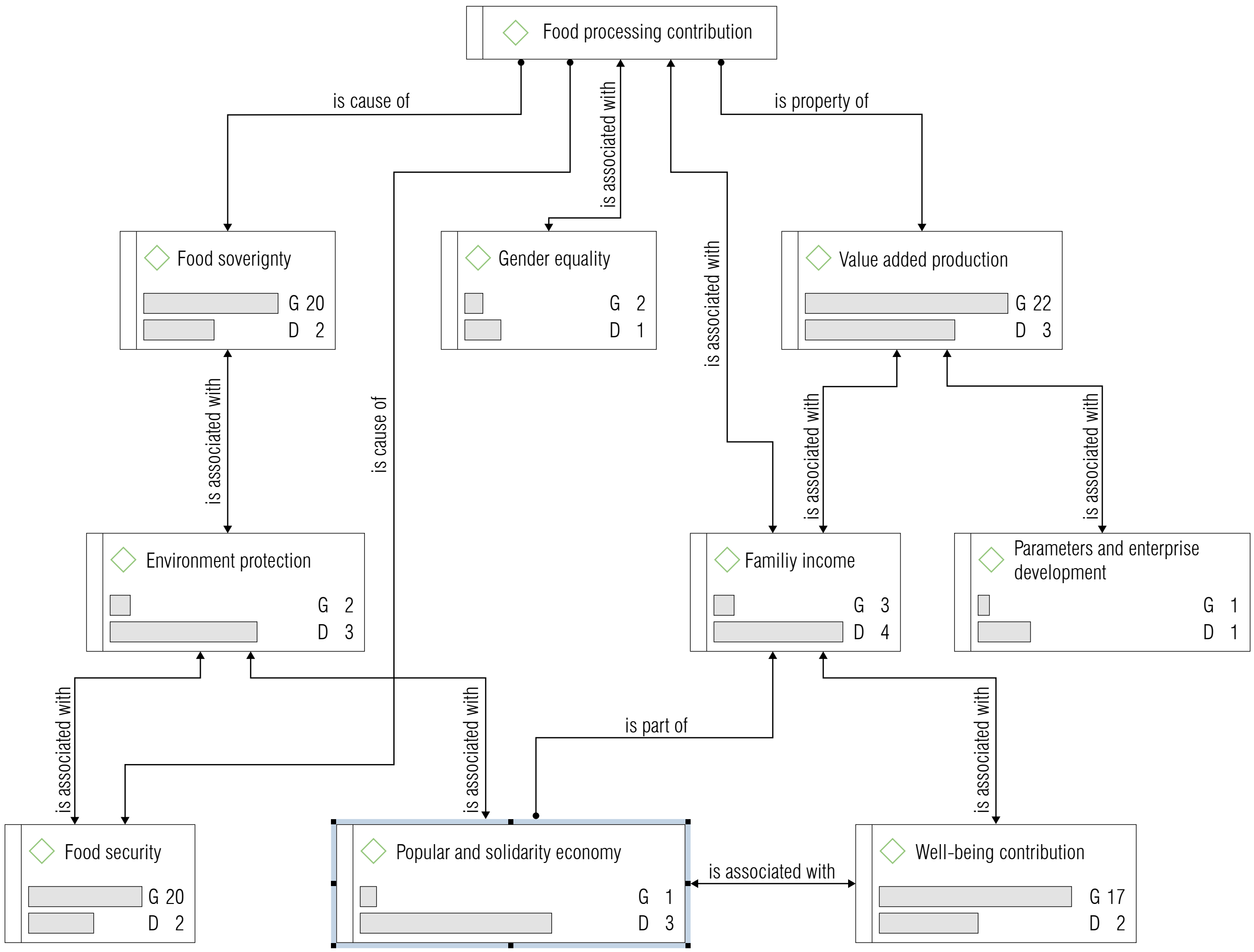

FIGURE 3. Illustration of the relationships between codes obtained from the qualitative analysis of the information. G and D are the indexes of frequency, where G represents groups in relationship and D is the linked document.

Various social, economic, and environmental impacts are observed as a result of the improvement of opportunities for both men and women through trade, employment, and wealth creation. This involvement of inhabitants contributes to productive activities and their well-being overall. Community enterprises also show a sense of environmental responsibility in terms of the protection, conservation, and preservation of natural resources. Finally, their contribution to food sovereignty is evident in the extent of the production of food for self-consumption.

A text analysis showed that CBCs contribute to rural development, in terms of food security and sovereignty, and to social and environmental protection impacts, which generate added value in food production and processing (Fig. 3).

In Figure 3, each bar graph provides a specific amount of information about the fit of the proposed model, according to the variables observed.

Figure 4 shows that the observed cases are distributed above the average of the residuals. In the Q-Q plot, the regression is expressed, reaching a slope greater than 1. From these diagnostic graphs, we can say that the model fits the assumption of homoscedasticity.

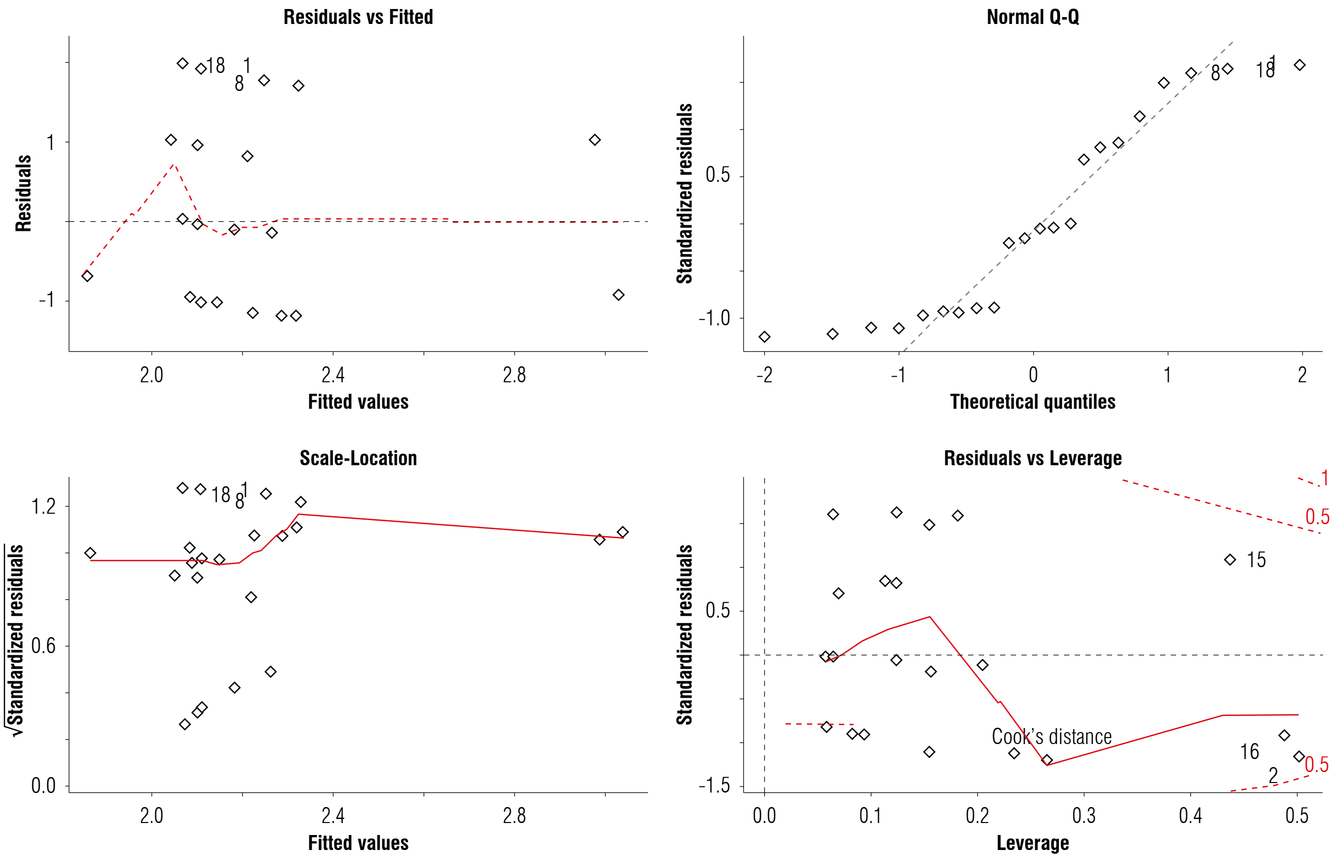

FIGURE 4. Diagnostic plots showing the unexplained variance (residuals) over the entire range of observed data.

Discussion

Community enterprises represent an instrument for rural development. In Ecuador, progress in rural areas has been made through the promotion of agro-industrial enterprises and companies that provide opportunities and help address malnutrition, especially in Indigenous communities (Villafuerte & Represa, 2017). This is in line with Vázquez *et al.* (2018), who point out that social enterprises represent a social innovation that promotes entrepreneurship, while the added value improves the commercial value of products, benefiting stakeholders through the mechanisms of employment, sustainable exploitation of resources, and positive social and environmental impacts.

In the province of Tungurahua, Ecuador, the generation of added value by popular and solidarity economy (PSE) organizations made up of inhabitants is concentrated in communities in the cantons of Ambato, Tisaleo, Píllaro, Pelileo, and Quero. There is evidence of a specialization in products such as vegetable oil, snacks, dairy products, meat, cereals, beverages, and preserves, with 83.3% of food products contributing to food security. In addition, food security is ensured through the application of efficient management in terms of safety and hygiene measures that relate to the control, cleaning, and disinfection of equipment and the use of personal protective equipment, which is essential to avoid contamination during food processing.

Regarding the classification of food and beverages, there is a considerable production of processed food products (77.8%) consisting of dairy, beverages, preserves, and snacks. These products contribute to food security, provided that their consumption is moderate, due to their high content of salt, sugar, and fat, and that they are balanced in terms of macro- and micro-nutrients (FAO, 2013). Weaver *et al.* (2014) emphasize that both natural and processed foods contribute to food security by promoting the consumption of vital nutrients such as vitamins, minerals, and fiber. However, consumption of foods that contain excess sodium, sugars, and saturated fats must be limited. Minimally processed foods are 5.5% of total production. This category contributes significantly to food security since these products have not undergone extreme changes from their original state. No production of ultra-processed foods was observed; this is positive, as the nutritional composition of these is relatively unbalanced, which generates disadvantages for the health of consumers, including obesity (AESAN, 2020).

Based on the pillars of food sovereignty, which emphasize healthy food for all, the valorization of producers, direct marketing at fair prices, and positive impacts on nature (Food Secure Canada, 2012), this study has found that community companies contribute significantly to food sovereignty in the province of Tungurahua, Ecuador. By taking advantage of the use of raw materials from local areas, as well as agroecology, fair trade, a solidarity economy, direct marketing, care for the environment, and production of food for self-consumption, these enterprises provide sustainable development and improve the living conditions of the communities in which they operate.

Direct marketing between producer and consumer through different marketing channels, including entrepreneurship fairs and short marketing circuits, has advantages in terms of greater commercial prices for food products and positive impacts on the ecosystem by minimizing transport across long distances (ECLAC, 2014). Marketing channels allow CBCs to reach people of all ages and promote consumption habits that are beneficial for the health of the people of Tungurahua.

For the twenty-one community enterprises in our sample, capital was found to be a significant production factor (for 85% of enterprises), as capital is required to ensure competitiveness, security, and good performance (Kongtanajaruanun, 2017). Technology and access to credit were reported by 45% of the CBCs as being very important. A study conducted by García and Chávez (2020) obtained similar results when identifying the needs of 64 organizations in the Imbabura province (North of Ecuador). The results show that the main needs of organizations include sources of financing, implementation of technology, advice on business and marketing management, liquidity, and physical infrastructure. Undoubtedly, insufficient factors of production can cause many difficulties in terms of administrative processes, production, and marketing. There is evidence of cooperation with public institutions for economic resources, supplies, and machinery, as evidenced by Evert-Jan (2017), who found that social enterprises receive both financial and non-financial support such as machinery, training, and technology from governmental and non-governmental institutions. Wawire and Nafukho (2010) show that each person involved in a CBC fully fulfills their role through the implementation of strategies to generate and guarantee greater productivity in the productive processes, to propel the development of the community enterprise and the communities themselves.

Through their social, economic, and environmental contributions in the province of Tungurahua, companies generate positive impacts in terms of employment, trade, gender equality, decreased emigration, and care for the environment. As Ruiz (2015) points out, solidarity economy organizations promote the development of rural communities facing problems of poverty and marginalization, guaranteeing protection, equity, and well-being for community members.

Conclusions

The results suggest that the current trends in food production by CBCs are encouraging. Sustainable practices and production of foods that maintain a relationship with traditions and that are nutritionally adequate set a pattern for food production in the future. Although there are limitations in terms of the professionalization of CBCs, their organizational practices contribute to reducing food insecurity. In addition, the proliferation of by-products from Andean crops opens the door for new CBCs to be created in the future, thereby generating alternative economic channels for rural households.

Conflict of interest statement

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

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Author's contributions

CFC: conceptualization, data curation, supervision, and validation; LT: formal analysis, investigation, and methodology. All authors reviewed the final version of the manuscript.

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Performance of agri-food organizations in the Colombian Central Region supplying food to Bogotá

Desempeño de organizaciones agroalimentarias de la región Central de Colombia que abastecen alimentos a Bogotá

Miguel Augusto Delgado-López[1]*, Juan Carlos Barrientos Fuentes[1], and Dursun Barrios[1]

ABSTRACT

The city of Bogotá requires approximately 2.7 million t of food products annually, of which 84% comes from the territories associated with the Central Region of the country (Bogotá, Boyacá, Cundinamarca, Meta, and Tolima). Small agricultural producers, who supply food to the main urban centers through intermediaries, are now targeted by a new marketing strategy promoted by the district. The objective of this strategy is to transform *agroredes* (organizations of small agricultural producers, in Spanish) into participants in the food market in the city of Bogotá. However, these organizations need to enhance their operations; measuring their performance is fundamental for this purpose. The objective of this research was to assess the performance of 60 agroredes in the Central Region of Colombia using the organizational performance index (OPI). For this, a quantitative, transversal and descriptive research was carried out, evaluating agricultural organizations across various dimensions, including administration and finance, associativity and network management, marketing, information and communications technology (ICT), management and logistics, and storage. The organizational performance of the agroredes in the Central Region of Colombia is currently in a consolidation stage. The performance dimensions that have been identified are associativity and network management. However, the most significant challenges are found in ICT management.

Key words: agricultural markets, agricultural enterprises, agricultural management, rural economy.

RESUMEN

La ciudad de Bogotá requiere anualmente aproximadamente 2.7 millones t de productos alimenticios, de los cuales el 84% proviene de los territorios asociados a la región Central del país (Bogotá, Boyacá, Cundinamarca, Meta y Tolima). Los pequeños productores agrícolas, que abastecen de alimentos a los principales centros urbanos a través de intermediarios, son ahora el objetivo de una nueva estrategia de comercialización impulsada por el distrito. El objetivo de esta estrategia es transformar a las agroredes (organizaciones de pequeños productores agrícolas) en participantes del mercado de alimentos de la ciudad de Bogotá. Sin embargo, estas organizaciones necesitan mejorar sus operaciones; medir su desempeño es fundamental para este propósito. El objetivo de esta investigación fue determinar el desempeño de 60 agroredes de la región Central de Colombia mediante el índice de desempeño organizacional (IDO). Para tal fin, se realizó una investigación cuantitativa, transversal y descriptiva, evaluando las organizaciones agrícolas en diversas dimensiones, incluyendo administración y finanzas, asociatividad y gestión de redes, marketing, tecnologías de la información y las comunicaciones (TIC) y gestión y logística, y almacenamiento. El desempeño organizacional de las agroredes de la región Central de Colombia se encuentra actualmente en una etapa de consolidación. Las dimensiones de desempeño que se han identificado son la asociatividad y la gestión de redes. Sin embargo, los desafíos más importantes se encuentran en la gestión de las TIC.

Palabras clave: mercados agrícolas, empresas agrícolas, gestión agrícola, economía rural.

Introduction

Recently, citizens from various regions of Colombia have been migrating to Bogotá due to the job opportunities and support benefits offered by the capital city (Pinto *et al*., 2019). According to the 2018 census, Bogotá had approximately 7.1 million inhabitants, which translates to an annual food demand of 2.7 million t (DANE, 2018).

The food supply of the city of Bogotá relies on the production from nearby territories (Reina-Usuga *et al*., 2020). It is estimated that the internal supply of food production reaches 60 thousand t, accounting for a dependency rate of 97.9% (RAPE, 2020).

The territories of the Central Region of Colombia produce approximately 84% of the food supply for Bogotá. This

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region, with its diverse thermal floors, also possesses favorable geographical conditions that highlight its agricultural potential for the food supply. Thirty three percent of the essential food items consumed in the capital city are sourced within a distance of 40 km from the capital. Among these, 58% of the vegetables, legumes, tubers, and plantain come from municipalities in the department of Cundinamarca, 22% from the department of Meta, and 12% from the department of Boyacá (Mejía *et al*., 2021).

The consumption of the basic food basket in the Central Region amounts to approximately 4,996,619 t, where 55.6% is consumed in Bogotá. Additionally, the region is home to around 15.7 million inhabitants, and its annual food production reaches 15 million t (DANE, 2020).

In 2019, the Central Region had a multidimensional poverty rate of approximately 34.6%. This figure breaks down into an average of 10.1% for urban areas and 23.5% for rural areas. This condition is heterogeneous in each department that makes up the region (Arredondo Sánchez, 2021). Food marketing is marked by inequity in distribution, with producers assuming economic risks due to price changes and market instability. The distribution of these products is characterized by high production costs, long transportation times, inadequate road infrastructure, low levels of associativity, frequent reshipments, and underutilized vehicle capacity. Furthermore, limited opportunities for direct market access and the low administrative, logistical, financial, and associative capacity of growers further exacerbate their dependence on intermediaries (Romagnoli *et al*., 2018).

Approximately 750,000 persons are involved in food production and supply, with 67.7% of them being elderly individuals who earn less than a monthly minimum wage ($289,24 USD). Additionally, their products pass through up to eight intermediaries before reaching the supply centers. This results in long marketing circuits and minimal value addition to the final product (Borsellino *et al*., 2020).

To address the limitations in the food supply, the creation of the Master Plan for Food Supply and Food Security for Bogotá Capital District (PMASAB) has been established, along with the implementation of the *agrored* strategy. An "agrored" is defined as a collective of food producers aiming to enhance the efficiency of food supply to urban areas. It also strives to optimize production costs and boost the income of the rural population (Castellanos & Parrado, 2020). Its primary purpose is to streamline the supply and processing within a subregion, as per Decree 315 of 2006 by the Mayor's Office of Bogotá.

This strategy focuses on establishing networks that integrate rural production and neighborhood management. Its objective is to organize the supply and processing of food within subregions of the Central Region, thus, reducing input costs and expenses associated with production dispatch, product distribution, and intermediation (Mejía & García-Díaz, 2018). Additionally, the agroredes are part of the regional integration policy, which promotes their contribution to regional rural development (Ussa Garzón *et al*., 2020).

The agrored strategy involves aligning supply policies with the capacities of producers to generate value-added transformed products. It also contemplates a long-term vision to avoid the centralization of resources in short-term projects without proper monitoring (Novoa & Aranda Camacho, 2019).

In the light of this perspective, government entities endorsed strengthening of associative processes among producers in production areas. However, limited understanding of organizational challenges and cultural barriers diminishes the effectiveness of the objectives set by rural agricultural organizations (Minh, 2019). Organizations of small growers face limitations in achieving the desired results, due to the lack of strategic plans and collective decision-making, deficiencies in task distribution among the participants, and low participation of the associate members (Morris *et al*., 2017). In this context, an organizational performance tool emerges, which evaluates the extent to which objectives have been accomplished (Singh *et al*., 2016). Its measurement enables continuous assessment of strategies, improvement of decision-making, implementation of new management approaches, and the assurance of long-term profitability (Barrios *et al*., 2020).

The objective of this research was to assess the organizational performance of the agroredes of the Central Region of Colombia that supply food products to the city of Bogotá.

Materials and methods

The research was of quantitative, cross-sectional, and descriptive nature. The use of a quantitative approach was necessary to evaluate numerical data for subsequent statistical analysis. The research was cross-sectional and descriptive to allow describing a phenomenon and its characteristics at a specific moment in time, using surveys for data collection (Nassaji, 2015).

The study was conducted in the territories comprising the Central Region of Colombia, which include Boyacá,

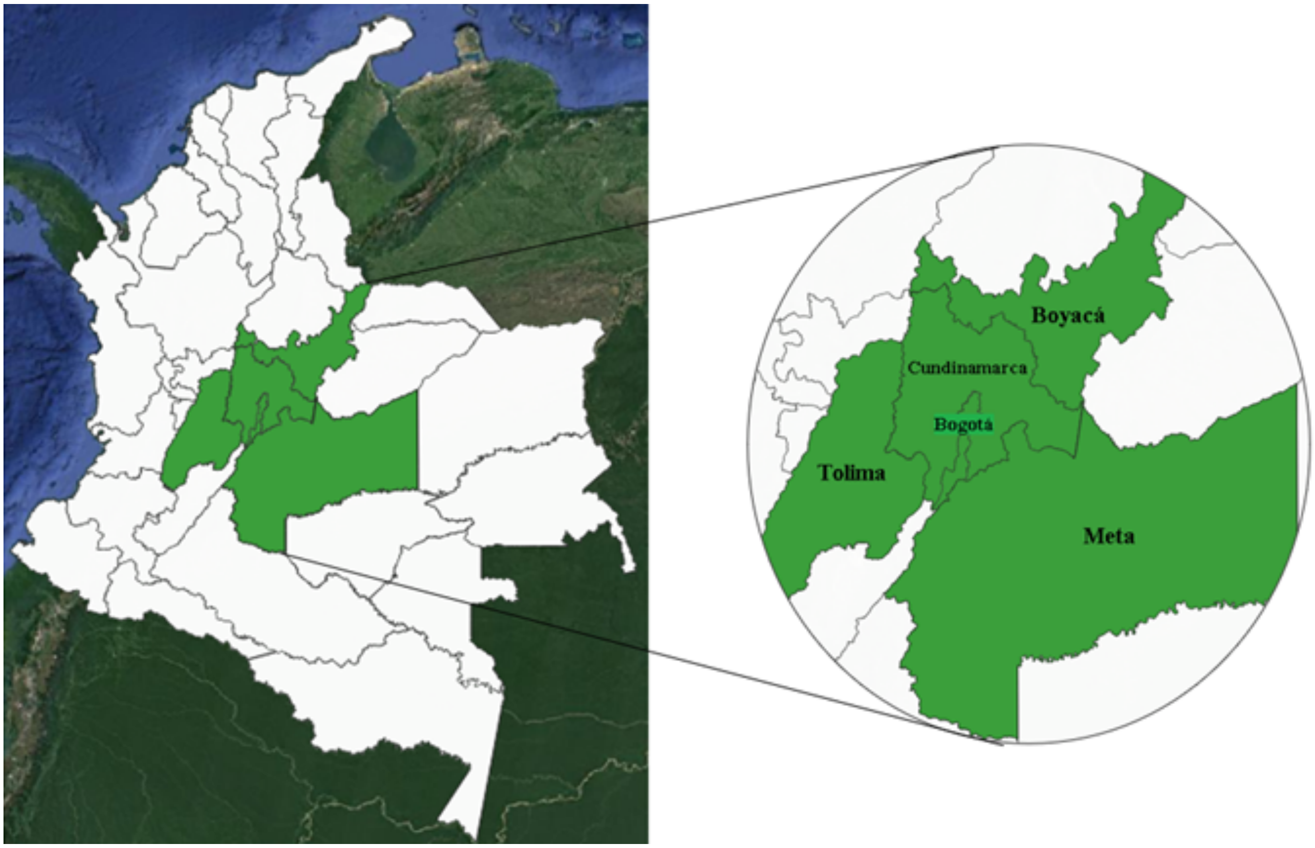

FIGURE 1. Central Region of Colombia and the departments that comprise it. Source: RAPE (2021).

Tolima, Meta, Cundinamarca, and the capital district of Bogotá (Fig. 1).

The sample consisted of 60 agroredes, selected through convenience sampling using a call and subsequent selection process. The criteria for selection included active operation, greater supply capacity and commercial coverage, agro-ecological production, differential population linkage, and seniority. For this study, it was not possible to consider the size of the population.

A diagnostic instrument was utilized to examine the organizational characteristics of the agrored and its level of performance across the components of marketing, administration and finance, associativity and network management, information and communication technologies (ICT) and logistics and storage (Giudici *et al.*, 2018; Cristobal-Fransi *et al.*, 2020; Santos *et al.*, 2020). Data collection was conducted through face-to-face interviews with the leaders of each agricultural organization from December 2021 to June 2022. The interview questions consisted of closed-ended, multiple-choice statements rated on an ordinal scale ranging from 0 to 3 points. According to SDDE (2020), the following data were presented (Tab. 1).

An Organizational Performance Index (OPI) was constructed using 45 items, which were divided into five dimensions (Tab. 1). The weighting factor for each dimension was determined based on the proposal by SDDE (2020). The OPI was calculated with the following equation:

$$OPI = \sum_{j=1}^{5} \left(\sum_{i=1}^{n} \frac{vi}{n} \right) * P \qquad \text{(Eq. 1)}$$

where:

OPI: Organizational Performance Index;

j: number of dimensions;

i: number of items per dimension;

vi: value obtained in item i of dimension j;

n: number of items in dimension j;

P: weighting of each dimension.

The OPI considered a nominal value of 50 points to represent the ideal state of organizational performance. The performance level of each agrored was determined based on the OPI value, as outlined in Table 2 according to SDDE (2020).

TABLE 1. Dimensions and items for measuring the organizational performance of agroredes.

Dimension	Item
Marketing	Products offered
	Portfolio availability
	Marketing channels
	Business plan implementation
	Issuance of legal invoice
	Use of bank account
	Commercial alliances
	Participation in trade fairs or business roundtables
	Marketing through electronic platforms
	Logo usage
	Access roads
	Commercial and logistic services to members
	Record
	Certified properties
Administration and finance	Working capital
	Budget preparation
	Accounting
	Update of financial statements
	Socialization of financial tools
	Knowledge of tax obligations
	Knowledge of the Special Tax Regime
	Sources of resources for the operation
	Microcredits
	Access to formal associative credit
	Access to supplier credit
	Financial services to members
	Financial management instruments
Associativity and network management	Operation of committees and work areas
	Defined functions known by members
	Attendance at meetings
	Network management
	Conflicts
	Structuring of projects or profiles of productive projects
	Participatory strategic planning exercises
	Articulation of the organization with other organizations
	Productive services with members
	Other services of the organization with members
	Management tools
Information technology and communication (ICT) management	Communication channels for product promotion
	Effective use of the computer
	Creation and use of email, social networks, and website
Logistics and storage	Quality certificates
	Logistics processes
	Postharvest handling
	Infrastructure, machinery, and equipment

TABLE 2. Classification of organizational performance in agroredes.

Score	Organizational performance level	Stage of development
Under 15	Low	Initiation
Between 15 and 30	Medium	Consolidation
Between 30 and 50	High	Maturity

Results and discussion

Out of the 60 participating agricultural organizations, 56.7% (34 agroredes) were from Cundinamarca, 16.7% (10 agroredes) from Boyacá, 16.7% (10 agroredes) from the capital district Bogotá, 6.67% (4 agroredes) from Tolima, and, finally, 3.33% (2 agroredes) from Meta (Tab. 3).

Among these organizations, 50% were not legally constituted. This lack of legalization can be attributed to the expensive procedures and high costs associated with legal registration and taxation which, in turn, limit the growth of these productive units (Rodríguez-Soto & Alvis, 2018). Benhassine *et al.* (2018) also indicate that legalization of an agricultural company leads to greater commercial skills training, while Tijdens *et al.* (2015) affirm that legalization provides access to credit, financial education, social protection, economic growth, and job creation.

The most prevalent type of agricultural organization among the agroredes was the peasant agricultural association, accounting for 80% of the total. Regarding the time of creation, most of the agroredes had less than 25 years of existence. This could be seen as a strength for these organizations, since, according to Messeni Petruzzelli *et al.* (2018), companies older than 15 years tend to have greater organizational maturity and are able to take advantage of their accumulated knowledge. This can be attributed to the learning experience gained over extended periods of time which allows for a more effective examination and utilization of knowledge.

The greatest benefit generated by associativity of growers is the generation of economies of scale (Melo Torres *et al.*, 2017). These economies enable productive efficiency, lower product costs and facilitate access to inputs, market information, and financial resources. They also ensure the use of new technologies and improve the quality of food production (Fischer & Qaim, 2014). As suggested by Orsi *et al.* (2017), collective actions promote social, technological and innovative development, adding value to products and facilitating access to productive resources for commercialization of agricultural products.

TABLE 3. General distribution of agroredes in the Central Region of Colombia.

Territory associated with the Central Region	Number of agroredes	Legally constituted (%)		Years of existence (%)			Type of agrored according to the offer of products (%)		
		Yes	No	Less than 25	Between 25 and 50	Over 50	Agricultural	Livestock	Agricultural and livestock
Cundinamarca	34	44	56	44	38	18	50	3	47
Boyacá	10	30	70	100	0	0	90	0	10
Meta	2	100	0	100	0	0	0	50	50
Tolima	4	75	25	75	25	0	100	0	0
Bogotá	10	30	70	100	0	0	40	0	60

Organizational performance

The average Organizational Performance Index (OPI) for the agroredes was 24.4, indicating a medium level of performance that aligns with the consolidation stage. This result suggests that organizations acknowledge the importance of process management across different areas of the company and the generation of productive projects, as highlighted by King *et al.* (2019). Similarly, Solarte-Pazos and Sánchez-Arias (2014) suggested that at the medium level of organizational performance, each worker within the company has well-defined roles and the fundamental processes are communicated effectively. Additionally, Savanevičienė *et al.* (2021) state that at the average performance level, experienced employees recognize the values of the organization, maintain positive associative relationships, exhibit job satisfaction, acknowledge the importance of process changes and rely on the organization image to collaborate with other organizations (Tab. 4).

Each dimension of organizational performance yielded a score below 30 points, indicating that no component has reached a maturity level. This result highlights the need for increased efforts in process management to achieve the objectives framed by the agrored. It is important to emphasize the significance of the ICT management dimension, especially after the pandemic, since organizations must leverage technological resources for production and marketing purposes. Those agri-food organizations that embraced digital marketing during the pandemic experienced a threefold increase in sales volume. This reflects a shift in consumer behavior towards online purchases, without overlooking the importance of voice-to-voice interaction (Csordás *et al.*, 2022).

At the territory level, the performance of the agroredes was found to be at a medium level, corresponding to the consolidation stage (Tab. 4). These results could be associated with the basic conditions of the Competitiveness Index, which indicated that territorial gaps (institutions, infrastructure, market size, education, health, and environment) were at an intermediate level across all associated territories in the Central Region (RAPE, 2016).

In the dimensions analyzed by territory, results ranging from 20 to 30 points were observed, confirming the average performance level in each dimension. However, there were atypical scores, with low scores in ICT management in the department of Cundinamarca (19.1) and Tolima (15.3) and high scores in Associativity and Network Management in the department of Meta (31.1) (Tab. 5).

TABLE 4. Organizational performance index by dimension and territory in the Central Region of Colombia.

Territory	Marketing	Administration and finance	Associativity and network management	ICT management	Logistics and storage	Global Organizational Performance Index
Bogotá	22.0	26.0	27.9	22.2	26.3	24.9
Boyacá	23.2	23.1	25.5	23.3	22.1	23.4
Cundinamarca	24.2	23.9	26.8	19.1	25.1	23.8
Meta	26.2	28.2	31.1	27.8	27.1	28.1
Tolima	22.3	26.9	23.5	15.3	20.8	21.8
Central Region	23.6	25.6	26.9	21.5	24.3	24.4

ICT - Information technology and communication.

TABLE 5. Organizational performance of agroredes in the Central Region of Colombia by dimension.

Dimension	Item	Score
Marketing	Products offered	1.15
	Portfolio availability	1.34
	Marketing channels	1.42
	Business plan implementation	1.49
	Issuance of legal invoice	1.32
	Use of bank account	1.62
	Commercial alliances	1.32
	Participation in trade fairs or business roundtables	1.83
	Marketing through electronic platforms	1.30
	Logo usage	2.34
	Access roads	1.51
	Commercial and logistic services to members	0.94
	Record	1.19
	Certified properties	1.11
Administration and finance	Working capital	1.57
	Budget preparation	1.30
	Accounting	1.58
	Update of financial statements	1.52
	Socialization of financial tools	1.75
	Knowledge of tax obligations	2.10
	Knowledge of the Special Tax Regime	1.67
	Sources of resources for the operation	1.85
	Microcredits	1.47
	Access to formal associative credit	1.43
	Access to supplier credit	1.55
	Financial services to members	0.50
	Financial management instruments	0.78
Associativity and network management	Operation of committees and work areas	1.08
	Defined functions known by members	2.08
	Attendance at meetings	2.51
	Network management	1.34
	Conflicts	2.62
	Structuring of projects or profiles of productive projects	2.13
	Participatory strategic planning exercises	1.58
	Articulation of the organization with other organizations	1.32
	Productive services with members	0.96
	Other services of the organization with members	0.87
	Management tools	1.43
ICT management	Communication channels for product promotion	1.38
	Effective use of the computer	1.15
	Creation and use of email, social networks, and website	1.19
Logistics and storage	Quality certificates	1.70
	Logistics processes	1.64
	Postharvest handling	1.81
	Infrastructure, machinery, and equipment	0.66

ICT - Information technology and communication.

Marketing

Brand positioning emerged as the item with the highest value, which could be attributed to the fact that the agroredes participated in at least three trade fairs or events over the last year to increase visibility of their products. This finding aligns with those of Santos *et al.* (2021), in which producers acknowledged the significance of participating in commercial events for establishing networks with groups of interest. Additionally, participating in such events facilitates access to innovation opportunities and contributes to the creation of value in the products offered, as indicated by Rinallo *et al.* (2017).

Furthermore, the majority of the agroredes had a representative logo but they did not use it. The lack of a logo limits the potential positive correlation with the development of competitive advantages. A logo is crucial for enhancing corporate identity, instilling a sense of belonging, cultivating skills for monitoring market trends and adopting best practices (Cannas, 2023).

Weak subcomponents were identified, particularly in terms of the limited commercial and logistic services the organization offered to its members. These findings differ from those of Kirezieva *et al.* (2016), which showed a positive relationship between the promotion of the organization services among the associates and the quality of the product as well as the effectiveness of the supply chain. However, each associate offered food products with different quality levels, which made it difficult to standardize the processes (Pereira *et al.*, 2022). Similarly, the organizations that had contracts for marketing the products exhibited higher levels of logistics and supply chain capabilities (Uddin, 2017). These findings confirm that the anonymity of producers in the market can lead to lower quality indices compared to competitors (Bizikova *et al.*, 2020).

Likewise, less than 33% of the properties where the agroredes operated had some certification demonstrating the quality of the products, registration of the harvested areas, and production volumes. These findings are consistent with the research by Furumo *et al.* (2020), which examined the factors influencing compliance with quality certifications. The study concluded that high initial costs posed a significant limitation and that characteristics of the cultivation area, household size, and access to extension services were decisive factors in implementing a certification (Kassa *et al.*, 2021).

Administration and finance

The tax subcomponent obtained higher scores, indicating that the representatives of the agroredes were aware of tax obligations. However, the organization was not fully compliant with these obligations. This discrepancy may be attributed to a reluctance to undergo audits for the calculation of taxes. In other words, the likelihood of being audited decreases the willingness of producers to fulfill their tax obligations (Mensah *et al*., 2021).

On the other hand, financial services and financial management tools received lower scores, indicating that the organizations made limited use of financial tools and offered few financial services to their members, such as electronic transfers, microcredits, and financial support. These findings align with those reported by Ankrah Twumasi *et al*. (2020), which highlighted that only educated farmers possessed the necessary skills to understand the financial market, emphasizing the need to strengthen financial literacy among small producers. Likewise, Bizikova *et al*. (2020) found that small producer households had varying incomes, placing them at the margin, with limited resources to bear the high transaction costs associated with accessing banking services.

Associativity and network management

The subcomponents related to decision-making, management, and participation obtained the highest scores, indicating that the organizations did not experience conflicts that negatively affected the operation during the last year. Additionally, assemblies were held in which approximately 50% of associates participated. Participatory methodologies with partners to identify needs and propose alternative solutions could improve their commitment to intervention processes (Espinosa *et al*., 2018). These methodologies also allow the creation of social capital to strengthen governance and generate control mechanisms for conflict resolution (Saz-Gil *et al*., 2021).

The importance of attendance by producers at meetings aligns with the findings described by Skaalsveen *et al*. (2020), with regular meetings proving instrumental in enhancing organizational planning by keeping associates informed and sharing responsibilities. The scheduled meetings facilitated cooperation within the group and encouraged active member participation in collective decision-making and the distribution of functions.

Challenges were identified in the subcomponents of other services, productive services, and articulation as the organizations offered limited productive, cultural, educational, and health services to the producers. These findings align with the research by Bizikova *et al*. (2020) who suggested that the services offered by the organizations generated positive impacts on the development of skills, knowledge, and information. However, implementing these services can often be costly and may require infrastructure for their application. In addition to capital limitations, organizations may prefer to allocate resources to other activities that they consider more important, despite the fact that most of the associates recognize the importance of these services.

The agroredes made limited efforts to establish collaborations with other organizations, not heeding the results reported by Santos *et al*. (2021). Their research indicates that establishing relationships with stakeholders enhanced innovation capabilities. Additionally, a high level of network creation among farmers fosters a favorable environment for interaction with interest groups and promotes a learning environment.

The subcomponent of organizational management tools revealed a lack of strategies for creating documents, such as statutes, a strategic plan, and management reports, not heeding the results presented by Namugenyi *et al*. (2019), who identified that organizations that implemented a business plan enhanced product market access, underscoring the significance of strategic planning in optimizing resources for effective business management.

ICT management

The agroredes made limited use of technological and digital tools for communication with their clients, which is similar to the results presented by Morris and James (2017) in their study on the use of social networks and platforms for food marketing in agricultural organizations in the United Kingdom. Their research revealed that less than 6% used social networks to promote their products. However, they recognized the crucial importance of these tools, which are more commonly used by the younger members of the organization. Bernal Jurado *et al*. (2019) showed that organizations offering organic food products had a greater interaction on digital platforms and social networks. Horská *et al*. (2020) also found that, in the dairy sector, producers who used digital platforms had sales four times higher on average compared to those who did not. In the subcomponent ICT, it was evident that organizations made limited use of computers to enhance process efficiency. This is consistent with the findings of Bowen & Morris (2019), in which small producers refrained from using computers for resource management, primarily due to associated costs and poor internet quality in rural areas. Barriers included a fear of technology, advanced age, slow typing skills, and a perception that computers were not essential for communication and information.

Logistics and storage

The lack of machinery, equipment, land, and infrastructure for the efficient product logistics was evident, aligning with the results presented by Mottaleb *et al.* (2016), which showed that the availability of labor can affect the ownership of equipment. This suggests that the effective use of machinery requires a demand for skilled labor.

Access to electricity is another key factor influencing the adoption of machinery and equipment. According to Mottaleb *et al.* (2016), on average, 3% of producer households operated at least one farm machine that required electricity. Similarly, it was stated that road improvements could accelerate the adoption of machinery and enhance the flow of information among producers. Therefore, households with larger land holdings, a higher number of cattle, and access to water sources were more likely to own machinery.

The agroredes have implemented quality systems to prevent failures in food products, such as safety and health issues, consumer complaints, and costs associated with these failures. The adoption of a quality system improves competitiveness and provides strategic advantages in the market. It also ensures the quality of the operations and, consequently, the quality of the products (Liu *et al.*, 2021).

The quality subcomponent revealed that the agroredes did not possess certificates for activities in the processing plant, and only carried out a limited number of post-harvest processes, with the participation of between 33% and 66% of the associates. These results align with research conducted by Meemken (2020), who emphasized that implementing a quality system is usually expensive and can be a determining factor for small companies in underdeveloped countries when deciding whether to adopt such systems. Also, limited education can make the certification process difficult for small producers. However, there have been discussions about the possibility for small producers to meet certification requirements if they collaborate and receive assistance from intermediaries (Soundararajan & Brammer, 2018).

This research underscores specific limitations that must be considered when interpreting the results. One of these limitations is the utilization of a convenience sample, which complicates the generalization of the findings. Furthermore, this study is based on the perceptions of the leaders within each organization regarding their performance. Notwithstanding these constraints, it contributes to a deeper understanding of the organizational dynamics within food supply agroredes. The outcomes of this research provide critical information for the development of public policies aimed at enhancing the performance of these organizations and, as a result, ensuring a dependable food supply to urban centers.

Conclusions

The organizational performance of the agroredes in the Central Region of Colombia is currently in a consolidation stage, which presents challenges in various dimensions such as administration, technology, logistics, marketing, and association to achieve the necessary organizational maturity for long-term sustainability. ICT management stands out as the dimension that requires further development.

The areas with the lowest performance within the organization are those related to the services provided to its members, infrastructure, and financial management. This indicates the need to promote plans and programs aimed at enhancing and implementing assistance services for the well-being of the associates, as well as facilitating access to financing for technological adoption.

Despite its limitations, such as relying on leaders' perceptions and using a convenience sample, this study provides valuable information about the organizational dynamics of food-supplying agroredes. Its results offer crucial information for crafting policies that can improve these organization performance and ensure food security in urban areas.

We recommend replicating this study in regions that supply food to major urban centers. This replication would generate valuable information that can be used as input for public policy development and the enhancement of the agroredes operation and the subsequent food supply to the local communities.

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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

MDL collected the information, wrote the manuscript, and processed and analyzed the data. JBF reviewed the manuscript. DB conceptualized and revised the final version of the manuscript. JBF and DB designed the research. All authors reviewed the final version of the manuscript.

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Cost analysis of a blueberry producing farm in the Cundiboyacense highlands, Colombia: A case study

Análisis de costos de finca productora de arándanos ubicada en el altiplano cundiboyacense, Colombia: un estudio de caso

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ABSTRACT

The blueberry industry is growing significantly in Colombia. For investors, cost analysis plays a key role in project planning for any agribusiness. This paper is an exploratory study, with a descriptive scope to identify components of establishment and production costs of growing blueberries in Colombia. Data were collected and analyzed from a project aimed at establishing a 1 ha blueberry crop in Zipaquirá and 18 blueberry small farms in the Cundiboyacense highlands. Components were applied to estimate the capital investment and to identify the establishment, production, and maintenance costs of this project. Decision investment analysis, including discount rates for the capital asset pricing model (CAPM), were used to compute the net present value (NPV), internal rate of return (IRR), and the return on investment (ROI). Production costs are still expensive due to the Colombian peso (COP) exchange rate compared to the US dollar (USD). Results show advantages for promoting the establishment of blueberry crops because of suitable climatic conditions for continuous production throughout the year in Colombia. Labor costs and utilities are cheaper compared to other countries and represent a competitive advantage in the investment. Intermediation suppliers affect establishment costs for commercial reasons. Blueberry production cost analysis in Colombia may facilitate agribusinesses to promote projects in Colombian agriculture.

Key words: cost of production, net present value, internal rate of return, capital asset pricing model, weighted average cost of capital.

RESUMEN

La industria de los arándanos está creciendo significativamente en Colombia. Para los inversores, el análisis de costos juega un papel clave en la planificación de proyectos para cualquier agronegocio. Este trabajo es un estudio exploratorio, con alcance descriptivo para identificar componentes de los costos de establecimiento y producción del cultivo de arándanos en Colombia. Los datos fueron recopilados y analizados de un proyecto destinado a establecer un cultivo de arándanos de 1 ha en Zipaquirá y 18 pequeñas fincas de arándanos en el altiplano cundiboyacense. Se aplicaron componentes para estimar la inversión de capital e identificar los costos de establecimiento, producción y mantenimiento de este proyecto. Se utilizaron análisis de decisiones de inversión, incluidas las tasas de descuento para el modelo de valoración de activos de capital, para calcular el valor presente neto, la tasa interna de rentabilidad y el retorno de la inversión. Los costos de producción aún son elevados debido a la tasa de cambio del peso colombiano (COP) en comparación con el dólar estadounidense (USD). Los resultados muestran ventajas para promover el establecimiento del cultivo de arándanos debido a las condiciones climáticas adecuadas para una producción continua durante todo el año en Colombia. Los costos laborales y los servicios públicos son más baratos en comparación con otros países y representan una ventaja competitiva en la inversión. Los proveedores de intermediación afectan los costos de establecimiento por motivos comerciales. El análisis de costos de producción de arándanos en Colombia puede facilitar que los agronegocios impulsen proyectos en la agricultura colombiana.

Palabras clave: costo de producción, valor actual neto, tasa interna de rendimiento, modelo de valoración de activos de capital, costo promedio ponderado del capital.

Introduction

A demand for blueberries has been growing significantly and at a faster rate than production. As a consequence, importation from abroad has also been increasing (Carrera, 2012). The blueberry market offers competitive advantages at a productive and commercial level for Colombia for geographical reasons, soil conditions, and for characteristics

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and properties of the fruits. Resources are available such as environmental conditions with optimal temperatures, soil humidity, soil types, pH, and water quality, etc. Blueberry production in Colombia is an attractive scenario for investors due to its weekly production without strong seasonality. Growing interest in blueberry production worldwide is due to its functional food properties, such as anthocyanin and antioxidant content (Maldonado *et al*., 2017). However, a lack of financial information in the formulation and evaluation of productive blueberry projects in Colombia reduces investor interest in decisions about establishing crops.

Industrial blueberry production began in Colombia in 2019 (Red Agrícola - Asocolblue, 2020). Various authors underline that Colombia occupies the sixth place in blueberry production among South American countries with an average of 25 ha cultivated; however, there is still not enough formal information on cultivation and on public demand (Brazelton *et al*., 2011; Fernández-Gutiérrez, 2014; Cortés-Rojas *et al*., 2016). Cundinamarca and Boyacá are the departments with the most planted areas; they equaled an estimated 550 ha in the country in 2020. The average planting is 2 ha per project (Red Agrícola - Asocolblue, 2020). The blueberry in Colombia is one of the most outstanding emerging products for 2021 (ProColombia, 2021).

An analysis of capital budgeting practices explains and supports decision-making for investors interested in blueberry agribusiness projects. There is a number of different methods for estimating the cost-effectiveness of agribusiness projects, but the most used are the net present value (NPV) and the internal rate of return (IRR) indicators in the cost analysis. The weighted average cost of capital (WACC) and the capital asset pricing model (CAPM) are indicators for risk, and the cost of capital affects corporate investment. In the present study, data were obtained and presented from blueberry growers, suppliers, and intermediates by building a 1 ha sample plot of blueberry cultivation in order to identify the opportunities for blueberry production in Colombia.

We identified the requirements for blueberry production, as well as the areas of the Colombian highlands suitable for cultivation (from 2,200 to 3,000 m a.s.l.) and the best soil characteristics (Red Agrícola - Asocolblue, 2020). As a result of this project, for the next four years the potential for foreign sales of blueberries could exceed 100 million US dollars (USD) as long as production and commercial processes are adjusted to international regulations (Asohofrucol, 2018). To achieve competitiveness and sustainability in the blueberry agricultural sector, it is essential to have reliable, timely, and pertinent information on land use and aspects such as appropriate location, extension, spatial and temporal distribution, costs of production and infrastructure, initial investment, and labor costs, etc. Therefore, it is important to develop a case study focused on project evaluation and blueberry production marketing in Colombia to identify financial viability.

Materials and methods

Literature review

A theoretical perspective in the development of this case study is both a process and a product. A process of immersion in existing and available knowledge is linked to our approach to the problem. A product (theoretical framework) is part of a larger product, *i.e.*, the research report. However, the case study is focused on presenting estimated capital investment in a financial scenario in order to make decisions for investors interested in blueberry cultivation in Colombia.

Estimated capital investment

In financing, complex methods related to capital budgeting theories are used by the management in the decision-making process related to capital budgeting theories in order to prevent risk factors and uncertainty (Dikerson, 1963; Mao, 1970; Arnold & Hatzopoulos, 2000; Cooper *et al*., 2002; Byrne & Davis, 2005; Bock & Trück, 2011; Keršytė, 2011; Zhang *et al*., 2011; Singh *et al*., 2012; Kaczmarek, 2015; Kengatharan & Lanka, 2017). Some authors have defined capital budgeting as follows: "capital budgeting practices are the methods and techniques used to evaluate and select an investment project" (Verbeeten, 2006). For many reasons, small and medium business enterprises (SMEs) and investment projects that focus on agriculture crops lack financial information because of the uncertainty and risks in the industry. However, national or international investors seek the most up-to-date information relating to budgeting of the most optimal and productive locations for blueberry production. An increase in financial uncertainty is associated with the use and importance of sophisticated capital budgeting practices. Simple capital budgeting practices generally consider the time value of money, and they systematically incorporate risk. However, advanced capital budgeting practices (IRR and NPV) consider cash flows (Verbeeten, 2006). The Colombian blueberry industry has experienced significant growth in recent years with different scenarios and characteristics in continuous production throughout the year that grants a competitive advantage and a financial opportunity for national and foreign investors. Thus, capital investment methods incorporated as a financial analysis are important for investors for future growth.

Methodology

This research was carried out between October and December 2021 in a blueberry crop located in Zipaquirá, Cundinamarca, Colombia, located at 4°59’00.001’’N, 73°59’26.94” W at 2,569 m a.s.l. A 1 ha blueberry-producing plot was established as a case study as a standard measure for installation cost.

We established the initial structure of the blueberry plot, data, and information by establishing and implementing 1 ha of blueberry crop var. Emerald. The research was an exploratory study with descriptions to identify components of production costs based on data collected from 18 blueberry farms in the area. Cost data were collected using three processes: First, a blueberry farmer from the case study was identified in Zipaquirá. The farmer submitted information and data on the costs for the infrastructure of 1 ha equipped with anti-hail mesh for 7,680 plants. Second, in a diagnosis and characterization process with the Colombian Association of Blueberry Growers - ASOCOLBLUE, data were obtained from the 18 blueberry producers surveyed. Data were collected through face-to-face interviews and surveys with farmers and administrators for 2 months in 2020. Finally, national economic data for WACC and CAPM analysis were obtained from the Colombian government websites and the National Administrative Department of Statistics – DANE.

According to Ericsson *et al.* (2009), a case study implies the following components of production cost: 1) estimated capital investment, 2) cost of establishment, 3) cost of land, 4) NPV, IRR, and 5) investment analysis. These components were used to summarize the establishment-production costs and the potential profitability of ‘Biloxi’ cultivar among Colombia’s blueberry crops. This methodology has become an interesting and effective tool for a business project to guarantee implementation from data collection to the determination of a corporate strategy and the corporate budget planning (Amir *et al*., 2016).

Assumptions

Assumptions used for the cost analysis were included in the structure for the plot, the plants, maintenance costs, and external factors focused on political and economic issues.

Anti-hail mesh structure

A structural model was presented according to the characteristics and minimum conditions for sustainable blueberry production such as land properties, altitude (m a.s.l.), and the Cundinamarca and Boyacá climate. Soil characteristics are optimal for blueberry cultivation in this region (Salgado *et al*., 2018); however, the cost analysis was organized and structured for a 1-ha blueberry planting in 25 L bags (due to the root development of the species) with fertirrigation monitoring management (Hart *et al*., 2006). The optimal altitude for a blueberry production in Colombia is between 2,100 and 3,000 m a.s.l. (Cleves, 2021). The structure for the plot was designed for 1 ha of flat land in the defined region, and it was distributed every 0.6 m among plants (240 plants per row) and 2.2 m between rows (32 rows) (Ojeda *et al*., 2016). The structure was covered with anti-hail mesh for hail protection due to the positive effects on blueberry production performance and crop yield at high altitudes (Milivojević *et al*., 2017). For the productive units established in greenhouses, the covering used was polyethylene plastic with mechanical properties of high resistance to tearing and impact, thus increasing investment costs. For this case study, owners invested in the anti-hail mesh structure to reduce costs.

Plants (seedlings)

A US nursery supplier company (Fall Creek Farm & Nursery, Inc., Oregon, USA), through its representatives in Colombia, offered quotes on the plants; and 7,680 plants were established for the farm in Zipaquirá. This supply company produces blueberry seedlings of high quality as well as varieties adjusted to the climate conditions of Colombia. This commercial nursery allows the growth of many varieties of blueberries, and the varieties suitable to be cultivated in Colombia in the present case were Biloxi and Emerald (Tab. 1) (Fall Creek Farm & Nursery, 2021). Blueberry yield was approximately 500 g fruits per plant in the first year (2022), with an increase of 30% in the second year (2023) and so on (Muñoz-Vega *et al*., 2017). The calculation of yield per plant is shown in the NPV as well as the pruning intensity effect on average fruit weight per plant. The unit costs for 7,680 plants are presented in Table 2. The substrate used was coconut fiber + perlite. Coconut fiber is a waste product of the coconut industry that provides good aeration to plant roots and a high rewetting capacity (Michel, 2010). This byproduct of the coconut fruit has been widely used as a substitute for peat in different horticultural, floriculture and fruit growing systems (Schmilewski, 2008). We transplanted the plants in 25 L polypropylene bags (Salgado *et al*., 2018). Other costs about of the anti-hail mesh structure and services are presented in Table 3.

Maintenance costs

According to experts, the first harvest after being cultivated takes twelve months for the plants to reach full production, and the opportunity for the possibility of continuous production could supply domestic and North American demand (Cortés-Rojas *et al*., 2016). So, costs may vary according to the maintenance of the structure, the irrigation system, production cost, and costs per harvest (four harvests per month approximately). On the other hand, land is not owned, and investors require a monthly lease of the land to establish a productive farm. Monthly costs may vary according to several factors in the Cundinamarca and Boyacá region such as location, water resources, flat land, proper roads, property extension, access to the farm, and distance for getting supplies and points of sale.

Cost per harvest is focused on labor costs and training. The operator's costs should be incorporated in different ways: labor seasonal cost, labor part-time cost, and labor full-time cost. Production costs are focused on harvest, pruning, herbicide, pesticide, fungicide, tools maintenance, transportation, and gasoline for water pumps (Tab. 4).

Financial assumptions

The weighted average cost of capital (WACC) is often used as the appropriate discount rate to value companies or projects and measure a company's cost to borrow money (Frank & Shen, 2016). WACC is an appropriate discount rate for valuing a company or for projecting using the discounted cash flows modality since it reflects the financial structure of the company and the cost of the resources used by it for the case study of its corporate purpose. WACC was evaluated using the formula:

$$WACC = ((Ke * (E / D + E) + ((kd * (1 - tx)) * (D / D + E))$$

where *Ke* is the cost of equity, *E* is equity, *D* is debt, *Kd* is the cost of debt (interest rate), and *Tx* is the tax rate. *Ke* is the cost of capital, *i.e*., a rate of profitability that investors (partners) would expect; it is known as TIO (opportunity interest rate) or expected profitability to invest in the project and not in other options. *E* is a proportional amount of contributions corresponding to "equity" or equity contribution generated by the partners and the absolute value; *D* is a proportional amount of contributions obtained by debt or credit "debt" or liabilities generated by third parties to constitute the total value of assets together with equity contributions and absolute value; *Kd* is a cost of credit or loans and is an interest rate of the aforementioned resources; and *Tx* is a current tax rate in Colombia. Parts of the assets come from resources provided by third parties and their cost. Cost is given by the current interest rates for such loans or credits (*kd*). Part of the assets come from the resources provided by its partners or shareholders when consolidating the company's equity. WACC is usually more subjective to determine since it is considered as the shareholder's opportunity interest rate (OIR). It reflects the rates expected to grant resources to the company instead of investing in other options. The risk of investing in the company is offset by higher profitability (Tab. 5).

To generate a more objective determination of the cost, the capital asset pricing model (CAPM) is used to establish the relationship between the risk and expected return for assets. It is widely followed for the pricing of risky securities like equity, generating expected returns for assets given the associated risk, and calculating costs of capital (Chen, 2021). Sharpe (1964) and Lintner (1975) present the CAPM as *"an equilibrium rate of returns on individual assets adjusted to levels that reflect the risk which each asset contributes to a market portfolio of all assets"* (Barry, 1981). Therefore, the case study presents a comparison in the blueberry market from the farming sector between the USA and Colombia. On this case, the formula to obtain CAPM is:

$$CAPM = Rf + (\beta * (Rm - Rf))$$

where *Rf* = risk free, β = Beta, and *Rm* = market risk (*Rf* is the interest rate or return of risk-free reference bonds; Beta is the level of sensitization of the model regarding movements in the market interest rate where evaluated; it is usually a value between -1 and 1. *Rm* is the market risk premium of the country under analysis to compensate with respect to risk-free bonds. In the case study, a parallel comparison of the CAPM model is presented for an agricultural project of two reference countries, the USA and Colombia. It is taken as a reference for the risk-free rate, the rate of government bonds of each country at 10 years as a reference par excellence to reflect the absence of risk, considering payment capacity of the nations and their risk rating. Bond rate for November 30, 2021, is taken as a reference by the cited information provider (Investing.com, 2021). In the case of a 10-year project, bonds for the stipulated date reflect an interest rate of 8.2% (Tab. 6).

For Beta, the information is published by Deamodaranonline.com (2021). The website presents the current information for estimating the discount rate of Beta adjusted to reflect a firm's total exposure to risk rather than only the market risk component. It is a function of the market beta and the portion of the total risk that is the market risk. These Beta might provide estimated costs of equity for undiversified owners of businesses. Total Beta by industrial sector for the global case includes countries such as Colombia. Beta for the "Farming / Agriculture" sector for the global area is at the level of 0.89.

The OIR expected by the shareholders is taken as a reference, and the 14% rate is accepted as a reference according to inquiries made by investors in the sector. From the calculation of the CAPM model under these conditions, the cost of equity adjusted under these conditions amounts to 13.3 %. As previously reviewed in the case currently studied, resources from debt are not contemplated; therefore, the *Ke* as a source of total resources for the company is taken as the WACC.

If an agribusiness in the future considers borrowing or acquiring financing from financial entities in Colombia, it could evaluate the cost of the resources reflected in the interest rates of loans for the sector. It may be of the same level or lower that the cost of equity and that they will also be adjusted by the tax shield if deducted from its tax base. The tax base is a proportion of the resources paid for financial expenses reflected in the WACC formula (1-tx for the WACC calculation formula, the tax shield is included, as part of the interest, is a deductible element for income purposes; *tx* is the tax rate). If these conditions are met, it could reflect a lower WACC which will have a favorable impact on the NPV of the same, providing a higher valuation (Tab. 7).

Other assumptions

Some products and materials for blueberry production are covered by a value added tax. However, some of them are exempted due to the last tax reform on the agricultural sector (Ministerio de Agricultura y Desarrollo Rural, 2020). The general income tax rate applicable to national companies is 32% for taxable year 2020, 31% for taxable year 2021 and 30% as of taxable year 2022.

Projections were applied with the average increase of the last 5 years of consumer price index (CPI acronym in English and IPC acronym in Spanish) in Colombia (Banco de la República, 2021a). The CPI index was used for the adjustment values of the fixed and variable costs on projections.

The fruit weight (kg) at the first harvest was determined for each plant after 12-months production. Therefore, the first year will not have a harvest until 10 or 12 months after having planted the seedlings (Cleves, 2021). The first blueberry harvest on one year production of a 1 ha weighed approximately about 500 g (Jorquera-Fontena *et al*., 2017; Muñoz-Vega *et al*., 2017). Weight increases on average until each plant reached 2,000 g after 10 years are based on several factors such as cross pollination, fertilizers, pruning, and good farming practices, and climate (Muñoz-Vega *et al*., 2017). This results in the fruits having a higher caliber, number of seeds and fruit weight.

According to the blueberry kilogram price index, ASOCOLBLUE determined on report of May 2021 the final average price for the last 12 months (Asocolblue, 2021) and it was used to determine the sales forecast in grams.

Results and discussion

Blueberry seedlings exported from the USA have a standard price compared to the national seedlings cultivated and reproduced by the propagation method. The varieties have a different performance in the Cundiboyacense region and distributors recommend cultivating specific varieties for the climate conditions found in Colombia. Seedling prices are high; however, the blueberry plant is a perennial plant with long productivity.

Initial structural costs are around USD $58,606. Benefits for the structure are high performance in production. Greenhouses are more efficient, although they duplicate costs. Around 70% of the materials purchases for the structure in our case study came from abroad and 50% of the distributors were found in Bogotá city (close to the farm). Annual maintenance costs are focused on the structure, utilities, fert-irrigation system, costs per season harvest, production, transportation, and labor cost. The cost is around USD $22,800 at the beginning of the first year. On many of the cases, land purchases for agriculture are expensive and involve generational legal problems in Colombia. So, investors prefer a lease contract rather than the purchase of the property allowing them low maintenance costs. The cost of land lease is cheap compared to a lease contract for buildings or houses for living in cities. Labor cost is the highest cost and is proportional to the production season. In Colombia, the agricultural sector is still using an informal wage according to the activities of the farmer, and this process even reduces costs. Minimum informal wage oscillates between USD $10 and $12, according to the farm activities. The fert-irrigation system maintenance is the cheapest cost of the production year. Also, control of the different diseases for the several climate conditions represents a high price on maintenance costs; however, that is still cheap for annual maintenance costs.

Following, NPV was adjusted according to the exchange rate: COP to USD (November 21rst, 2021). With a WACC of the 13.4%, the IRR was obtained at 19% and NPV of about USD $33,500. As a result, ROI turned positive on year 3 (Fig. 1). According to the methodology, differences between a pessimistic, real and an optimistic scenario are not particularly wide. Based on the different scenarios, the IRR is dominant and adapted from a conservative net production estimate that could be affected by climate conditions. However, some authors estimate increased production for the next 5 years.

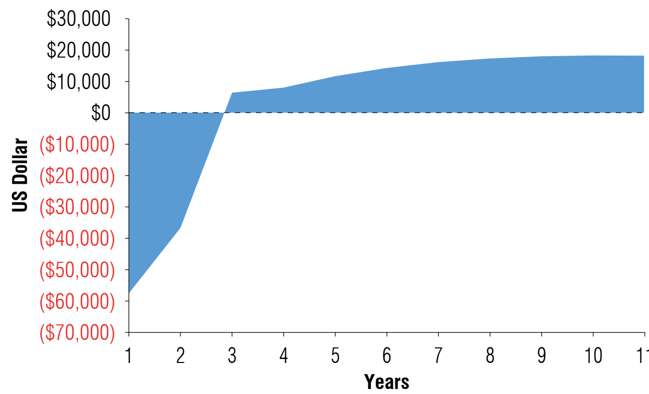

FIGURE 1. Return of the investment (ROI). Colombian peso exchange rate on November 11th, 2021 was $1 USD = COP$ 3,875.38 (Banco de la República, 2021b).

TABLE 1. Units cost of blueberry varieties per plant (seedling).

Blueberry varieties	Unit cost (COP$)	Unit cost (USD$)
Biloxi	12,584.90	3.17
Legacy	12,584.90	3.17
TH-929-Victoria *	15,086.00	3.80
Ventura *	15,086.00	3.80
Emerald	12,584.90	3.17

* Licensed to Fall Creek; royalties should apply. COP$ corresponds to Colombian peso; USD$ corresponds to USA dollar.

TABLE 2. Costs of plants (seedlings) for 1 ha (7,680 plants) blueberry plantation establishment.

Blueberry varieties	Unit cost (COP$)	Unit cost (USD$)	Seedling quantity	Total cost (COP$)	Total cost (USD$)
Biloxi	12,584.90	3.17	3,840	48,326,016.00	12,172.80
Legacy	12,584.90	3.17	3,840	48,326,016.00	12,172.80
		Total	7,680	96,652,032.00	24,345.60

Colombian peso exchange rate on November 11th, 2021 was $1 USD = COP$3,875.38 (Banco de la República, 2021b). COP$ corresponds to Colombian peso; USD$ corresponds to USA dollar.

TABLE 3. Initial structure costs for 1 ha (7,680 plants) blueberry plantation establishment.

Initial structure costs					
Item	**Description**	**Quantity**	**Unit value (COP$)**	**Total (COP$)**	**Total (USD$)**
Structure					
Wood poles (wooden beam)	Wood poles between 3 and 4 m long according to the ground	824	25,000.00	20,600,000.00	5,188.92
Steal wire rope	Wire rope of 1/8" inch and 18,000 m selling by cable rolls	18,000	714.29	12,857,220.00	3,238.59
Cement	24 lumps	24	28,000.00	672,000.00	169.27
Gravel	6 m	6	90,000.00	540,000.00	136.02
Rebar steel	0.635 cm (1/4" inch)	100	8,600.00	860,000.00	216.62
Staples	24 kg	24	12,000.00	288,000.00	72.54
Anti-hail mesh	3,000 m or 25 rolls on the top coverage	3,000	4,873.95	14,621,850.00	3,683.09
Anti-bird mesh	400 m for perimeter coverage	400	3,921.57	1,568,628.00	395.12
Padded beds	5 rolls - 5,200 m	5,200	1,790.00	9,308,000.00	2,344.58

Continued

TABLE 3, continuation. Initial structure costs for 1 ha (7,680 plants) blueberry plantation establishment.

Initial structure costs					
Item	**Description**	**Quantity**	**Unit value (COP$)**	**Total (COP$)**	**Total (USD$)**
Structure					
Australian water tank	Dimension of 9.69 m in diameter and 84,600 L of capacity with geomembrane	1	8,990,000.00	8,990,000.00	2,264.48
Construction sand	By lumps for supporting tank-base	8	62,500.00	500,000.00	125.94
Irrigation system	Irrigation system for 7,680 seedlings		38,000,000.00	38,000,000.00	9,571.79
Plastic bags	Bags of 25 L each	7,680	525.20	4,033,536.00	1,016.00
Pre-mixed substrate	Lumps of washed, solarized and buffered coconut fiber for 7,680 seedlings	2,200	26,500.00	58,300,000.00	14,685.14
Substrate transport	Transportation of 32 t from San Juan de Urabá, Antioquia (884 km).	4	2,625,000.00	10,500,000.00	2,644.84
Labor structure cost	8 persons	8	38,000,000.00	38,000,000.00	9,571.79
Motor pump	Arvek diesel of 10 HP	1	2,280,000.00	2,280,000.00	574.31
Windbreaker net	Green windbreaker wind in m according to the property	200	3,781.52	756,304.00	190.50
Property additions and improvements					
Postharvest room adaptation	Room improvements	1	1,500,000.00	1,500,000.00	377.83
Roof improvements	Roof tiles	1	124,500.00	124,500.00	31.36
	Roof trestles, staples, and buckets	1	679,000.00	679,000.00	171.03
	Metal doors and padlocks	1	700,000.00	700,000.00	176.32
Electrical adaptations		1	2,580,600.00	2,580,600.00	650.03
Materials for electrical installation	Breakers, cables, adaptors, switches, electrical outlets	1	190,400.00	190,400.00	47.96
Paint and brushes	Two different colors and brushes dimensions	1	311,400.00	311,400.00	78.44
Sanitary adaptations (2 toilets)	2 toilet adaptations	2	358,810.00	717,620.00	180.76
Sink faucets and paint	Toilet adaptations	1	435,300.00	435,300.00	109.65
Pipeline installation	Pipelines	1	74,700.00	74,700.00	18.82
Installation of motor pump	Hoses rolls, couplings, and adapters	1	1,339,600.00	1,339,600.00	337.43
	Materials hoses, staples, and base installation	1	1,339,600.00	1,339,600.00	337.43
Total				232,668,258.00	58,606.61

Colombian peso exchange rate on November 11th, 2021 was $1 USD = COP$ 3,875.38 (Banco de la República, 2021b). COP$ corresponds to Colombian peso; USD$ corresponds to USA dollar.

TABLE 4. Maintenance costs for 1 ha (7,680 plants) blueberry plantation.

Annual maintenance costs					
Item	**Description**	**Quantity**	**Unit value (COP$)**	**Total (COP$)**	**Total (USD$)**
Structure					
Property lease	Monthly rent of the blueberry-producing farm	12	500,000.00	6,000,000.00	1,511.3
Maintenance structure	Maintenance of the semi-annual structure	2	300,000.00	600,000.00	151.1
Utilities					
Electric power	Electricity services	12	250,000.00	3,000,000.00	755.7
Water supply and sanitation (WASH)		12	150,000.00	1,800,000.00	453.4
Fertirrigation system					
Irrigation system maintenance	Hoses and system washing	2	400,000.00	800,000.00	201.5
Water analysis	Chemical analysis of water (quarterly)	4	150,000.00	600,000.00	151.1

Continued

TABLE 4, continuation. Maintenance costs for 1 ha (7,680 plants) blueberry plantation.

Annual maintenance costs					
Item	**Description**	**Quantity**	**Unit value (COP$)**	**Total (COP$)**	**Total (USD$)**
Costs per harvest (seasonal cost)					
Blueberry baskets	Baskets used for postharvest	30	50,000.00	1,500,000.00	377.8
Harvest training program	Training program biannual	2	370,000.00	740,000.00	186.4
Equipment for operators	Boots, garden overalls, hats, glasses, and gloves (per person COP$ 190,000)	8	190,000.00	1,520,000.00	382.9
Production					
Pruning labor	Every 12-months pruning	12	100,000.00	1,200,000.00	302.3
Agricultural tools	Purchase of agricultural tools: shovel, pick, drill, wheelbarrow, hammer, pruning shears, pliers, meter, wrench set, padlocks, gloves, high pressure washer, stapler, and hooks	1	2,200,000.00	2,200,000.00	554.2
Fertilizers	Fertilizers	6	150,000.00	900,000.00	226.7
Herbicide, pesticide, and fungicide	Every 6-months bottles of herbicides, pesticides, and fungicides	8	64,900.00	519,200.00	130.8
Transportation	Transportation to the point of sale (distributor)	12	200,000.00	2,400,000.00	604.5
Fuel and lubricant	Fuel and lubricant for the water pump	12	50,000.00	600,000.00	151.1
Transportation					
Production transportation	Transportation of production and supplies	12	600,000.00	7,200,000.00	1,813.6
Labor cost					
Labor costs (labor full-time cost)	1 manager	1	2,200,000.00	26,400,000.00	6,649.9
Labor costs (labor part-time cost)	2 operators	2	691,142.66	16,587,423.84	4,178.2
Labor costs (seasonal harvest)	6 operators for the seasonal harvest (four times per month, approximately)	6	55,000.00	3,960,000.00	997.5
Labor cost of agricultural engineer	Monthly advice on the crop and its yields in the production of seedlings and dosage of fertilizers	12	1,000,000.00	12,000,000.00	3,022.7
Total				90,526,623.84	22,802.68

Colombian peso exchange rate on November 11th, 2021 was $1 USD = COP$ 3,875.38) (Banco de la República, 2021b).

TABLE 5. Weighted average cost of capital (WACC), net present value (NPV), and internal rate of return (IRR) for different scenario assumptions.

Rate	Pesimistic	Real	Optimistic
WACC	13.4%	13.4%	13.4%
NPV	$15,143.3	$33,558.9	$51,974.6
IRR	15.97%	19.04%	22.04%

TABLE 6. Capital asset pricing model (CAPM) in the USA *vs*. Colombian (Col) currency. Rf, risk free; Beta, the level of the sensitization of the model regarding movements in the market interest rate where is evaluated; Ke, the cost of capital.

10 year Treasury bonds (November 30, 2021)	
RF Col	8.26%
RF USA	1.45%
Farming/Agriculture	
Beta global	0.89
Beta USA	0.87
Ke (ROI) USA	6.0%
Ke (ROI) Col	14.0%
CAPM USA	5.41%
CAPM Col	13.37%

TABLE 7. Net present value - NPV for a real scenario (USD$) of 1 ha blueberry plantation.

Production by year	0	1	2	3	4	5	6	7	8	9	10
Components											
Initial investment	$57,763.30	$25,188.92									
Plants (seedlings)		7,680	7,680	7,680	7,680	7,680	7,680	7,680	7,680	7,680	7,680
Production in g x plant x year			600	700	900	1,100	1,300	1,500	1,700	1,900	2,100
Net production in g x year		0	4,608,000	5,376,000	6,912,000	8,448,000	9,984,000	11,520,000	13,056,000	14,592,000	16,128,000
Net production in kg		0	4,608	5,376	6,912	8,448	9,984	11,520	13,056	14,592	16,128
Sale price x kg USD (average 2021 approximately)	$7.48	$0.00	$8.69	$10.13	$13.03	$15.92	$18.82	$21.71	$24.61	$27.50	$30.40
Total estimated net production USD per year (sales per kg)	$57,763.30	-$25,188.92	$35,863.99	$41,841.33	$53,795.99	$65,750.65	$77,705.32	$89,659.98	$101,614.65	$113,569.31	$125,523.98
Fixed costs											
Structure		$2,418.14	$2,515.06	$2,615.88	$2,720.73	$2,829.78	$2,943.21	$3,061.18	$3,183.89	$3,311.51	$3,444.24
Fertirrigation system		$352.64	$366.78	$381.48	$396.77	$412.68	$429.22	$446.42	$464.32	$482.93	$502.29
Costs per harvest (seasonal cost)			$947.10	$985.07	$1,024.55	$1,065.62	$1,108.33	$1,152.76	$1,198.96	$1,247.02	$1,297.01
Production			$1,969.57	$2,048.52	$2,130.63	$2,216.03	$2,304.86	$2,397.25	$2,493.34	$2,593.28	$2,697.22
Labor cost		$13,850.74	$15,443.38	$16,062.41	$16,706.24	$17,375.88	$18,072.37	$18,796.77	$19,550.20	$20,333.84	$21,148.89
Total, fixed cost		$16,621.52	$21,241.90	$22,093.35	$22,978.92	$23,899.99	$24,857.99	$25,854.38	$26,890.71	$27,968.58	$29,089.65
Variable costs											
Utilities			$1,209.07	$1,257.53	$1,307.94	$1,360.36	$1,414.89	$1,471.61	$1,530.59	$1,591.94	$1,655.75
Transportation			$1,813.60	$1,886.30	$1,961.91	$2,040.55	$2,122.34	$2,207.41	$2,295.89	$2,387.92	$2,483.63
Total, variable cost		$0.00	$3,022.67	$3,143.83	$3,269.84	$3,400.91	$3,537.23	$3,679.01	$3,826.48	$3,979.86	$4,139.39
Profit before taxes	-$57,763.30	-$41,810.43	$11,599.42	$16,604.15	$27,547.22	$38,449.75	$49,310.10	$60,126.59	$70,897.46	$81,620.88	$92,294.94
Taxes	31%	$0.00	$3,595.82	$5,147.29	$8,539.64	$11,919.42	$15,286.13	$18,639.24	$21,978.21	$25,302.47	$28,611.43
Profit after taxes	-$57,763.30	-$41,810.43	$8,003.60	$11,456.86	$19,007.58	$26,530.33	$34,023.97	$41,487.35	$48,919.25	$56,318.40	$63,683.51

Colombian peso exchange rate on November 11th, 2021 was $1 USD = COP$3,875.38 (Banco de la República, 2021b).

Conclusions

The case study provided costs and investment analysis for the decisions made by investors in the production of Colombian blueberries. Final information and analysis should be useful for foreign investors, new growers, and for current blueberry growers. For new growers, this represents an economic benchmark analysis on the average operation for a 1 ha plot. For international investors, the study estimates expenses and costs combined with the market price and estimates potential revenues and profits in this agribusiness.

The research identified an initial investment of about USD $58,000 in the first year and about USD $25,000 in the second year per 1 ha of blueberry crop in the Cundiboyacense highlands region. Consequently, blueberry production is profitable under the best conditions. However, a greenhouse infrastructure and different blueberry varieties for testing may represent better performance for future research.

The NPV underlined differences between the present value of cash inflow and the present value of cash outflow over 10 years. The IRR is still relevant and attractive in different scenarios. To guarantee a profitable scenario, plantation care during the first two years of growth is crucial when it comes to planting blueberries. It is still cheaper compared to other countries such as the USA, Perú, and Chile. Labor costs are cheaper and more attractive compared to other Latin-American countries. Profitability depends on the quality of the fruit that results from each harvest.

Intermediate suppliers affect establishment costs for different commercial reasons. Resource imports and raw materials for the initial blueberry crop affects the initial investment and rise of Colombian prices on the market. However, the weighted average cost of capital (WACC) shows an average rate where the case study is expected to pay for stakeholders to support assets. The capital asset pricing model (CAPM) shows little volatility in the initial investment. However, according to blueberry imports, market price, and marketing, the higher the CAPM, the higher the expected return.

In brief, several factors were concluded to consider the initial investment reasonable and timely in the infrastructure creating a favorable microclimate for the development of blueberries and increasing production focused on fruit quality.

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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

CAMO and FPB formulated the research goals, set up the methodology, obtained financial support for the project. FPB carried out activities to annotate scrub data. DRAF applied financial analysis and techniques to analyze or synthesize study data. CAMO formulated overarching research goals and aims. CAMO conducted the research, specifically performing the experiments or data/evidence collection, wrote/translated the initial draft, and prepared, created, and presented the published work and oversaw its visualization/data presentation. CAMO wrote/translated the initial draft. All authors reviewed the final version of the manuscript.

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Use of quinoa (*Chenopodium quinoa* Willd.) flour in a couscous-type product as a substitute for wheat couscous

Uso de harina de quinua (*Chenopodium quinoa* Willd.) en un producto tipo cuscús como sustituto del cuscús de trigo

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ABSTRACT

Quinoa (*Chenopodium quinoa* Willd.) couscous is a new nutritional product that can replace wheat couscous. The processes of agglomeration, steam-cooking, and drying for its preparation were analyzed. The output variable for the agglomeration process was yield and the factors studied were ratio of fine/coarse quinoa flour, moisture of the mixture, use of a binding agent, and temperature of the binding agent solution. The conditions for the highest agglomeration yield (48.62%) were: 70/30 flour ratio, moisture of 40%, no binding agent, and a temperature of 70°C. The output variables for the cooking and drying processes were Water Absorption Index (WAI), Swelling Power (SP), and Water Solubility Index (WSI). The factors studied were steam-cooking time and drying time and temperature. The conditions maximizing the WAI and SP and minimizing the WSI were: 30 min of steam-cooking time, 120 min of drying time, and 70°C for the drying temperature. Finally, a paired comparison was carried out between the functional, chemical, and sensory properties of the quinoa and a commercial wheat couscous. The chemical properties of the quinoa couscous were better and this product was preferred by 42% of the panelists. However, in the sensorial characteristics, the new product scored lower.

Key words: Andean cereal, agglomeration, new product technology.

RESUMEN

El cuscús de quinua (*Chenopodium quinoa* Willd.) se presenta como un nuevo producto nutricional que puede reemplazar al cuscús de trigo. Para su preparación se analizaron los procesos de aglomeración, cocción y secado. La variable de salida para el proceso de aglomeración fue el rendimiento y los factores estudiados fueron: relación harina de quinua fina/gruesa, humedad de la mezcla, uso de un agente aglutinante y temperatura de la solución aglutinante. Las condiciones para el mayor rendimiento de aglomeración (48.62%) fueron: 70/30 relación de harina, humedad de 40%, sin agente aglutinante, y temperatura de 70°C. Las variables de salida para los procesos de cocción y secado fueron: Índice de Absorción de Agua (IAA), Poder de Hinchamiento (PH) e Índice de Solubilidad en Agua (ISA). Los factores estudiados fueron: tiempo de cocción y tiempo y temperatura de secado. Las condiciones que maximizaron IAA y PH y minimizaron ISA fueron: 30 min de cocción, y 120 min y 70°C en el proceso de secado. Finalmente, se realizó una comparación por pares entre las propiedades funcionales, químicas y sensoriales del cuscús de quinua y un cuscús comercial de trigo. Las características químicas del cuscús de quinua fueron mejores y este producto fue preferido por el 42% de los panelistas. Sin embargo, en las características sensoriales el nuevo producto obtuvo una puntuación más baja.

Palabras clave: cereal andino, aglomeración, tecnología para nuevos productos.

Introduction

Couscous is a North African meal prepared with wheat flour and considered in some countries as a rice substitute with better nutritional qualities given its higher protein content. However, around 2% of the world population is intolerant to wheat main protein gluten (Kaushik *et al*., 2015).

Nowadays, consumers are interested in natural and unconventional foods as a consequence of the problems seen in human health and nutrition. There is a renewed interest in pseudo-cereal grains: amaranth, quinoa, buckwheat, and chia, which are highly appreciated for their nutritional content, especially by gluten-free consumers (Boukid *et al*., 2018). Additionally, from the farmer perspective, pseudo-cereals are known for their high adaptability and low needs in terms of irrigation, fertilization, and energy, turning them into attractive products to grow in the rural areas (Santra & Schoenlechner, 2017). These pseudo-cereals are nowadays being used as raw materials or as functional ingredients in modern diets (Boukid *et al*., 2018).

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Quinoa, an Andean pseudo-cereal with a protein content of around 14% (Navruz-Varli & Sanlier, 2016), has received attention as a wheat substitute for people with gluten intolerance (Jacobsen, 2003). Most importantly, it could satisfy the nutritional requirements of the population as it has all essential amino acids, a high content of a range of vitamins and minerals, and health-beneficial phytochemicals such as saponins, phytosterols, and phytoecdysteroids (Koziol, 1992; Jacobsen, 2003; Navruz-Varli & Sanlier, 2016). Quinoa is considered a high potential crop due to the rapid expansion of its harvested area. As a result of its nutritional status, its high resilience to agro-ecological extremes and its high tolerance to drought and salinity, quinoa is nowadays cultivated or tested in over 95 countries (Jacobsen, 2003; Ruiz *et al*., 2014; Bazile *et al*., 2015).

Traditional couscous is a mixture of wheat flour and water and can be made by hand or using specific machinery. Some research has been done aiming to mechanize couscous production and to improve its nutritional value. For example, Debbouz and Donnelly (1996) studied extrusion technology to obtain uniform couscous grains; Hafsa *et al*. (2015) studied the feasibility of the use of different equipment (*e.g*., vertical and horizontal mixers, fluidized bed reactors and spray dryers) as mechanical technologies for the agglomeration process. Other studies have focused on using different ingredients to improve the agglomeration process or the nutritional value of couscous. For example, Barkouti *et al*. (2012) analyzed the influence of different binding agents in the physicochemical properties of couscous. Their study demonstrated that any of the studied agents could be used for nuclei association during the agglomeration stage. In the nutritional area, Benatallah *et al*. (2008) analyzed the use of three different formulas to obtain gluten-free couscous. Their product was made of a mix of rice semolina with pea, chickpea, or fava bean. The results showed that the three mixtures could be used for couscous production, but a consumer acceptance test showed a preference for traditional wheat couscous followed by the rice-fava bean mix. Demir *et al*. (2010) replaced wheat flour with different percentages of chickpea flour and the results showed that a 50% replacement didn't affect the product's sensory and functional properties. More recently, Chemache *et al*. (2019) analyzed the agglomeration properties of manioc, corn, amaranth, buckwheat, and quinoa flours, obtaining positive results for the last four.

Jacobsen (2003) suggested that the advantageous properties of quinoa must be exploited through the development of new products and adequate process technologies; to date, its use as a substitute for couscous preparation has not yet been researched. This paper aimed to address this gap and offer an alternative gluten-free product with high nutritional value that could be broadly consumed by humans, including those with gluten intolerance. Quinoa alone was used as the main ingredient for the couscous preparation, given its high protein content, nutritional value, and agglomeration capacity. The study identified the best conditions for the agglomeration, cooking, and drying stages to obtain a suitable product and compared the final product's functional, chemical, and sensorial properties with those of a commercial wheat couscous.

Materials and methods

The commercial quinoa (*Chenopodium quinoa* Willd., Tunkahuan variety) grains (without saponin) provided by Cereales Andinos Cia. Ltda (Ecuador) were used to obtain the flour for the couscous preparation. Polysorbate 20 (Sigma Aldrich) at different concentrations was used as a binding agent for the flour-water mixture during the agglomeration process. The study used a commercial non-flavored local wheat couscous for chemical and sensorial comparisons.

Preparation and characterization of the quinoa flour

Fine and coarse quinoa flours were obtained using two milling processes. For the fine flour, clean grains (without saponin as certified by Cereales Andinos Cia. Ltda.: certification code: QUI02-5152301) were milled using a pin mill (ALPINE, UPZ 160, Germany). The flour passed through a vibrating sieve (WS TYLER, TX-29, USA), and the material collected between sieves 70 and 140, with a particle size between 106 and 250 µm, was used as fine flour (INEN, 2013). For the coarse flour, grains without saponin were milled using a disc mill (ELECTROLUX, N4, Sweden) and passed through ASTM sieves 20 and 40 to collect the material with a particle size between 425 and 850 µm. Proximal analysis of the quinoa flour was carried out according to the Association of Official Analytical Chemists (AOAC, 2005) for moisture (925.10), ash (923.03), protein (2001.11), fat (920.39), and the International Association for Cereal Science and Technology for crude fiber (ICC-STD#113) (ICC, 1972).

Conditions for the agglomeration process

The agglomeration process is a critical step-involving wetting and mixing the fine and coarse flour, leading to a particle size enlargement. According to Silva Castro (2014), this process has three stages: a first stage where the liquid comes into contact with the flour and forms the agglomerate's nuclei; a second stage where these nuclei are compacted and enlarged due to the collisions against each other, against dry flour particles or the against the container surface; and a final stage where the agglomerates start breaking down due to particle impacts. The agglomeration process was carried out according to the procedure of Barkouti *et al.* (2012), with some modifications. First, 150 g of fine and coarse flour (ratio according to the experimental design) were homogenized for 2 min at 136 rpm using a mixer with a flat beater (HOBART, N50, USA). With the mixer switched on, the polysorbate solution was sprinkled on the mixture at 0.32 g s^{-1} using a compressor device (THOMAS, AS-06, China).

After this wetting process, the product was mixed for 30 additional sec. Finally, once the agglomerates were formed, they were sieved (ASTM sieves 10, 20, and 40) for 2 min to collect particles of the correct size. According to the Codex Alimentarius (FAO & OMS, 2007), the optimal size of the agglomerated particles should be between 850 and 2000 μm.

Four factors at two levels each were studied using a 2x experimental design to find the optimal conditions during the agglomeration process. The factors were: a) ratio of fine/coarse quinoa flour: 70/30 and 75/25, b) moisture of the mixture: 35 and 40%, c) polysorbate concentration in the solution used to wet the mixture: 0 and 3%, and d) temperature of the polysorbate solution: room temperature (approx. 20°C) and 70°C. The output variable for selecting the best agglomeration conditions was yield, calculated as the ratio of the weight of the particles between 850 and 2000 μm and the initial weight of the mixture.

An ANOVA test was performed ($P<0.05$) to evaluate the significant effect of the factors under study and their interactions on the yield of the agglomeration process. Additionally, mean values of all treatments were compared using a Duncan multiple comparison test.

Conditions for the steam-cooking and drying processes

The steam-cooking and drying of the agglomerates aim to improve their stability. The use of heat promotes the starch gelatinization and the cross-linking of the proteins, which in turn reinforce the product's structure (Cuq *et al.*, 2011).

The agglomerates obtained under the best conditions selected in the previous stage were placed in a thin layer on a perforated stainless-steel tray, which was put into a steam cooker (MIRMEG, Ecuador) at 92°C. The cooked product was then dried in a stove (POL-EKO, SLW 115 ECO, Poland) and sieved using an ASTM sieve 20.

Three factors at two levels each were studied using a 2x3 experimental design with one central point to find the optimal cooking and drying conditions. The factors were: a) cooking time: 15 and 30 min, b) drying time: 60 and 120 min, and c) drying temperature: 50 and 70°C. The output variables were the functional properties of the final product: Water Absorption Index (WAI), Swelling Power (SP), and Water Solubility Index (WSI), as described by Anderson *et al.* (1969). The selected conditions maximized the WAI and SP and minimized WSI, which help preserve the structure of the quinoa flour agglomerates.

Multiple ANOVA tests ($P<0.05$) were performed to evaluate the significant effect of the factors under study on the functional properties of the final product (*e.g.*, WAI, SP, WSI). A multiple comparison test was used to compare the means of the functional properties obtained for the different treatments. The technique of multiple response variable optimization was used to identify the optimal cooking and drying conditions that maximize WAI and SP and minimize WSI.

Functional, chemical and sensory properties of quinoa vs. wheat couscous

The functional (*e.g.*, WAI, SP, WSI), chemical, and sensory properties of quinoa couscous prepared at the selected agglomeration, cooking, and drying conditions were analyzed and compared with the properties of commercial wheat couscous.

Additionally, a micrograph showing the morphological characteristics of the quinoa couscous was obtained with a scanning electron microscope (ASPEX, PSEM eXpress, USA) and compared with the micrograph for wheat couscous published by Hafsa *et al.* (2015).

For the chemical properties that quantify the nutrients in the product, a proximal analysis was carried out according to the Association of Official Analytical Chemists (AOAC, 2005) and the International Association for Cereal Science and Technology (ICC, 1972).

For the sensory analysis, 12 semi-trained panelists tasted quinoa and commercial wheat couscous (Martino Prestige Couscous) samples and stated their overall preferences. Additionally, applying a paired comparison between the two samples, the panelists evaluated the color, flavor, odor, texture, and the presence of odd flavors or aromas (Hernández, 2005). Couscous samples were prepared using hot water at 92°C (relation water/couscous of 1:1). The selected panelists were persons involved in the food field that had taken at least one course on sensory analysis. Panelists were trained to detect, recognize and describe the characteristics of interest of this product. For this training, two couscous samples (different from the ones analyzed in this study) were used.

Results and discussion

Characterization of the quinoa flour

The results for the proximal analysis of the quinoa flour (Tunkahuan variety) are presented in Table 1, together with published data reported by Navruz-Varli and Sanlier (2016) for quinoa, wheat, and rice used for comparison purposes.

TABLE 1. Chemical characterization of quinoa flour (author data), compared to other grains highly consumed in developing countries (values are based on dry matter).

	Quinoa flour Tunkahuan variety g 100 g^{-1}	Quinoa* g 100 g^{-1}	Wheat* g 100 g^{-1}	Rice* g 100 g^{-1}
Ash	2.61 ± 0.00	2.70	1.13	0.19
Protein	14.62 ± 0.29	14.12	13.68	6.81
Fat	6.92 ± 0.04	6.07	2.47	0.55
Crude fiber	2.79 ± 0.49	7.00	10.70	2.80
Carbohydrate	66.34 ± 0.10	64.16	71.13	81.68

±σ; n = 2.

* Navruz-Varli and Sanlier (2016).

The results for ash, protein, fat and carbohydrate obtained for the quinoa flour used in this study are in a similar range of the values reported by Navruz-Varli and Sanlier for quinoa grain (2016). When comparing quinoa with the most consumed staples in developing countries, its protein content (14.62%) is higher than that of wheat (13.68%) and rice (6.81%) (Navruz-Varli & Sanlier, 2016), and also than the protein of corn (8.50%), manioc (0.50%) and buckwheat (12.00%) (Chemache *et al*., 2019). The amount of fiber in the quinoa flour sample is the lowest because the grain already underwent a saponin elimination process that removed its outer layer. According to Li and Zhu (2018), starch is the main carbohydrate present in quinoa (30 to 70 %) and is composed of amylose (27.7%) and amylopectin (7.1%).

Conditions for the agglomeration process

The results of the agglomeration yield for the different treatments are shown in Table 2. The ANOVA test showed a statistically significant effect (P<0.01) for the variables of moisture of the mixture, polysorbate concentration, and their interaction. In general, a higher moisture content had a significant positive effect on the agglomeration yield. Using polysorbate in the solution to wet the flour had a significant negative effect. The ratio of fine/coarse flour and the temperature of the solution did not have any effect on the outcome variable.

Higher agglomeration yields are observed in the quadrants H2-S1 and H2-S2 and under the condition P1 (Tab. 2: 50.61% ± 0.31 and 48.62% ± 1.25, and 48.18% ± 3.13 and 47.52% ± 0.94, respectively). On average, the use of polysorbate in the solution slightly decreased the yields for the P1 condition treatments. Therefore, polysorbate was not used in the following stages. Although the variable temperature of the polysorbate solution had no significant effect, the level T2 (*e.g.*, 70°C) was selected. A higher temperature promotes the glass transition of the flour particles, and the resulting partial fusion could strengthen the inner structure of the agglomerates (Cuq *et al*., 2013). Arzapalo Quinto *et al*. (2015) showed that the starch gelatinization temperature for three quinoa varieties was between 66 and 69°C; therefore, we expect that the use of a warm solution will allow for a partial gelatinization process to take place. With these considerations, the selected conditions for the

TABLE 2. Agglomeration yield (%) obtained under different treatments.

Parameter		S1^{+++}		S2^{+++}	
P1		P2	P1	P2	
H1***	T1	23.94±6.09^{c}	22.75±3.05^{bc}	14.13±1.02^{a}	15.80±1.35^{a}
	T2	23.23±0.34^{bc}	23.94±0.68^{c}	15.80±0.68^{a}	17.24±1.35^{ab}
H2***	T1	50.61±0.31^{e}	45.75±7.19^{de}	48.18±3.13^{e}	41.99±1.25^{d}
	T2	48.62±1.25^{e}	47.96±3. 44de	47.52±0.94^{de}	47.74±0.63^{de}

±σ, n=2.

Different letters among all treatments show significantly different yields. Letter □ corresponds to the lowest yield, and letter □ to the highest yield.

H1: Moisture of the mixture 35%, H2: Moisture of the mixture 40%.

T1: Polysorbate solution at room temperature (~20°C), T2: Polysorbate solution at 70°C.

P1: Fine/coarse flour: 70/30, P2: Fine/coarse flour 75/25.

S1: Polysorbate solution: 0%, S2: Polysorbate solution: 3%.

ANOVA-test results: *** significant at the 1% level for Moisture of the mixture (H); +++ significant at the 1% level for Polysorbate solution (S).

quinoa agglomeration process were: a) 70/30 ratio of fine/coarse flour, b) 40% moisture of the mixture, c) no polysorbate added to the solution, and d) a temperature of 70°C for the solution used to wet the flour. Under these conditions, the yield of the agglomeration process was 48.62% ± 1.25%. A similar result was reported by Silva Castro (2014) for couscous prepared with durum wheat flour using moisture of 40% in the mixture.

Conditions for the couscous steam-cooking and drying processes

The results for the output variables for the second and third stages are shown in Table 3. The ANOVA test for WAI showed a statistically significant effect ($P<0.05$) for the variable drying temperature and the interaction drying time–drying temperature.

According to Debbouz and Donelly (1996) and Giovanelli *et al.* (2023), a high WAI is a good quality factor for raw couscous, because it is an indicator of its ability to absorb water during cooking and to maintain its firmness, while WSI is a negative attribute because it indicates the amount of soluble materials leached out during cooking, affecting its stickiness. Water absorption is the result of the interaction of water with protein, due to its hydrophilic properties, therefore these indexes are affected by the couscous protein concentration and its structural characteristics (Butt & Batool, 2010). Laya *et al.* (2022) indicate that processing methods, such as crushing, grinding and sieving carried out during flour production, have an effect on the flour protein content.

The SP was also significantly influenced by the variable drying temperature ($P<0.05$). A higher drying temperature (70°C) caused a slight increase in the WAI and SP. Cerón-Fernández *et al.* (2016) also reported higher WAI for higher extrusion temperatures in a quinoa product. The WSI was significantly influenced by the variable drying time and the interaction drying time-drying temperature (0.05). Based on the WSI data, a longer drying time and a higher drying temperature resulted in a lower WSI. However, when the couscous underwent a shorter drying period at a lower temperature, the WSI decreased.

In summary, the variables that had a significant influence on the functional properties of the quinoa couscous were drying temperature and drying time. The supply of energy to the agglomerates improves their stability through starch gelatinization and the cross-linking of the proteins, which in turn reinforce the product's structure (Cuq *et al.*, 2011).

The multiple response optimization methodology (*e.g.*, maximize the WAI and SP and at the same time minimize the WSI) was further used to determine the range of conditions to be selected for the cooking and drying processes. The surface graph for the multiple response optimization, when the drying time is 70°C, is presented in Figure 1. The possible combinations of the best conditions (cooking time–drying time) that can be used to obtain the highest desirability are in the blue surface area of the graph. With the drying temperature fixed at 70°C, the highest desirability is observed for a cooking time of at least 27 min (if the drying time is 120 min) or for a drying time of at least

TABLE 3. Water Absorption Index (WAI), Swelling Power (SP), and Water Solubility Index (WSI) for different steam-cooking and drying conditions of quinoa agglomerates.

Conditions			Steam-cooked agglomerate properties		
td (min)	Td (°C)	Tc (min)	WAI (g g^{-1})	SP (g g^{-1})	WSI (%)
60	50	15	4.54±0.03^{cd}	4.72±0.03^{cd}	8.06±0.13^{bcd}
		30	4.31±0.03^{ab}	4.48±0.04^{ab}	8.02±0.13^{bc}
	70	15	4.35±0.07^{abc}	4.55±0.55^{abc}	9.47±0.11^{f}
		30	4.53±0.18^{bcd}	4.72±0.19^{cd}	8.94±0.06^{ef}
90	60	22.5	4.52±0.09^{bcd}	4.68±0.09^{bcd}	7.31±0.33^{a}
120	50	15	4.22±0.09^{a}	4.39±0.10^{a}	8.51±0.21^{cde}
		30	4.45±0.11^{bc}	4.64±0.11^{bc}	8.73±0.77^{de}
	70	15	4.53±0.11^{bcd}	4.69±0.11^{bcd}	7.50±0.24^{ab}
		30	4.71±0.07^{d}	4.88±0.07^{d}	7.20±0.12^{a}

$x\pm\sigma$, n=2.

Different letters in each column show a significant difference for the analyzed property. Letter *a* corresponds to the lowest index observed, and letter *d* (WAI and SP) or *de* (WSI) to the highest index.

Tc: cooking time (min); td: drying time (min); Td: drying temperature (^{0}C).

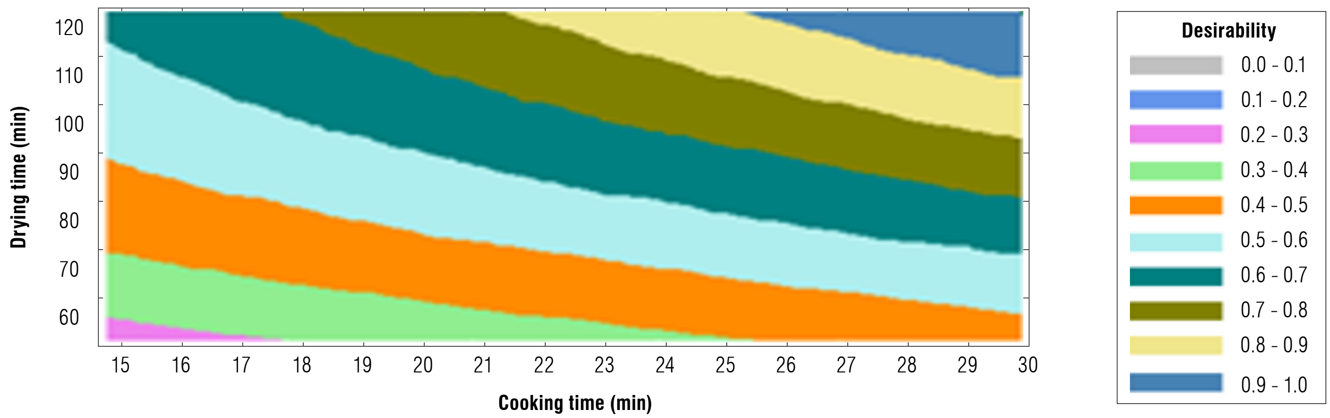

FIGURE 1. Surface graph for the multiple response optimization, when the drying temperature was 70°C.

106 min (if the cooking time is 30 min). The overall highest desirability is observed for a cooking time of 30 min and a drying time of 120 min.

Functional, chemical and sensorial properties of quinoa vs. wheat couscous

Functional and physical properties

A comparison between the results for the functional properties (*e.g.*, WAI, SP, and WSI) for the quinoa and commercial wheat couscous is shown in Table 4. A high WAI is considered a desired quality attribute because high-water absorption improves the firmness of the couscous grain (Debbouz & Donnelly, 1996). The values of WAI for the quinoa (4.72 ± 0.06) and wheat couscous (5.04 ± 0.17) are not significantly different, and both are higher than the minimum WAI required reported by Coskun (2013). The SP of wheat couscous (5.23 ± 0.16) is 7% higher than that of quinoa couscous (4.88 ± 0.07). This result is in line with the existing literature, which reports that products with a higher gluten content have a higher SP than those with lower gluten content (Debbouz & Donnelly, 1996).

Regarding the WSI, the value for the quinoa couscous (7.20% ± 0.12) is twice as high as that for the wheat couscous (3.53% ± 0.28), which implies that the structure of the first sample is less stable. According to Hafsa *et al.* (2015), partial leaching of starch grains out of the amylose chains can happen during the gelatinization process. These starch grains are dissolved in water which increases the WSI. Aboubacar and Hamaker (1999) report a WSI of 6.2% for sorghum (*Sorghum bicolor* L. Moench) couscous, which is also significantly higher than the WSI reported for wheat couscous. WSI was acceptable, as it normally ranges between 4 and 16% (Giovanelli *et al.*, 2023). Finally, Cankurtaran and Bilgiçli (2021) report that the use of pseudocereal flour (*e.g.*, amaranth, buckwheat and quinoa) in pasta-like products, causes an increase in their WSI because of the weak protein-starch matrix.

TABLE 4. Functional and physical properties and chemical characterization of quinoa couscous and wheat commercial couscous.

Functional and physical properties	Quinoa couscous	Commercial wheat couscous
WAI (g g^{-1})	4.72 ± 0.06[a]	5.04 ± 0.17[a]
SP (g g^{-1})	4.88 ± 0.07[a]	5.23 ± 0.16[b]
WSI (%)	7.20 ± 0.12[b]	3.53 ± 0.28[a]
Chemical constituents (g 100 g^{-1})	**Quinoa couscous**	**Commercial wheat couscous**
Humidity	8.19 ± 0.01	9.23 ± 0.04
Ash	2.55 ± 0.01	0.97 ± 0.03
Protein	14.05 ± 0.02	13.95 ± 0.06
Crude fiber	4.04 ± 0.01	0.45 ± 0.02
Fat	6.44 ± 0.65	0.21 ± 0.04
Remaining total carbohydrates	69.19 ± 0.07	75.65 ± 0.02

$x \pm \sigma$, n=2.

Figure 2 shows the micrograph from our quinoa couscous sample. The granule is spherical and as seen in the graph, flour particles appear to be strongly joined together and show irregularities and pores on their surface. Additionally, smooth areas also appear on the surface, which are a product of partial fusion of flour particles that have been gelatinized. Partial starch gelatinization occurs during agglomeration and cooking processes (Hafsa *et al.*, 2015). The quinoa granule structure is similar to the one found by Hafsa *et al.* (2015) for wheat couscous, but in their study the wheat granule appeared slightly more elongated and formed by smaller flour particles.

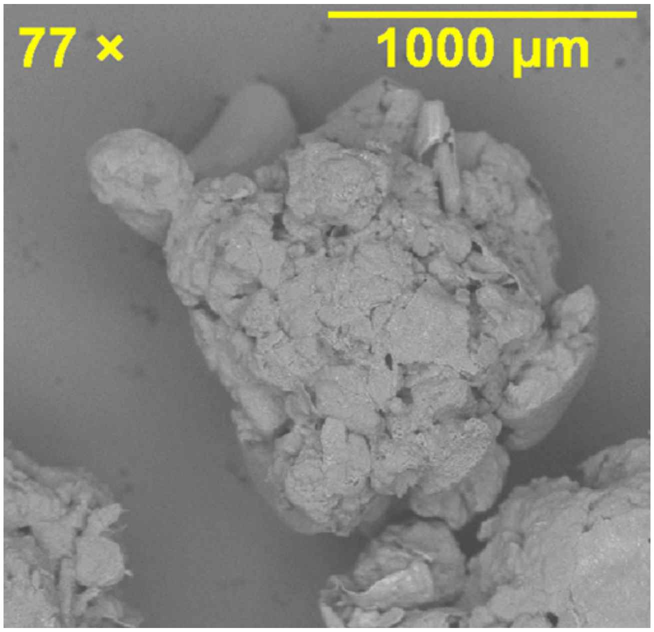

FIGURE 2. Micrograph from our quinoa couscous sample obtained at x77.

Chemical properties

The results for the proximal analysis of both samples are presented in Table 4. The moisture in both samples is lower than the maximum value of 13.5% set in the Couscous Codex for this product type (FAO & OMS, 2007). The ash content in the quinoa couscous (2.55% ± 0.01) is 2.5 times higher than in the wheat product (0.97% ± 0.03). According to the Couscous Codex, the maximum ash content in wheat couscous should be 1.1% (FAO & OMS, 2007). This value was set to avoid any adulteration of the product, given that the wheat's maximum ash content is 1.3% (FAO & OMS, 2007). However, the ash content in the quinoa flour used as raw material (2.62%) is higher than the ash in wheat flour, thus, the higher amount of ash in quinoa couscous. The ash content is related to the minerals in the sample. Koziol (1992) mentions that quinoa has 2.9 times more calcium than wheat, which increases its nutritional value.

There is no significant difference in the amount of protein in both samples when a t-student test for mean differences was applied (*P* value=0.258). However, in terms of protein quality, quinoa flour has more lysine (7.4%) and histidine (3.9%) than reported in wheat (*e.g.*, lysine 3.6% and histidine 2.4%) (Abugoch James, 2009). Regarding crude fiber and fat, the amount found in quinoa couscous is 9 and 30 times higher respectively than that in the wheat couscous sample. The higher percentage of fiber in quinoa couscous could bring health benefits by improving bowel movements (Sánchez Almaraz *et al.*, 2015). According to Repo-Carrasco *et al.* (2003), 87% of the fatty acids found in quinoa are unsaturated, with a high amount of Omega 3, 6, and 9, which play an essential role in maintaining cellular membrane functions and are, therefore, desirable in human nutrition.

Additionally, the carbohydrate content of quinoa couscous is lower than that of wheat couscous. According to Hernández Rodríguez (2015), quinoa carbohydrates have a low glycemic index and are therefore suitable for people suffering from diabetes mellitus.

Sensory properties

Fewer panelists chose quinoa couscous as their favorite when evaluating the following parameters: color, flavor, aroma, and texture. In detail, only 25% of the panelists preferred the color and texture of the quinoa couscous and 33.3% preferred its flavor over the wheat couscous. Some panelists even mentioned the presence of off-flavors and aromas in the quinoa product. This points in the direction of the possible need of additional ingredients or additives to improve the flavor and aroma of the product. Nevertheless, 42% of the panelists did choose quinoa couscous as their *overall favorite product* (42% for quinoa vs. 58% for wheat), probably because they related it with a higher nutritional quality. Care has to be taken when looking at these results, given that the difference in the panelist choices is not statistically significant. In a future study, more panelists should be added to the pair comparison test. Cankurtaran and Bilgiçli (2021) report that a highly acceptable couscous sample was obtained when 25% of the wheat flour was replaced with quinoa flour and that the use of an increasing quantity of quinoa decreased the overall acceptability and appearance of the product.

Conclusions

When using quinoa (*Chenopodium quinoa* Willd.) for the preparation of couscous, the highest yield in the agglomeration process was achieved under the following conditions: ratio of fine/coarse quinoa flour of 70/30, a moisture of the mixture of 40%, no bidding agent (polysorbate), and a 70°C water temperature. For the second and third process stages, the best conditions to obtain a stable agglomerated sample were: a steam-cooking time of 30 min, a drying time of 120 min and a temperature in the drying process of 70°C.

The obtained quinoa couscous showed acceptable values for WAI, SP, and WSI. Compared to a commercial wheat couscous sample, the WAI and SP were similar, but the WSI was approximately double. Regarding the chemical properties, the protein amount of the quinoa and wheat couscous samples were similar. However, quinoa does not contain gluten; therefore, the new product could be consumed by gluten-intolerant people. Additionally, the amount of ash, fat, and crude fiber reported for the quinoa product was higher. Finally, quinoa couscous scored lower on the stated preferences of a sensory panel, yet 42% of the panelists did choose quinoa couscous as their favorite product. This result could improve if, in a future study, additional ingredients were added to improve the flavor of the product.

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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

CBA carried out the laboratory experiments; CSG and JAV designed and supervised the experiments, obtained the financial support for the material, reagents, laboratory samples, instrumentation, and other analysis tools, and wrote the article. CRG contributed to the data analysis, wrote and translated the manuscript. All authors reviewed the final version of the manuscript.

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Influence of temperature and solute concentration during osmotic dehydration of apple (*Malus domestica*) cubes on the stability of probiotics

Influencia de la temperatura y concentración de solutos durante la deshidratación osmótica de cubos de manzana (*Malus domestica*) sobre la estabilidad de probióticos

Maritza Yola Ccaza–Cari [1] and Alex Danny Chambi-Rodriguez[1]

ABSTRACT

Currently, there is an upsurge in preference for the consumption of probiotic-rich foods. Besides their nutritive function, these compounds have demonstrated, in some instances, medicinal properties. The purpose of this study was to evaluate how temperature and sucrose concentration influenced the stability of probiotics (specifically *Saccharomyces boulardii)* during the osmotic dehydration of Granny Smith apple (*Malus domestica*) cubes. We prepared osmotic solutions with different sucrose concentrations (40, 50, and 60°Brix). We inoculated *S. boulardii* (250 mg each) into these solutions, followed by immersion of 1 cm-cubed apple cubes. We exposed these cubes to varying temperatures (37°C, 42°C, and 47°C) for a duration of 80 min. Various parameters were calculated, including the percentage of weight loss, percentage of solid gain, number of generations, and the doubling time. Results indicated that the apple cubes with more extreme dehydration were those treated at 50°Brix and 47°C exhibiting a weight loss of 40%. The treatment at 60°Brix and 42°C stood out, showing an increase of 350% of solid gain compared to other groups. Additionally, the highest number of generations of the strain occurred in the group treated at 50°Brix and 37°C, with a value of 9.32 ± 0.11 CFU/g and a doubling time of 7.50 ± 0.09 min. In conclusion, we deduced that under conditions of elevated temperatures and high solute concentrations, the *S. boulardii* strain might undergo inhibition and fail to develop adequately in the apple cubes subjected to osmotic dehydration.

Key words: *Saccharomyces boulardii***,** heat treatment, dehydrated fruit, cell viability.

RESUMEN

En la actualidad, hay un aumento en la preferencia por el consumo de alimentos ricos en probióticos. Estos compuestos, más allá de su función nutritiva, han demostrado en algunos casos poseer propiedades medicinales. En este sentido, el propósito de este estudio consistió en evaluar cómo la temperatura y la concentración de sacarosa influyen en la estabilidad de los probióticos (específicamente *Saccharomyces boulardii*) durante el proceso de deshidratación osmótica de cubos de manzana (*Malus domestica*) de la variedad Granny Smith. Para esto, se prepararon soluciones osmóticas con distintas concentraciones de sacarosa (40, 50 y 60°Brix). En estas soluciones se inoculó *S. boulardii* (250 mg cada una), seguido de la inmersión de cubos de manzana de 1 cm de lado. Estos cubos fueron expuestos a diferentes temperaturas (37, 42 y 47°C) durante un período de 80 min. Se procedió a calcular diversos parámetros, como el porcentaje de pérdida de peso, el porcentaje de ganancia de sólidos, el número de generaciones y el tiempo de duplicación. Los resultados indicaron que los cubos de manzana que experimentaron una mayor deshidratación correspondieron a los tratados a 50°Brix y 47°C, registrando una pérdida de peso del 40%. En cuanto al porcentaje de ganancia de sólidos, se destacó el tratamiento a 60°Brix y 42°C, alcanzando un aumento del 350% en comparación con los demás grupos. Además, el mayor número de generaciones de la cepa ocurrió en el grupo tratado a 50°Brix y 37 °C, con un valor de 9.32 ± 0.11 UFC/g y un tiempo de duplicación de 7.50 ± 0.09 min. Se concluyó que en condiciones de temperaturas elevadas y altas concentraciones de soluto, la cepa de *S. boulardii* podría sufrir inhibición y no desarrollarse adecuadamente en los cubos de manzana sometidos al proceso de deshidratación osmótica.

Palabras clave: *Saccharomyces boulardii*, tratamiento térmico, fruta deshidratada, viabilidad celular.

Introduction

Most foods that contain probiotics are derived from milk (Vinderola *et al*., 2017). This creates a big problem for some consumers since it involves the consumption of allergens and lactose. In addition, it implies the consumption of products of animal origin that are not suitable for a vegetarian or vegan diet and have a short shelf life (Makinen *et al*., 2016). Because of this the demand for non-dairy probiotic products has grown because of an increased incidence of dietary restrictions on dairy products for reasons including lactose intolerance, allergic reactions to milk proteins, and veganism (Kumar *et al*., 2015; Neffe-Skocińska *et al*., 2018).

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Probiotics are defined as live microorganisms that, when administered in sufficient amounts, confer a health benefit on the consumer (Hill *et al*., 2014). *Saccharomyces boulardii* is included among the microorganisms with probiotic effect. This microorganism can remain viable and active so that when ingested it can give pharmacodynamic effects similar to the physiological effects of normal intestinal flora (Peña, 2007; Zamora Vega *et al*., 2015); it also acts as a transporter, releasing enzymes, proteins, and trophic factors during its intestinal transit, improving the host's immune defenses, digestion, and nutrient absorption. So, this yeast is an important source for probiotic products (Mejía *et al*., 2016; Sen & Mansell, 2020). Its probiotic activity has been linked to multiple pathways, including enhancement of gut barrier function, competitive pathogen exclusion, antimicrobial peptide production, and immunomodulatory and nutritional effects (Zhang *et al*., 2021). This probiotic strain helps in maintaining the balance of intestinal flora by stimulating the production of lactic acid and acetic acid, lowering intestinal pH, and preventing the proliferation of pathogenic bacteria (Li, Xia *et al*., 2021; Li, Zhu *et al*., 2021).

Fruits and vegetables are an essential part of the human diet, since they are a source of bioactive compounds such as vitamins, minerals, phytosterols, dietary fiber, etc. Generally, fruits and vegetables are marketed fresh. However, their shelf life is limited by metabolic activity, and they are susceptible to mechanical damage and the presence of microorganisms that accelerate their senescence and death (Yousuf *et al*., 2018; Al-Tayyar *et al*., 2020). Osmotic dehydration is a technique used to reduce the water content and to include solutes in fruits and vegetables by immersion in an osmotic solution. This technique has been widely used in the food industry to produce dehydrated fruits with a longer shelf life and nutritional value (Yousuf *et al*., 2018).

The apple fruit matrix has been proven to be highly applicable for probiotics, possibly due to its high porosity and, therefore, the easy incorporation of probiotics. Espírito Santo *et al*. (2012) attribute this to the cellulose content in apples that is not digested and could serve as a protective matrix for probiotics through the intestinal tract (Kourkoutas *et al*., 2006). Rêgo *et al*. (2013) demonstrate the compliance of apple as a fruit matrix for probiotic survival over time. They studied hot-air-dried apple cubes with *Lactobacillus plantarum* incorporated during 65 d of storage and found a loss of viability of 1 log CFU/g.

Gupta and Garg (2009) suggest a dose of 5 billion colony forming units (CFU) for at least 5 d (5×10^9 CFU/d) to produce health benefits. Probiotics may be available in foods and dietary supplements (capsules, tablets, and powders). Also, to improve probiotic survival, the food should be dehydrated by lyophilization instead of hot air (Betoret *et al*., 2003; Rascón *et al*., 2018). Another methodology that is increasingly of interest in the impregnation of probiotics is osmotic dehydration. This consists of submerging the food in a hypertonic solution that contains microbial cells. The water migrates from the food to the solution, partially dehydrating it; and the solutes, including the probiotics, migrate towards the food (Rascón *et al*., 2018).

In a study carried out on plantain impregnated with *Lactobacillus rhamnosus,* the process was successful, maintaining levels of 10^7 CFU/g (Huerta-Vera *et al*., 2017). Using 50% w/w hypertonic sucrose solutions, Rascón *et al*. (2018) impregnated *L. rhamnosus* in banana slices by osmotic dehydration to subsequently freeze-dry them. The survival kinetics of the microorganism showed that its viability decreased significantly when water activity (aw) exceeded values of 0.327.

The purpose of this study was to evaluate how temperature and concentration influence the stability of probiotics (specifically *Saccharomyces boulardii)* during the osmotic dehydration of Granny Smith apple (*Malus domestica*) cubes.

Materials and methods

Raw material

Granny Smith apples were purchased at the local market in the province of San Román, department of Puno (Peru) and stored at 4°C. We washed them for 5 min in an aqueous solution of active chlorine at a concentration of 7500 mg L^{-1} and then cut them into cubes of 1 cm square. Freeze-dried strains of *S. boulardii* Hansen CBS 5926 from Mexico were inoculated into the solutions.

Maturity index

To determine the maturity index in apples, we measured °Brix and acidity. According to the AOAC (2005) method, we determined the acidity by preparing a 0.1N NaOH solution; then we weighed 10 g of apple pulp and added 50 ml of distilled water. The mixture was vigorously shaken for a few minutes, and we collected an aliquot of 25 ml of the solution. Once filtered, we added 3 drops of phenolphthalein. We titrated the solution with a standard 0.1 N NaOH solution until it reached the equivalence point by changing color to light pink. We used the volume spent to calculate the acidity percentage (Eq. 1). We determined Brix with an ATAGO range refractometer (0-40°Brix).

$$\%Acidity = \frac{(A \times B \times C) \times 100}{D} \quad (1)$$

where A represents the volume of NaOH spent, B signifies the normality of NaOH (0.097 meq ml^{-1}), C denotes the equivalent weight expressed in grams of the predominant acid in the fruits (citric acid 0.064 g meq^{-1}; malic acid 0.067 g meq^{-1}), and D stands for the weight in grams of the sample used.

The maturity index (MI) was determined with the following formula:

$$MI = \frac{°Brix}{Acidity} \quad (2)$$

Osmotic dehydration and inoculation of the strain

We prepared aqueous osmotic solutions with sucrose at different concentrations (40°Brix, 50°Brix, and 60°Brix). The apple cubes were immersed in 500 ml beakers with 200 ml of the prepared osmotic solution at three different temperatures (37°C, 42°C, and 47°C), and 250 mg of *S. boulardii* were immediately added. We applied treatments by shaking at 350 rpm. Each apple sample was removed every 10 min up to a total time of 80 min to conduct weight measurements on an OHAUS analytical balance and measure the soluble solids content in the liquid phase (Brix) using an ATAGO range refractometer (0-85°Brix). We conducted all the experiments in triplicate.

Weight loss and solid gain

During osmotic dehydration, we monitored mass transfer by the time variation in solid gains and weight loss (Della Rocca & Mascheroni, 2011; Wais, 2011).

Equation 3 was used to calculate weight loss.

$$WL\ (\%) = \left(\frac{M_o - M_f}{M_o}\right) \times 100 \quad (3)$$

where WL (%) is the percentage of weight loss, M_o represents the initial weight of the sample in grams, and M_f is the weight of the sample treated at time t.

The solid gains were calculated as indicated by Equation 4.

$$SG\ (\%) = \left(\frac{°B_f - °B_o}{°B_o}\right) \times 100 \quad (4)$$

where SG (%) were the solid gains percentages, $°B_f$ were the Brix degrees of the osmodehydrated sample and $°B_o$ were the Brix degrees of the fresh sample.

Microbiological kinetics

To assess the microbiological growth kinetics in the polynomial model, we employed several key statistical metrics. To gauge the model's fitness for survival, doubling time, and the number of generations, we utilized the coefficient of determination (R^2). We computed this coefficient using the following equation:

$$R_2 = 1 - \frac{SSE}{SST} \quad (5)$$

where SSE (Sum of Squared Errors) represented the sum of the squares of differences between actual observations and model predictions, and SST (Total Sum of Squares) was the sum of the squares of differences between actual observations and their mean.

Additionally, to evaluate the model's goodness of fit in terms of complexity, we employed the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). We calculated AIC using the following equation:

$$AIC = -2 \times Log - likelihood + 2k \quad (6)$$

where log-likelihood was the logarithmic likelihood function of the model and k was the number of estimated parameters in the model.

BIC was computed as follows:

$$BIC = -2 \times Log - likelihood + k \times Log\ (n) \quad (7)$$

where log-likelihood was the logarithmic likelihood function of the model, k was the number of estimated parameters, and n was the number of observations in the data.

We applied these statistical metrics in the study's methodology to assess the goodness of fit of the polynomial model and its ability to explain the observed data.

Survival, doubling time, and number of generations

To determine cell survival, we used a 10^{-1} dilution in 1% saline solution, and then Petri dishes were inoculated with 0.1 ml of Sabouraud Dextrose Agar and dispersed with a Drigalski spatula; we used a convection incubator at 27°C for 48 h; the readings were expressed in colony-forming units per gram (CFU/g).

To calculate the number of generations, we applied the following equation:

$$N_G = \frac{LogN_2 - LogN_1}{Log2} \quad (8)$$

where N_G was the number of generations, N_2 was the concentration of cells at time 2 and N_1 was the concentration of cells at time 1, expressed in colony-forming units per gram (CFU/g).

The doubling time was calculated with the following equation:

$$T_D = \frac{t_f - t_i}{N_G} \quad (9)$$

where T_D was the doubling time, t_f was the final incubation time, and t_i was the initial incubation time in minutes.

Results and discussion

Osmotic dehydration

In Figure 1, the treatments with the highest percentage of weight loss were the treatments 50°B47°C, followed by 50°B37°C. In contrast, the treatment 60°B42°C obtained an unfavorable result. All other treatments had a positive slope indicating that weight loss was continuous over time.

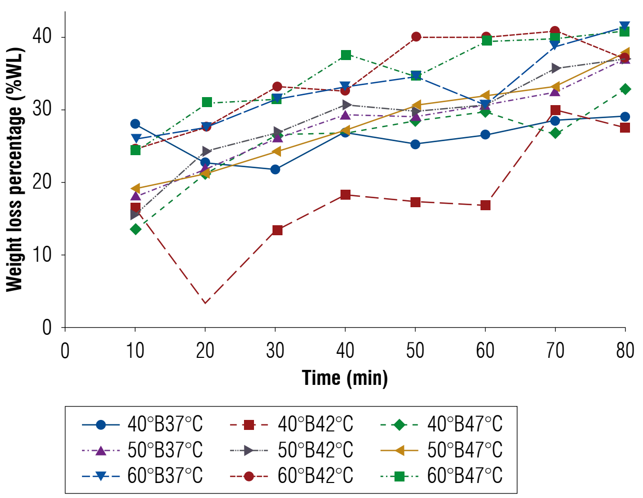

FIGURE 1. Weight loss percentage (%WL) as a function of time of apple cubes inoculated with *Saccharomyces boulardii* at different concentrations and temperatures.

The statistical analysis of the regression (Table 1) revealed that 50°B47°C was the treatment with the best fit with an R^2 of 0.98 in contrast to the others. Likewise, it showed the highest value in the R^2 adjusted (0.98) related to the typical error. The same treatment showed the smallest value of typical error (0.91). On the other hand, all the treatments were significant with a *P*-value less than 0.05. However, two had higher values, 40°B42°C with 0.21 and 50°B42°C with 4.60.

TABLE 1. Statistics of the linear regression of the percentage of weight loss in apple cubes inoculated with *Saccharomyces boulardii*

Treatments	Regression statistics				
	Determination coefficient (R^2)	R^2 adjusted	Typical error	MSE	Probability
40°B37°C	0.26	0.13	2.45	12.84	0.00
40°B42°C	0.61	0.54	5.56	291.84	0.21
40°B47°C	0.72	0.68	3.41	188.00	0.00
50°B37°C	0.93	0.92	1.65	239.76	0.00
50°B42°C	0.87	0.85	2.58	278.38	0.00
50°B47°C	0.98	0.98	0.91	289.51	4.60
60°B37°C	0.82	0.79	2.37	158.26	0.00
60°B42°C	0.75	0.70	3.22	188.75	0.00
60°B47°C	0.85	0.83	2.36	200.46	0.00

Note: In each treatment, B corresponds to degrees Brix in the medium followed by the treatment temperature. MSE – Mean Square Error.

Figure 2 shows that the gain of solids increased as the treatment time increased. This was probably due to the incorporation of soluble solids in the fruit. Also, the rate of gain of solids increased as the concentration increased.

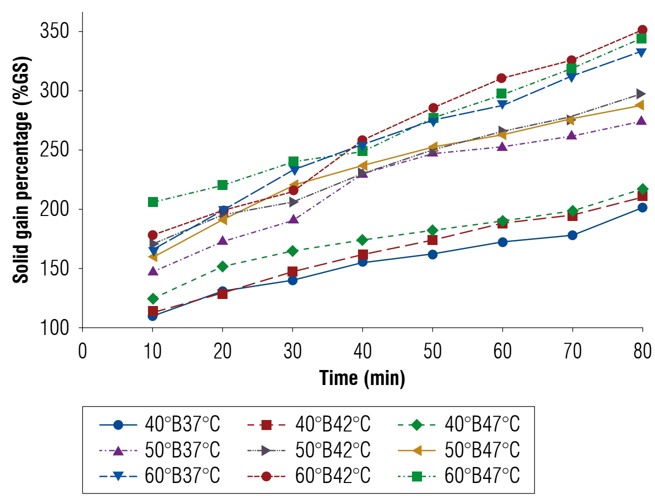

FIGURE 2. Solid gain percentage (%GS) as a function of time in apple cubes inoculated with *Saccharomyces boulardii* at different concentrations and temperatures.

Table 2 presents the results of the statistical regression analyzes, where all the treatments had a good adjustment (R^2>0.93) ranking between 0.94 and 0.99. The error 40°B42°C and 50°B42°C had the smallest values of all the treatments.

TABLE 2. Statistics of the linear regression of the percentage solid gain in apple cubes inoculated with *Saccharomyces boulardii.*

Treatments	Regression statistics				
	Determination coefficient (R^2)	R^2 adjusted	Typical error	MSE	Probability
40°B37°C	0.97	0.97	4.66	5562.16	1.23
40°B42°C	0.99	0.99	3.14	8353.05	1.33
40°B47°C	0.95	0.94	6.77	5503.31	4.04
50°B37°C	0.94	0.93	11.6	14276	5.08
50°B42°C	0.99	0.99	3.91	13438.68	3.82
50°B47°C	0.95	0.94	10.15	12804.23	1.07
60°B37°C	0.98	0.97	8.29	22189.70	3.60
60°B42°C	0.99	0.98	6.71	28141.88	1.27
60°B47°C	0.99	0.98	5.34	16432.79	1.04

Note: In each treatment, B corresponds to degrees Brix in the medium followed by the treatment temperature. MSE – Mean Square Error.

Microbiological kinetics

The microbiological kinetics of each of the treatments revealed intriguing findings. Specifically, *S. boulardii* experienced a significant increase in its development at a temperature of 42°C across all evaluated concentrations. This strongly suggested that this temperature represented the optimal condition for cellular reproduction of this microorganism. This discovery holds significant relevance as it provided valuable insights into the ideal conditions for the cultivation and stability of *S. boulardii* in osmotic dehydration. Furthermore, when analyzing the kinetic curves in 60°Brix media, we noted a distinct behavior. These curves exhibited significantly lower values compared to the other two graphs. This phenomenon could be associated with the influence of sugar concentration in the growth medium on the activity and growth of microorganisms. These results open new avenues of research to better understand how environmental variables, such as temperature and nutrient concentration, impact the growth kinetics of biotechnologically relevant microorganisms. This, in turn, could lead to improvements in the production and application of *S. boulardii* in apple cubes.

Survival of *Saccharomyces boulardii*

The survival of *S. boulardii* during osmotic dehydration exhibited divergent responses across the various treatments. In certain instances, such as in the case of 60°B47°C and 40°B47°C, we observed limited development with values of 1.75 and 1.72 CFU/g. Conversely, two treatments yielded the highest number of generations: 50°B37°C with 9.32 CFU/g and 60°B37°C with 8.08 CFU/g. Furthermore, these two treatments demonstrated the shortest doubling times, indicating a more rapid increase in biomass.

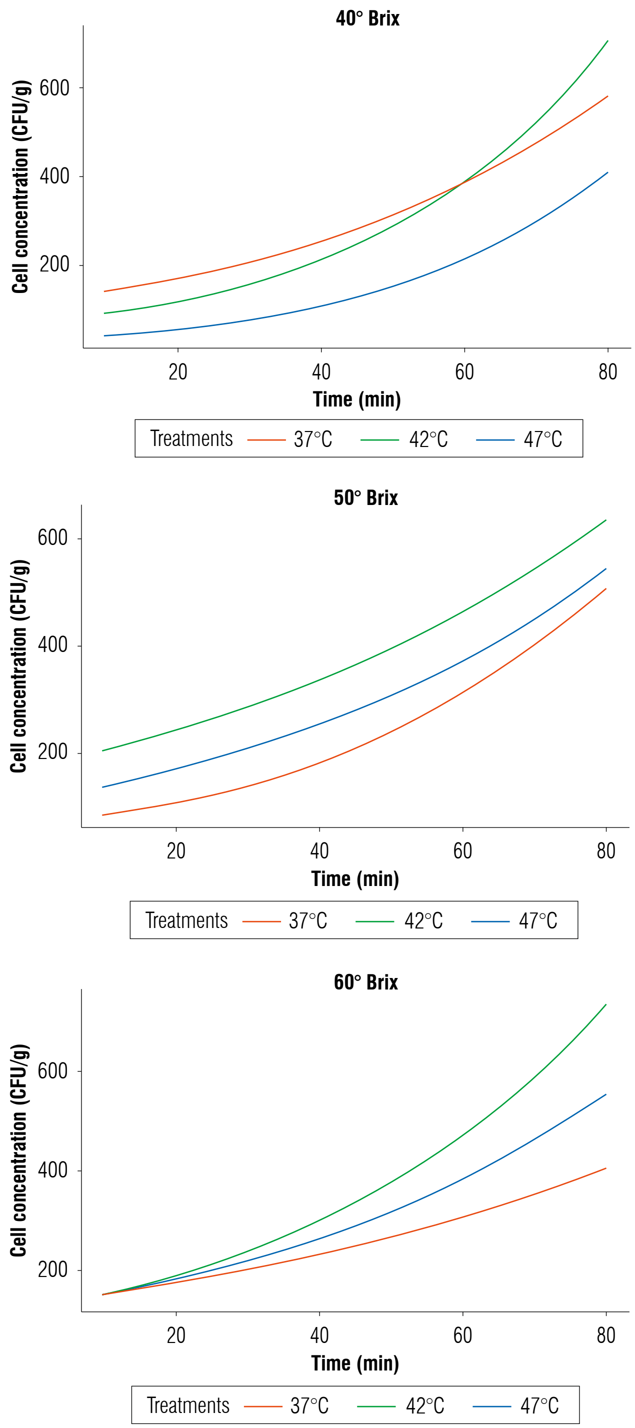

FIGURE 3. Microbiological kinetics of *Saccharomyces boulardii* in apple cubes at different concentrations of solids and temperatures.

Specifically, 50°B37°C had a doubling time of 7.50 min, whereas 60°B37°C had a doubling time of 8.69 min (Tab. 3). These findings underscored the significant influence of temperature and solute concentration conditions on the survival and growth of *S. boulardii* during osmotic dehydration. Treatments conducted at lower temperatures, such as 50°B37°C, facilitated greater bacterial growth and more abundant biomass compared to treatments conducted at higher temperatures, such as 60°B47°C. These results emphasized the critical importance of judiciously selecting osmotic dehydration conditions to achieve desired outcomes in terms of microbial survival and growth.

TABLE 3. Viability of *S. boulardii* in apple cubes inoculated during osmotic dehydration at different concentrations and temperatures.

Treatments	Number of generations (CFU/g)			Doubling time (min)		
	$\bar{x}$	±	σ	$\bar{x}$	±	σ
40°B37°C	4.38	±	0.58	16.12	±	2.14
40°B42°C	2.40	±	0.01	29.14	±	0.17
40°B47°C	1.72	±	0.13	40.70	±	3.12
50°B37°C	9.32	±	0.11	7.50	±	0.09
50°B42°C	1.91	±	0.01	36.62	±	0.26
50°B47°C	2.15	±	0.38	33.05	±	5.86
60°B37°C	8.08	±	0.07	8.69	±	0.07
60°B42°C	2.33	±	0.34	30.31	±	4.49
60°B47°C	1.75	±	0.72	39.89	±	1.72

Note: All means are expressed as mean ± σ (n = 3). In each treatment, B corresponds to degrees Brix in the medium followed by the treatment temperature.

In the analysis of the response surface graph for the number of generations, increasing the total solids concentrations (°Brix) had a noticeable impact on biomass concentration. This impact varied with temperature. Initially, as we increased the solids concentration, the biomass concentration also increased, and this relationship held true up to a certain point. However, beyond that point, there was a decline in biomass concentration. This trend is clearly illustrated in the cont our graph, where the green region represents the range of conditions that result in the highest biomass concentrations.

Likewise, when examining the response surface for doubling time, at higher concentrations of solids and temperatures, the rate of cell doubling increased. The contour graph further corroborated this observation. It highlighted the blue area that signified those lower solid concentrations (specifically 40 and 45°Brix) lead to the shortest doubling times within the temperature range of 40°C to 47°C. This means that under certain conditions, cells doubled more rapidly. This could be crucial information for optimizing the process.

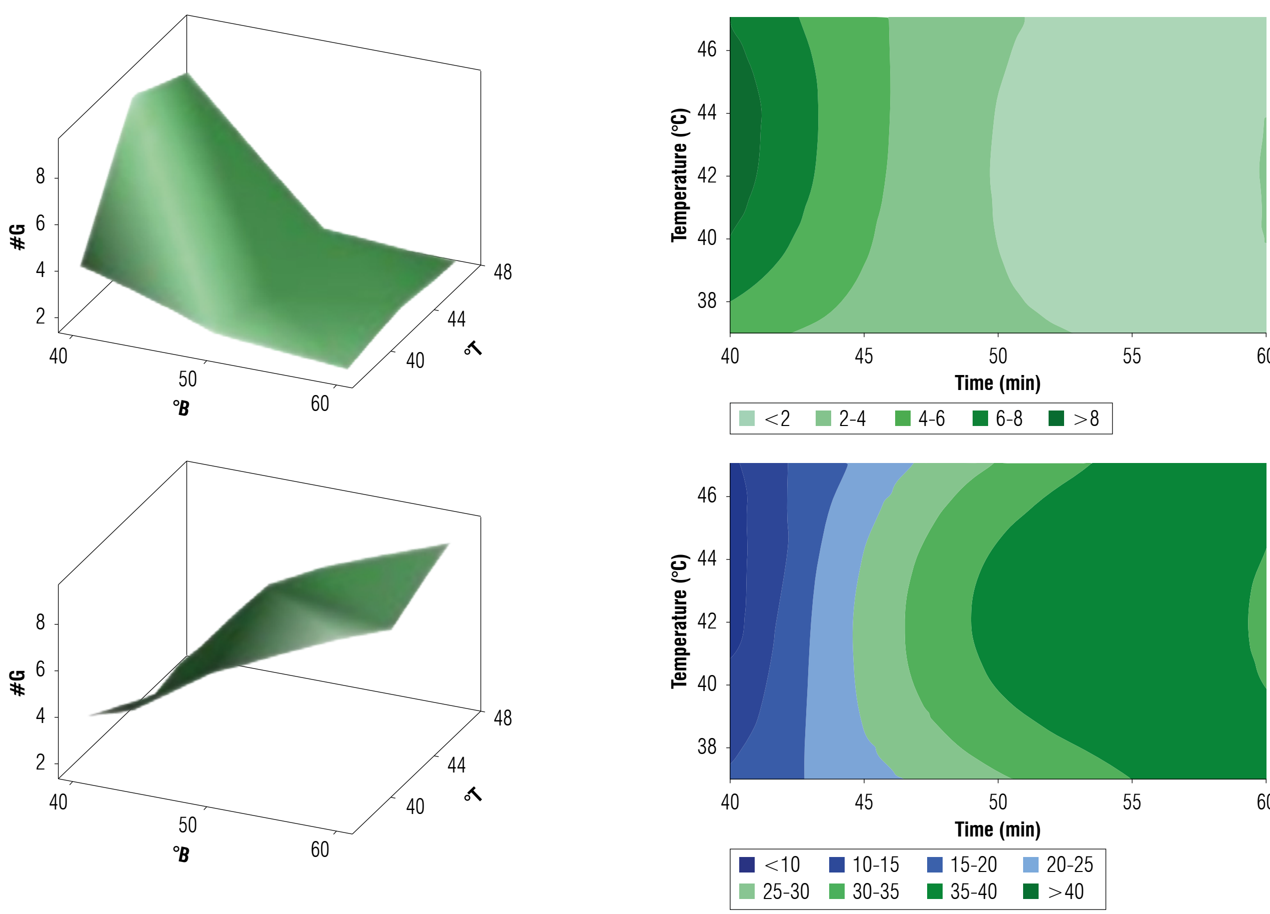

FIGURE 4. Response surface and contour plots for survival of *S. boulardii* in osmodehydrated apple cubes. #G is the number of generations expressed in CFU/g, °B is °Brix, T is the temperature in C° and TD is the doubling time in minutes.

Discussion

Cui *et al.* (2018) evaluate weight loss during osmotic dehydration of pears in solutions of sucrose and sodium chloride at different concentrations. Their results show that weight loss increases with the concentration of the solution and with dehydration time, agreeing with the data shown in Figure 1. This also agrees with the findings of Ayala-Aponte *et al.* (2010) in mangoes with sucrose solutions, where weight loss increased with the concentration of the solution and with dehydration time.

Parra-Palacios (2020) mentions that the increase in weight loss in pineapple slices shows that when the concentration and temperature increase there is a 19.50% weight loss.

Giraldo *et al.* (2005) point out that the weight loss in mango at different concentrations is due to the amount of water transferred from the mango to the osmotic medium that is greater than the quantity of soluble solids that migrate from the hypertonic medium. Likewise, Della Rocca and Mascheroni (2011) argue that temperature is one of the most significant variables since it will modify the kinetics in the osmotic dehydration process, hence weight loss. Weight loss is more affected than a gain in soluble solids; because of the use of high temperatures, the syrup solute cannot easily diffuse.

In Figure 2, the results of a gain of solids with respect to time are shown. It is possible to observe that weight loss increases as the treatment time increases. This is probably due to the incorporation of soluble solids in the fruit; and, also, the rate of solid gains increased as the concentration of the osmotic solution and the temperature increased. This agrees with what is found by Ochoa and Ayala (2009) who state that in slices of yacón an increase in solids is proportional to the concentration and temperature. Huerta-Vera (2021) reveals that at higher concentrations of the osmotic solution, the solid gain increased during osmotic dehydration in chayote slices, while an increase in the temperature of the osmotic medium also causes an increase in an increase in solids of chayote. Likewise, the results Arias *et al.* (2017) indicate that as the temperature of the osmotic solution increases, there is a greater alteration of the cellular structure, which favors the entry of solutes into the interior of the food. To limit the impregnation, it is convenient to use high solute concentrations and short osmotic dehydration times (Della Rocca & Mascheroni, 2011).

The survival of *S. boulardii* under osmotic dehydration conditions in the different treatments showed that under adverse conditions these could be inhibited, generating an increase in the biomass concentration (Tab. 3, Fig. 4). This is corroborated by Rascón *et al.* (2018) who affirm that the addition of *Lactobacillus rhamnosus* to banana slices causes an inhibition after 300 min, achieving a concentration of 9.40 ± 0.23 $\log_{10}$ CFU/ml. Likewise, Rodrigues *et al.* (2018) agree that a prolonged time for *Lactobacillus casei* in apples dried at 60°C generates a decrease in biomass, which shows that the treatments carried out at low temperatures (37°C and 42°C) do not exceed the adverse conditions for the normal development of the biomass.

Conclusions

In this study, microbiological growth kinetics were assessed under osmotic dehydration conditions. We observed that treatments 50°B47°C and 50°B37°C exhibited the highest weight loss and displayed an excellent fit in the regression analysis, with R^2 values of 0.983 and 0.980. Furthermore, they stood out for their low typical errors (0.912).

Regarding microbiological kinetics, 42°C represented the optimal temperature for the growth of *S. boulardii* across all evaluated concentrations. Additionally, sugar concentrations significantly influenced the kinetics, resulting in reduced growth at 60°Brix.

Survival of *S. boulardii* during osmotic dehydration varied among treatments, with 50°B37°C and 60°B37°C demonstrating a higher number of generations and shorter doubling times. These findings underscored the critical influence of temperature and solute concentrations on microbial survival and growth during osmotic dehydration.

In summary, this study furnished valuable insights into the optimal conditions for the growth and survival of *S. boulardii* during osmotic dehydration. These discoveries hold significant implications for the production and application of *S. boulardii* in apple cube processing and pave the way for further research into environmental variables affecting microbial kinetics.

Conflict of interest statement

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

Author's contributions

MYCC: writing of original draft, research, validation. ADCR: conceptualization, methodology, formal analysis, research, writing of original draft. All authors reviewed the final version of the manuscript.

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SCIENTIFIC NOTE

Development of a web application for the resource management in the course "Cycle 2: Execution of production project" at the Universidad Nacional de Colombia, Bogotá

Desarrollo de una aplicación web para la administración de recursos en la asignatura "Ciclo 2: Ejecución de un proyecto productivo" en la Universidad Nacional de Colombia, Bogotá

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ABSTRACT

The development of online tools has provided solutions to different activities, including academia. The Agronomy program at the Universidad Nacional de Colombia has updated its competency-based teaching approaches by encouraging the development and application of computational tools for analysis, modeling, and interpretation of data. A registration and follow-up system was chosen to automate tasks in the course "Cycle 2: Execution of production project" developed at the university farm (Marengo Agricultural Center), where technical, financial, and administrative variables inherent to the selected crop must be managed with monitoring and support by teachers and monitors. The aim of this research was to develop a web application for the academic-administrative management of certain components. This was mainly developed with Python and a PostgreSQL database. As an open resource, the app was hosted on GitHub and, for user access, it was hosted on PythonAnywhere. The Alpha version was validated by several students and academic staff involved in the course. The development of a web application consisting of three administrative modules was achieved and included an inventory management of supplies, machinery, and available tools; the allocation and management of the schedule of activities and list of requests, and the tracking of resource use through project costs. The web application is simple to execute, and its use will adjust the relevant processes of the productive projects of Cycle 2.

Key words: resource management, productivity enhancement, process technification, monitoring and evaluation.

RESUMEN

El desarrollo de herramientas en línea ha brindado soluciones a diferentes actividades, incluida la academia. La carrera de Agronomía de la Universidad Nacional de Colombia ha actualizado sus enfoques de enseñanza basados en competencias fomentando el desarrollo y la aplicación de herramientas computacionales para análisis, modelado e interpretación de datos. Se optó por un sistema de registro y seguimiento para la automatización de tareas en el curso "Ciclo 2: Ejecución de proyecto productivo" desarrollado en la granja universitaria (Centro Agropecuario Marengo), donde se abordaron variables técnicas, financieras y administrativas inherentes al cultivo seleccionado con el seguimiento y apoyo por parte de docentes y monitores. El objetivo fue desarrollar una aplicación web para la gestión académico-administrativa de determinados componentes. Esta fue desarrollada principalmente con Python y una base de datos PostgreSQL. Como recurso abierto, la aplicación se alojó en GitHub y, para el acceso de los usuarios, se alojó en PythonAnywhere. La versión Alpha fue validada por varios estudiantes y personal académico involucrado en el curso. Se logró el desarrollo de una aplicación web que consta de tres módulos administrativos e incluyó un manejo de inventario de insumos, maquinaria y herramientas disponibles; la consignación y gestión del cronograma de actividades y lista de solicitudes, y el seguimiento del uso de recursos a través de los costos del proyecto. La aplicación web es sencilla de ejecutar y su uso ajustará los procesos pertinentes de los proyectos productivos de Ciclo 2.

Palabras clave: manejo de recursos, mejora de la productividad, tecnificación de procesos, seguimiento y evaluación.

Introduction

Only a few decades ago an environment of exchange of information as spectacular as the internet could not be contemplated. It was not possible to imagine anything that involved a set of communication networks, including its use in academia (Gómez *et al*., 2016). In 1995, Netscape introduced a client scripting language called JavaScript, and the history of web applications can be traced from that point on, though the term web application appeared later

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in 1999. Applications have had impressive advances from static pages to dynamic options, offering user personalized experiences (Odlyzko, 2012).

Web applications are used for a variety of purposes: medical appointment systems, instant messaging, domestic or international trade, entertainment, climate monitoring, human disease monitoring, animals and plants, business management, and others, for both academic and teaching purposes (Albokai *et al*., 2019). Almost every school has its own site and various administrative and academic tasks have been developed so that users can perform different tasks from their own mobile phones. Even the courses offered by the institutions from these sites are updated with some frequency, allowing, in many cases, the possibility to monitor a simple consultation from the site or a deeper navigation, document downloading, chat-box, and other consulting devices, and course management (Espinoza, 2017).

For the case of the Universidad Nacional de Colombia, particularly in the field of Agronomy, the course programs are updated frequently, adapting them to new approaches for generic and specific competencies involving aspects of communication, teamwork, autonomous learning, critical thinking and other generic competences, as well as involving students in the course skills, development and application of computational analytic tools, and modeling and interpretation of data from any source (Meriño *et al*., 2016).

Strategies for the development of a productive project in the course "Cycle 2: Execution of production project" (Cycle 2) at the Marengo Agricultural Center (Mosquera, department of Cundinamarca) of the undergraduate program in Agronomic engineering at the Faculty of Agricultural Sciences involve the formation of student teams for the execution of each project. This gives continuity to the previous work carried out in the course "Cycle 1" (Cycle 1), where the productive project is initially formulated. At the end of course Cycle 1, the students must submit a proposal for a farm project (usually vegetable crops of semi-annual growth cycle). Some of the most relevant parameters within this proposal that must be addressed during the execution of the project include technical-administrative parameters or specifications, such as the lot for project execution within the Marengo farm, required area, schedule of activities, required inputs and implements, general costs and project costs, etc. When beginning each project, student teams must have a constant dialogue with the academic staff and instructors in charge to coordinate each of the activities, purchase the inputs and other required tools; these are generalized activities the students are responsible for; they are considered crucial and are addressed in the present project. The responsible academic staff and monitors require knowledge of these project-specific schedules and budgets, in addition to the inventory of inputs, machinery, tools, and wages available for the project execution.

This first stage (course Cycle 1), completed and evaluated by the academic instructors, paves the way for the next step, corresponding to Cycle 2. In Cycle 2, all this information, semester by semester, involves different crops and inputs and a series of entry or exit resources that change both in space and time at Marengo farm. This information is usually carried out in a dispersed manner; the objective of the current study consisted in the development of a web application that allows a more coordinated resource management for some of the fundamental activities required for the implementation of the Cycle 2 projects.

Materials and methods

Software

For the development of the web application, we decided to use the Python programming language (version 3.9.6) because of the simplicity of its syntax, availability of modules designed for specific tasks such as the creation of the interface, and the connections with databases, development community, etc. Among the modules available in Python, we worked with the Flask framework (version 2.2.2) for the BackEnd that is supported by web development languages such as HTML, CSS, and JavaScript for FrontEnd development. In addition, we used other modules for specific tasks, such as psycopg2 for connection to a relational database PostgreSQL (version 14), werkzeug for the encryption of passwords of each user, and Pandas for handling tables of data that enter from the different forms into the application.

Access and hosting

To achieve open-source development available free to the public, one of the objectives of the current web application, we chose to host all the source code in GitHub, a free repository widely known by the developer community. This repository is based on a version control system that allows not only the distribution of the source code but also the management of the changes that are made to each of the elements. In order to make the application available to end-users (involved in Cycle 2), a platform was sought to provide free hosting service for both the application and the database that would imply, in most platforms, a limitation in the hardware and time of use of these services. The PythonAnywhere platform developed by Anaconda provides a free virtual machine that supports the hosting of web applications developed using Python frameworks like Flask and Django. This also makes it easier to deploy compared to others. This virtual machine has the following technical specifications: it is supported in the operating system Ubuntu 20.04.4 LTS, allowing 512 MB storage for application files and their databases, supports Python 3.x, and allows 100 s d^{-1} usage. Upon completion of a certain time period, the application still grants an access to the application, only decreasing the response speed when only one domain is accessed.

Feedback

The adjustment of the web tool according to the needs of the course Cycle 2 was based on an Alpha version designed with the information provided by several student teams developing their productive projects in the second half of 2022. This information allowed the collection of important data for use, safety, functionality, and aspects inherent to each crop such as responsible academic staff, agricultural inputs, and other administrative aspects related to the subject.

Results and discussion

In software engineering, the life cycle of the software release is the set of the progress states of the creation of different computer applications (Khomh *et al.*, 2015); this identifies progress in the development of an application. Each version of an application or software, in general, usually goes through a phase in which new features are added (alpha stage), followed by another phase where errors are actively eliminated (beta stage). The final phase is where all relevant errors have been debugged (stable stage). Other software developers use different names for the intermediate phases; this depends on the magnitude of the project. Tests made by experts on the application and on the future or current users allow development to be considered as in beta phase. The formal application in the course during the following semesters (at least two semesters) will allow the discovery of errors and permit pertinent modifications in order to reach a stage considered to be stable to fulfill the objectives.

If the process is managed well, it is possible to develop a web tool that is attractive and has a user-friendly interface for any web browser. This will provide important capabilities in the management of all records associated with the above-mentioned characteristics of Cycle 1 and Cycle 2 during each semester and will simplify decision-making by the Cycle 2 course managers.

As the first module, the login component (Fig. 1) was developed mainly to access and to control permissions for each of the features that the application has from the assignment of roles for each user. This is due to the differentiation between the activities carried out by each of the different actors, whether monitors or students.

FIGURE 1. Screenshot of the web application login module (in Spanish). http://caariveram.pythonanywhere.com

The development of a warehouse module (Fig. 2) shows a list of products, inputs, and tools available for the execution of the student projects within the course. This allows the selection of inputs and their respective quantities in order to make the requests required for each of the activities programmed in each project. It also allows the user (depending on the role of the user) to register or delete the products and also to record purchases and to update the price and quantity available. In the case of the student teams, the application functionality includes new products that are not available in stock as well as damage reports related to machinery or tools that may be out of order. This allows for other student teams to be notified of this current situation, so that they can readjust their requests.

This module has a great advantage over what has traditionally been done; previously, the students and monitors faced uncertainty about the availability of inputs or equipment in the production unit as well as the respective prices. This strongly affected planning, start times, and cost estimations during the project execution. With this development of the updated inventory list, real-time inventory tracking helps to improve management and ensures optimal stock availability to meet orders. It also reduces costs, saves time, and improves the planning of each production project; this is translated into substantial improvement in customer service that, in this case, are the students and other production unit workers (Naliaka & Namusonge, 2015; Demizu *et al*., 2023).

Records that are properly kept facilitate calculations on the inventory management, such as semi-annual average consumption which, along with information on delivery date, allows the user to establish the reorder point. This can also facilitate the construction of other indicators, such as the calculation of the maximum inventory, physical space required, stowage capacity, etc. (Arvidsson, 2021).

Tracking prices and other indicators required for the management of the production unit, previously unavailable, can help answer the following questions: How much input is needed for the high and the low levels of the crop production? What crops should be cultivated in the same area in later semesters? What kind of agricultural products should be used? What equipment or machinery is available? How much time should be available to the student teams to execute the project? These questions can all be answered at the time of the soil preparation for the project execution or before the crop establishment in the field.

The following modules were developed to manage the planning, review, and developmental activities for each agricultural project. The first module was implemented as a priority to schedule (Fig. 3A) different activities (sowing, fertilization, phytosanitary management, etc.). Each of these activities must contain mainly the scheduled date of execution, time of duration and the estimated cost, in addition to a description of the agricultural products and their quantities required for the project execution. After this, the next developed module allows the respective requests (Fig. 3B) for inputs, equipment, and machinery to be made at the time of the execution of the activities, so that the quantities of products available could be selected from the warehouse. All requests are listed for review and response (rejection or approval) by the academic staff or monitors. Finally, the third module was developed in order to allow the supervisor (monitor), after approval, delivery of inputs or tools and performance of the activity, to conclude the activity with an approval date and, thus, record the final costs within the project costs (Fig. 3C).

The development of this group of modules focuses on the planning and control that every agricultural productive project requires during its execution. This is attained by making a schedule of each project, such as the one that allows a wide view of the distinct phases of the various crops cultivated. This allows better management of the inventory of inputs and equipment and thus better estimation of the number of workers to be contracted and for how long as well as better management of ecological or environmental indicators such as environmental impact indices or an environmental pressure index (Perevochtchikova, 2013). Although the projects implemented each semester appear to be independent since they are prepared by distinct groups

FIGURE 2. Screenshot of warehouse module of the web application, with example of agricultural products, their quantities and price per unit available in Marengo farm for the course Cycle 2 (in Spanish). $ corresponds to Colombian peso.

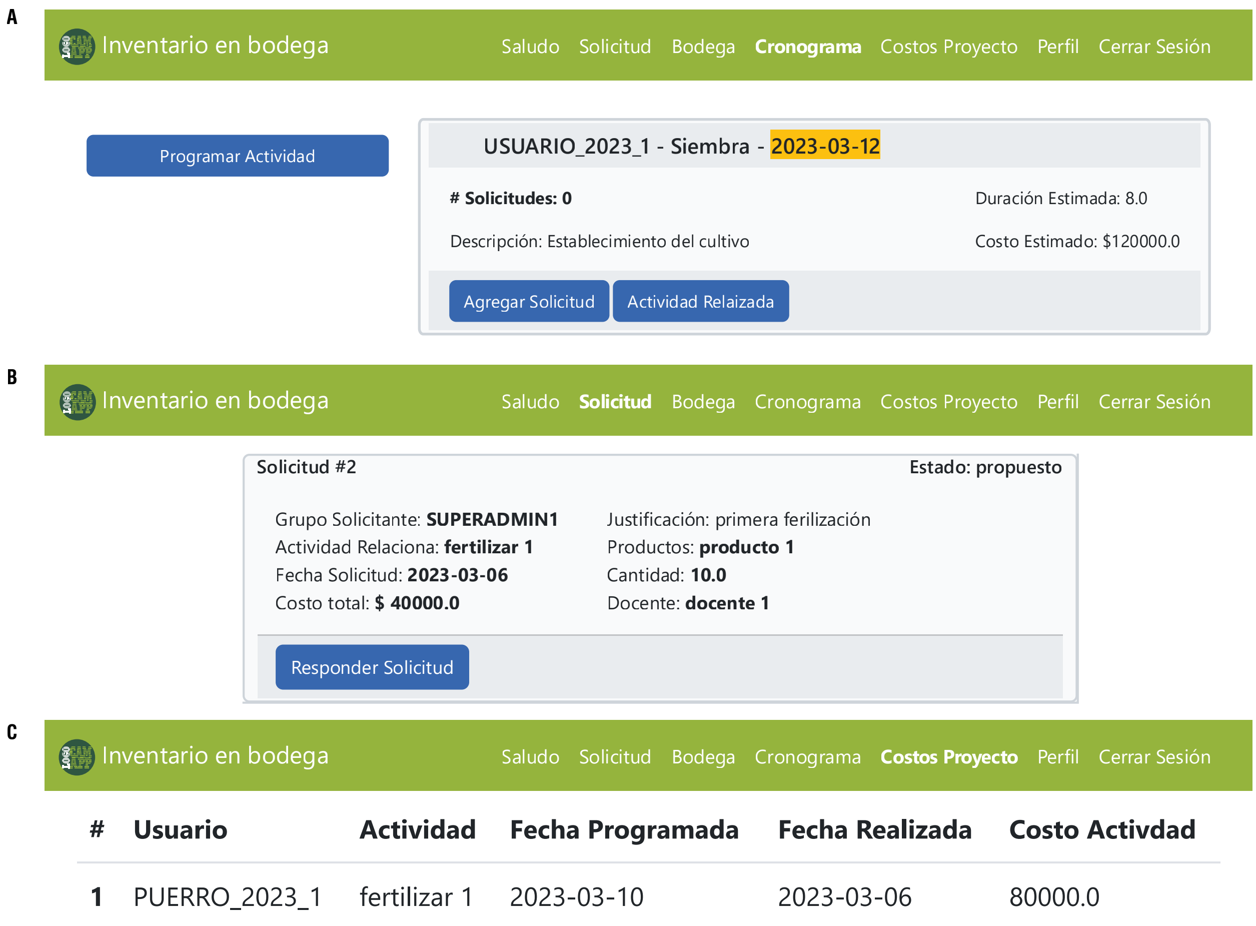

FIGURE 3. Screenshot of web application administration modules. A) List of activities schedule, B) Review of requests for the implementation of each activity, C) Project costs of activities for each Cycle 2 production project (in Spanish). $ corresponds to Colombian peso.

of students, many of these projects are done for the same production systems. Because they are implemented in the same soil lots, it is not enough to have a semi-annual crop observation window; the management information on the previously cultivated crop must be integrated. This information includes data on the soil mechanization and agricultural products used for pests, diseases, and fertility in the previous crops cultivated in the same soil. In this sense, the use of the application expands said observation window to the management that has been carried out, in accordance with the information provided by the database of continuous semesters focused on the generation of management history. This way, the project execution will succeed in overcoming adversities or problems and will mitigate the environmental impact caused by over-mechanization of the soil or the repetitive use of agricultural products in short periods of time (Santiteerakul *et al.*, 2020; Pérez-Pons *et al.*, 2021).

Finally, Figure 4 summarizes the sequence of activities each user must follow, according to their role, to access the application modules: a chief administrator is focused on user and database management, an administrator (academic staff and monitors) is focused on the supervision and management of the inventory and applications, and student teams manage the schedule and request inputs for the development of activities specific to each crop.

As an extrinsic benefit, this study sets a precedent aimed at how information and communication technologies can be applied to solve simple tasks. In this specific case, the open source allows for subsequent projects to be developed in various areas to solve specific situations. Being more precise, this tool allows for the development and incorporation of modules for collection of information on the sampling and monitoring of the phytosanitary crop status and meteorological monitoring for subsequent analysis of the crop life cycle, as well as allowing decision-making in management during project execution and the addition of modules focused on expanding technical and administrative aspects. In this way, the possibilities of this application are diverse, which is why this tool could also be integrated

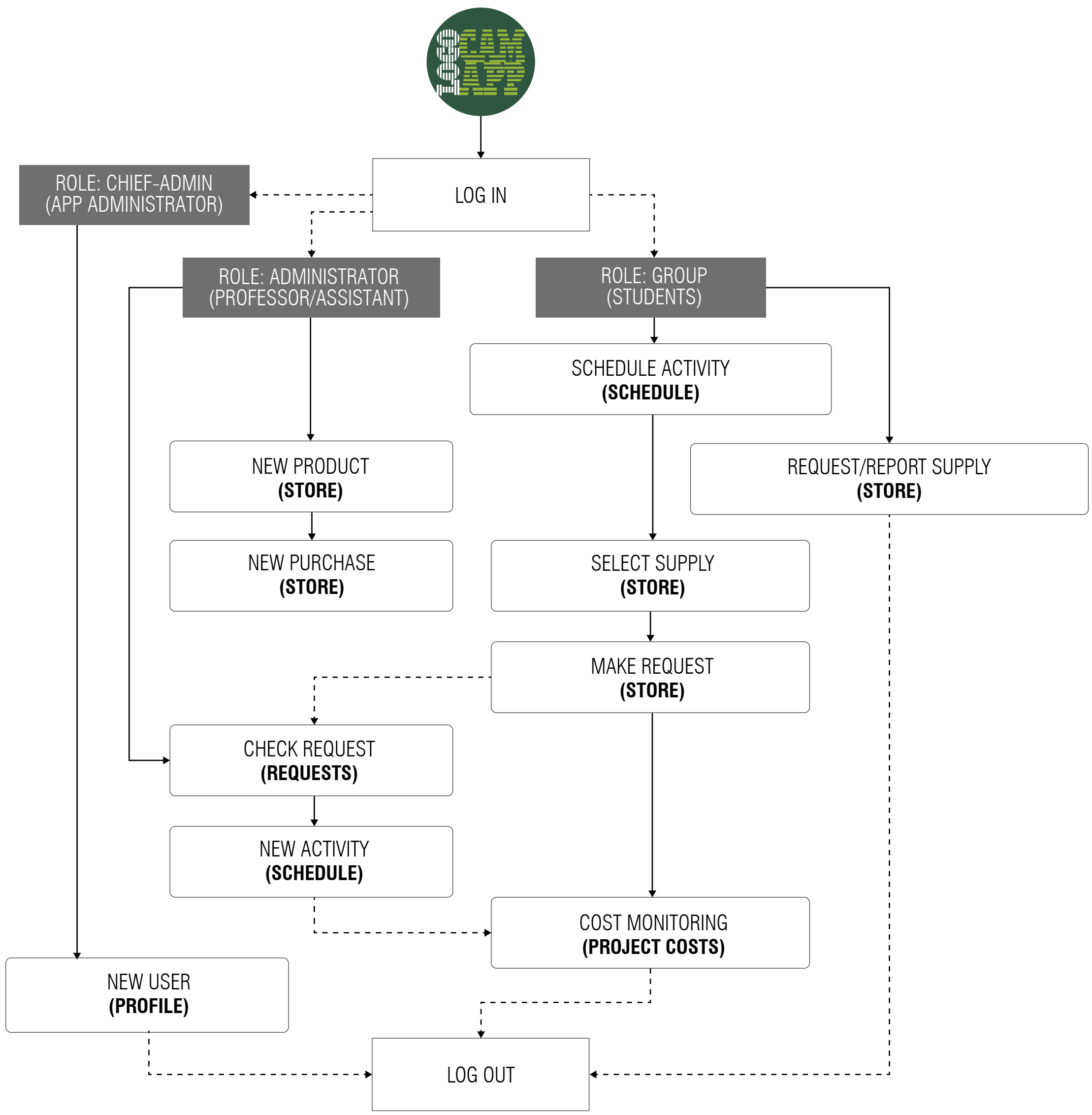

FIGURE 4. Flow diagram of the main route of activities to be carried out in the course Cycle 2.

into academic and pedagogical work, with other courses (for example, Cycle 1) as active users.

Conclusion

The development of the application made it possible to modernize some of the most relevant processes for developing the productive projects of Cycle 2, thus facilitating the professor-monitor-student dialogue. The collection and recording of information permitted the tracking of handling, price change over time, crops involved, equipment in use, or possible faults. This optimizes use of resources and helps reduce the environmental impact related to the use of agricultural inputs related to crop protection and production.

Resources

GitHub repository of project source code: https://github.com/CarlosRivera1212/CAM-APP.V2 Application deployment domain for user access: http://caariveram.pythonanywhere.com/

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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

CARM and AEDC: conceptualization, writing, and editing. CARM: software. AEDC: resources, supervision. All authors have read and approved the definitive version of the manuscript.

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SCIENTIFIC NOTE

Estimation of effective water vapour diffusion and mass transfer during quinoa (*Chenopodium quinoa* Willd.) drying

Estimación de la difusión efectiva de vapor de agua y de la transferencia de masa durante el secado de quinoa (*Chenopodium quinoa* Willd.)

Julia Luisetti[1], María Florencia Balzarini[1], and María Cristina Ciappini[1]

ABSTRACT

This study shows the modeling of the convective drying operation of quinoa (*Chenopodium quinoa* Willd. var. Hualhuas) grains implemented in the General Algebraic Modeling System (GAMS) software. The proposed model was based on Fick's second law. The drying experiences were carried out using a pilot-scale oven. The drying air conditions were: 40, 60, and 80°C and 0.2 and 0.7 m s^{-1}. The mathematical modeling was employed to describe the behavior of the drying operation according to variations of the average moisture over time. The effective diffusivity of moisture and mass transfer were studied for the different operating conditions. The model was validated by experimental data. It was possible to model the quinoa grains drying process, obtaining a high precision between the experimental and estimated values. Quinoa drying curves can be represented properly by the studied model. In the operating ranges tested, the effective diffusivity values of moisture were between 2.52 10^{-10} and 1 10^{-9} m^2 s^{-1} and the mass transfer values were between 7.20 and 11.47 cm s^{-1}. The effective diffusivity (D_{eff}) showed significant differences (P<0.05) with the speed of the drying air, while the mass transfer coefficient (k) was significantly affected (P<0.05) by the temperature of the drying air.

Key words: grain, drying, effective diffusion, GAMS.

RESUMEN

Este estudio presenta la modelización de la operación de secado convectivo de granos de quinoa (*Chenopodium quinoa* Willd. var. Hualhuas) implementado en el software GAMS (General Algebraic Modeling System). El desarrollo del mismo se basó en la ley de difusión de Fick. Las experiencias de secado se realizaron utilizando una estufa a escala piloto. Las condiciones del aire de secado fueron: 40, 60 y 80°C y 0.2 y 0.7 m s^{-1}. El modelado matemático se empleó para describir el comportamiento de la operación de secado en función de las variaciones de la humedad promedio con el tiempo. Se estudió la difusividad efectiva y la transferencia de masa para las diferentes condiciones operativas. El modelo propuesto se validó con datos experimentales. Se obtuvo un ajuste adecuado entre los valores experimentales y los estimados, lo cual demuestra que el modelo propuesto se puede aplicar a la descripción precisa de las curvas de secado experimentales para granos de quinoa. En los rangos operativos ensayados, se obtuvieron valores de la difusividad efectiva de la humedad comprendidos entre 2.52 10^{-10} y 1 10^{-9} m^2 s^{-1} y del coeficiente de transferencia de masa entre 7.20 y 11.47 cm s^{-1}. La difusividad efectiva (D_{eff}) presentó diferencias significativas (P<0.05) con la velocidad del aire de secado, mientras que la transferencia de masa (k) fue afectada significativamente (P<0.05) con la temperatura del aire de secado.

Palabras clave: grano, secado, difusividad efectiva, GAMS.

Introduction

Quinoa (*Chenopodium quinoa* Willd.) is an Andean seed widely cultivated by pre-Columbian communities. Its marginalization and replacement began with the conquest of America and the introduction of cereals such as barley and wheat (FAO, 2011).

One of the critical points in the cultivation of quinoa is the need to preserve the seeds to ensure that they are available over a long period of time. The start of the harvest is delayed until the seed reaches 14% moisture w.b. (wet basis), which is the marketing base and the seed moisture limit for temporary storage. The delay in harvesting increases the probability that the crop will be affected by rainfall and pests. The early seed harvest is used when its moisture is around 30% w.b. The alternatives for this practice are chemical desiccants or convective drying (Cuniberti, 2015).

In his study of convective drying, Garnero (2006) employed air temperatures between 40°C and 12°C for periods of 0.5 to 4 h. For wheat, the maximum air-drying temperature recommended is 90°C. The seed temperature must not exceed 50°C or 60°C to maintain an acceptable quality

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for seed use in industry. The optimum drying temperature should be lesser than 80°C because lysine content is destroyed by higher temperatures. As a general rule, in most countries, a maximum drying air temperature of 90°C has been adopted (Garnero, 2006). During quinoa drying, the thermal process modifies the digestibility of the seed protein and starches. This chemical alteration depends on various parameters: the variety and the processing conditions, temperature, pH, and the presence of oxygen (García Pacheco *et al*., 2019; Neji *et al*., 2022).

Different researchers (Wahengbam *et al*., 2019; Sozzi *et al*., 2021) designed mathematical models to study the moisture transfer process in the food structure using the classical theory of diffusion. These studies have generally been carried out in seeds whose geometry can be assumed to be spherical or cylindrical. The study of food matrices drying kinetics is important for the design and control of different drying process (Zhou *et al*., 2017; Chua *et al*., 2018; Moscon *et al*., 2020; Haripriya *et al*., 2021). The mathematical models that were validated with experimental data are appropriate alternatives to describe the operating conditions to design a dryer or to convert a batch process into a continuous process.

The present work was oriented to study the Hualhuas variety of quinoa, using a more industrial vision than the published antecedents. Estimated coefficients were the effective diffusivity (D_{eff}) and the mass transfer (k). The parameters obtained by the model are suitable to designing industrial equipment or to convert a batch process into a continuous process.

Materials and methods

Raw material and sample preparation

Five kg of quinoa seeds (*Chenopodium quinoa* Willd. var. Hualhuas), suitable for consumption, was purchased at the Zucchi Distributor (Rosario, Santa Fe, Argentina). The quinoa employed in this study was native to Trujillo, Peru (8°6'57.56" S, 79°1'47.93" W).

Drying experiments

The temperatures were selected based on the other studies. Vilche *et al*. (2003) tested the temperatures 30°C, 50°C, 70°C, and 90°C. Ramos Gómez and Peña Rivera (2019) dried quinoa grains at 40°C, 50°C, and 60°C. Paquita Ninaraqui (2015) studied the Salcedo Inia variety of quinoa and used temperatures of 50°C, 60°C, and 70°C. Carciochi (2014) used temperatures of 100°C, 145°C, and 190°C. As a generally accepted standard in most countries, a maximum drying air temperature of 90°C has been established (Garnero, 2006). Considering the aforementioned, temperatures of 40°C, 60°C, and 80°C were selected for this study.

During the drying, the speed of the air must not be so high as to cause the entrainment of solids. Typical drying air velocities in industrial dryers range from 0.7 to 3.0 m s^{-1} (Perry, 1992). Vilche *et al.* (2003) used a drying air speed of 0.3 m s^{-1}. The laboratory dryer selected for this research operated at two speeds: 0.2 and 0.7 m s^{-1}.

The drying treatments were carried out using a pilot-scale oven (Tecno Dalvo, Model CHC/F/I, Argentina). For each experiment, 0.200±0.001 kg of quinoa grains were weighed. The grains were placed in a stainless-steel mesh to allow the circulation of the air. The drying experiments were carried out in triplicate.

The sample moisture content was determined gravimetrically according to the AOAC 945.15 technique (AOAC, 1990); 0.002±0.001 kg of grains were weighed in aluminum capsules previously tared, then dried in an oven (Sific, Argentina) for 3 h at 103±2°C. The sample was cooled to room temperature in a desiccator before being weighed.

Flour milling and sieving

The seeds were processed for 15 s in a blade mill (IKA, Germany). The flour was kept in plastic bags until use.

Flours grading was determined by a Ro Tap sifter (Tyler, USA) equipped with 16 (1000 µm), 25 (679 µm), 50 (289 µm), 100 (149 µm), 200 (74 µm), and 270 (53 mm) U.S meshes. 0.1 kg samples were sieved and shaken for 5 min. Finally, the fractions of flour retained in each sieve were weighed and the retention percentage was calculated. The final mean value size was obtained in triplicate.

Mathematical modeling of the drying operation

Quinoa seed sphericity

Sphericity is the degree of approximation of a seed to a sphere and in any seed it is a function of its physical dimensions (length, width, and thickness). The quinoa seed sphericity was calculated using the following expressions (Gallegos Ramos *et al.*, 2022):

$$\phi = \frac{D_g}{L} \quad (1)$$

$$D_g = (L \cdot A \cdot e)^{\frac{1}{3}} \quad (2)$$

where:

ϕ is the dimensionless sphericity;

D_g is the geometric mean seed diameter in mm;

L is the seed length in mm;

A is the seed width in mm;

e is the seed thickness in mm.

Measurements of the seed length, width and thickness were made in triplicate with a caliper (precision 0.05 mm) as shown in Figure 1. For the determinations, a sample of thirty grains was taken at random.

Considerations for the drying model application in GAMS

Because the constant drying period in most foods is very short and the critical humidity is practically equal to the initial moisture, only the period of decreasing rate was considered.

A mathematical model based on the law of conservation of mass was proposed to describe the moisture profile of the samples during the drying process. The following hypotheses were considered:

- Water diffuses to the surface of each particle, according to Fick's second law;
- The diffusion phenomenon is the predominant mass transfer process;
- Water evaporation takes place only at the surface level;
- The moisture on the surface is in equilibrium with the drying air;
- The air temperature remains constant. There is enough airflow to evaporate the internal moisture content through the mesh used;
- Moisture diffusivity depends on moisture content and temperature;
- Drying takes place over the entire grains surface, since a stainless steel mesh sheet is used;
- The air flow is large enough to keep constant drying conditions (humidity, temperature) throughout the material. Grains reaches drying air temperature;
- Heat generation inside the material and radiation effects are negligible;
- The air is perfectly mixed inside the stove and the air flow characteristics are invariable;
- Due to the small size of the seed, its density is considered constant during the drying process;
- The seeds are considered as spheres with a diameter (D) of 1.99 mm.

Mass transfer model

The mathematical expressions used were written in spherical coordinates, which were fixed in the geometric center of the grain.

The radial diffusion model of moisture transfer was used to study the time evolution of the radial distribution of the local moisture content during drying.

FIGURE 1. Length, width, and thickness measurements of the quinoa grain.

Fick's second law was applied to describe the diffusion of moisture within quinoa grain with radio R, as in Equation 3.

$$\rho s \cdot \frac{\partial HS(r,t)}{\partial t} = D_{eff}(t) \cdot \rho s \cdot \left(\frac{\partial^2 HS(r,t)}{\partial r^2} + \frac{2}{r}\frac{\partial HS(r,t)}{\partial r}\right) \quad (3)$$

$t > 0, \quad 0 < r < R_s$

where HS is the grain moisture content; ρs is the grain density; D_{eff} is the effective diffusion of the moisture content; t is the drying time, and R_s is the radius of the solid grain.

The initial and boundary conditions that were used to solve the equation are:

a) Initial condition

At the start of the drying operation the moisture content of the grain was uniform.

$$HS(r, 0) = HSo \quad (4)$$

$t = 0, \quad 0 < r < R_s$

b) Symmetry condition

c) Due to symmetry, there was no moisture gradient. The boundary conditions at the center were:

$$-D_{eff}(t) \cdot \rho s \cdot \frac{\partial HS(0,t)}{\partial r} = 0 \quad (5)$$

$t > 0, \quad r = 0$

d) Boundary condition at the interface for convective drying

$$-D_{eff}(t) \cdot \rho s \cdot \frac{\partial HS(0,t)}{\partial r} = 0 \quad (6)$$

$t > 0, \quad r = R_s$

A convective mass transfer phenomenon was considered from the surface of the grain to the bulk air (Eq. 6). HS_{eq} and k are the equilibrium moisture content of the dry solid and the external mass transfer coefficient, respectively.

The mass transfer coefficient (*k*) was calculated using the Sherwood number (S_h). It was determined by Mills and Coimbra (2015) with Equations 7-12:

$$S_h(t) = \frac{D \cdot k}{D_w} = 2 + 0.6 \cdot Re^{0.5} \cdot Sc^{0.33} \quad (7)$$

$$Re = \frac{D \cdot G_a}{\mu_a} \quad (8)$$

$$Sc = \frac{\mu_a}{\rho_a \cdot D_w} \quad (9)$$

$$\mu_a = \frac{0.000001 \cdot 1.4592 \cdot (T+273)^{1.5}}{109.10 + (T+273)} \quad (10)$$

$$\rho_a = \frac{Ma \cdot Pa}{R \cdot (T+273)} \quad (11)$$

$$G_a = \frac{Ma \cdot \upsilon \cdot Pa}{R \cdot (T+273)} \quad (12)$$

where

D_w is water vapor diffusion in air ($cm^2\ s^{-1}$);

Re is Reynolds number;

Sc is Schmidt number;

G_a air mass flow rate ($g\ cm^{-2}\ min^{-1}$);

μ_a is air viscosity ($g\ cm^{-1}\ min^{-1}$);

Ma is air molecular weight ($g\ mol^{-1}$);

Pa is water vapor pressure in air (Pa);

υ is air velocity ($cm\ s^{-1}$).

An Arrhenius-type equation was employed to evaluate the effective diffusivity of the moisture:

$$D_{eff}(t) = A \cdot e^{\left(\frac{-B}{273.15+T}\right)} \cdot e^{(Cx<HS>(t))} \quad (13)$$

where D_{eff} is the effective diffusivity and the parameters A, B and C are predicted by the model.

The average moisture content <HS> at each instant was obtained by integrating the local moisture content over the volume (V). Specifically, the average moisture content was expressed as stated in Equation 14, which can be solved using the trapezoidal rule.

$$< HS > (t) = \frac{\int_0^V HS(r,t)\ dV}{\int_0^V dV}; \quad t \geq 0 \quad (14)$$

Problem solving strategies

The differential equations were discretized to become algebraic equations and then implemented in the GAMS software. Therefore, Equations 3 to 6 were discretized using the implicit central finite difference method (CFDM).

Spatial and temporal variations were defined by Equations 15 and 16 respectively, with M=9 and N=100. M and N were determined before and both guarantee the stability of the solution.

$$\Delta r = \frac{r}{M} \quad (15)$$

$$\Delta t = \frac{tf}{N} \quad (16)$$

The nonlinear programming model was executed in the software GAMS using the solver CONOPT (Singh & Heldman, 2008).

At the beginning, the model was used to calculated the parameters of the effective diffusion (Eq. 13). The objective of this step was to evaluate the model performance and the correlations used. Then, the obtained diffusion coefficient parameters values were fixed. An objective function (Fo) was implemented (Eq. 17), which was based on the minimization of the root mean square error (RMSE) of the experimental and predicted moisture content data.

$$\mathrm{Fo} = \mathrm{Min}\{\mathrm{ESMR}\} = \mathrm{Min}\left\{\frac{1}{\mathrm{N}}\left(\sum_{\mathrm{to}}^{\mathrm{tf}} < \mathrm{HS} >_{\exp}(\mathrm{t}) - < \mathrm{HS} > (\mathrm{t})\right)^{2}\right\} \quad (17)$$

where $<HS>_{exp}$ is the experimental average humidity, <HS> is the average humidity predicted by the model, and N is the number of experimental data.

To estimate the mass transfer coefficients, correlations reported in the literature were used in order to reduce the degrees of freedom of the model and to facilitate the resolution of the Non Linear Programming (NLP) models. The resulting model involved 4,052 variables and 3,547 constraints.

Statistical analysis of the data

Experimental results were obtained in triplicate and were presented as mean ± standard deviation (SD). The statistical analysis was carried out using the Minitab program (Pennsylvania, USA), performing analysis of variance, with comparison of treatment means using the Tukey test ($P<0.05$). For the resolution of the drying model, the GAMS software (Washington, USA) was used, which solves models based on algebraic equations.

Results and discussion

Drying model application

Table 1 presents the water diffusion coefficient averaged over time, the mass transfer coefficient, and the root mean square error (RMSE) for the assayed model. The MSE is acceptable. The model describes the operation of drying satisfactorily and has a high goodness-of-fit.

The water effective diffusion growth increased with increasing of the drying air temperature. As expected, the higher the temperature of the drying air, the greater the mobility of the water from the interior to the surface of the grains (Fabani *et al*., 2020). This results in an increase in the effective diffusivity of water and mass transfer. The effective diffusivity presented significant differences ($P<0.05$) concerning the drying air temperature. The results obtained were similar to those reported by other authors (10^{-11} and 10^{-8} $m^2\ s^{-1}$) (Bravo *et al*., 2009).

Noroña Gamboa (2018) evaluated the drying kinetics of barley, wheat, and corn seeds using drying temperatures of 40 and 60°C and an air flow rate (1.1 $m\ s^{-1}$). The diffusions coefficients obtained were in a range of 1.39×10^{-10} - 1.83×10^{-10} $m^2\ s^{-1}$ for corn, 2.32×10^{-11} - 7.84×10^{-11} $m^2\ s^{-1}$ for wheat and 4.30×10^{-11} - 1.41×10^{-10} $m^2\ s^{-1}$ for barley.

Janampa Arango (2017) investigated the effective diffusivity during convective drying at 60°C and an air speed of 4.5 $m\ s^{-1}$. The following values were obtained for the different varieties of quinoa: Black Collana (5.73 x 10-11), Black Ayrampo (5.73×10^{-11}), Pasankalla (1.05×10^{-10}), Yellow Composite (5.23×10^{-11}), and Rosada de Juli (5.13×10^{-11}).

According to Paquita Ninaraqui (2015), high Ea values indicate low sensitivity of the diffusion coefficient with respect to temperature. Lowest values were obtained for highest air drying temperature and velocity (80°C and 0.7 $m\ s^{-1}$) (Tab. 1). Similar values were reported by Vega Gálvez *et al*. (2010), Noroña Gamboa *et al*. (2018), and Aravindakshan *et al*. (2021).

The mass transfer coefficient increased with increasing temperature and velocity of the drying air. It was significantly influenced ($P<0.05$) by the drying air velocity. A

TABLE 1. Adjustment data obtained by applying the drying model.

T (°C)	v (ms^{-1})	D_{eff} x 10^{-10} (m^2s^{-1})	RMSE x10^{-3}	A	B	C	Ea ($Jmol^{-1}$)	R^2	k x 10^{-2} (ms^{-1})
40	0.2	2.52^a	1.00	0.05	3100	0.03	17035	1.00	7.20^d
40	0.7	3.02^a	6.00	0.06	3100	0.03	16728	0.99	11.20^e
60	0.2	5.47^b	1.00	0.06	3100	0.03	15956	1.00	7.76^d
60	0.7	5.93^b	1.00	0.07	3100	0.03	14373	1.00	11.33^e
80	0.2	9.27^c	2.00	0.06	3100	0.03	13724	1.00	7.83^d
80	0.7	0.10^c	4.00	0.07	3100	0.03	12521	1.00	11.47^e

Different letters indicate significant differences using Tukey's test ($P<0.05$). T: drying temperature; v: air velocity; D_{eff}: effective diffusion coefficient; RMSE: root-mean-square error, A, B, and C: parameters from effective diffusivity equation; Ea: activation energy; R^2: coefficient of determination; k: mass transfer coefficient.

higher speed of the drying air promotes the renewal of the drying air, avoiding its saturation; it produced an increase in the mass transfer coefficient.

Model validation

Figures 2 shows the experimental and model-estimated average moisture values for quinoa grains at different temperatures (40°C, 60°C, and 80°C) and drying air speeds (0.2 m s^{-1} and 0.7 m s^{-1}). The proposed model accurately describes the drying kinetics for quinoa grains for all the operating conditions tested.

The development of the experimental design resulted in six runs. A portion of the data set (3 runs) was independently acquired to obtain A, B, and C from equation 13. The other experimental runs were implemented to validate the proposed model using the estimated coefficients.

Grading quinoa flour curve

Figure 3 shows flour grading curves obtained by grinding the dried seeds.

Table 2 shows the performance of quinoa flour retained in a 50-mesh sieve. According to the PROINPA (2011) classification, the flour obtained for 50 mesh (minimum granule size of 0.297 mm) corresponds to bran (granule size between 0.487 and 0.23 mm).

This permits its use for balanced foods, whole meal bread, bakery, biscuits, pasta, purees, soups, and creams.

In grinding performance for the two drying air speeds or the three drying temperatures tested, significant differences (P>0.05) were not found.

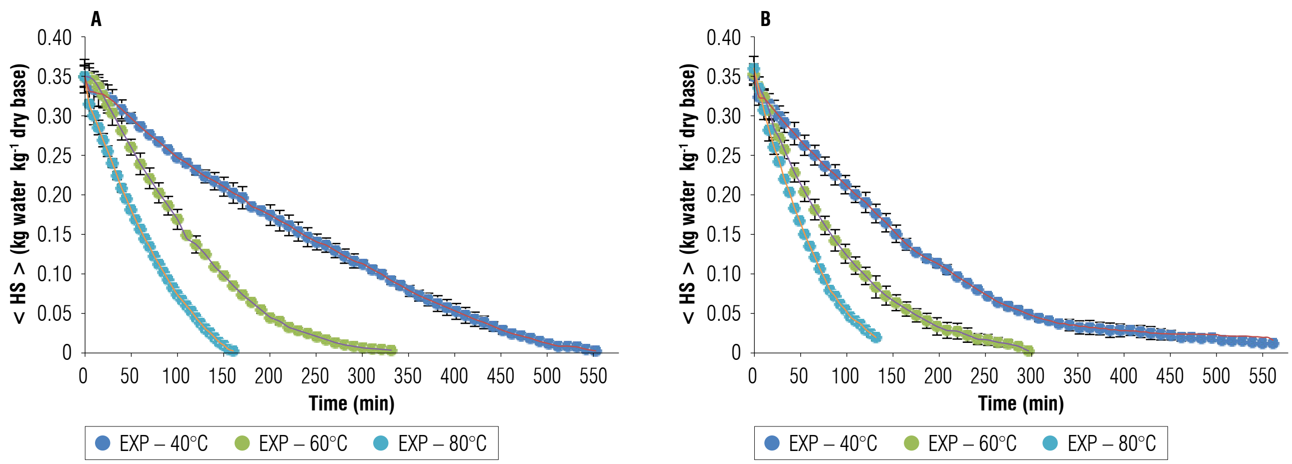

FIGURE 2. Experimental and model-predicted drying curves for the three drying temperatures tested. A) speed of the drying air, v_1= 0.2 m s^{-1}; B) speed of the drying air, v_2= 0.7 m s^{-1}. < HS >: average humidity. Error bars is standard error.

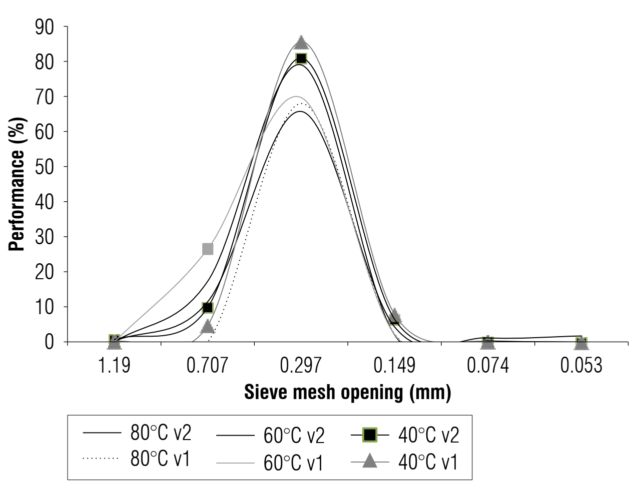

FIGURE 3. Grading quinoa flour curves.

TABLE 2. Quinoa flour (50-mesh) performance to different treatments.

Drying temperature °C	Drying air velocity (m s^{-1})	Performance (%)
40	v_1	85.6 [a]
	v_2	81.0 [a]
60	v_1	69.7 [a]
	v_2	79.0 [a]
80	v_1	68.0 [a]
	v_2	65.8 [a]

Different letters indicate significant differences according to Tukey's test (P<0.05).

Ortega Guerrero *et al*. (2013) used a series of Tayler sieves (14, 30, 60, 100, 200, and bottom) and reported 45.35% retained for 100 mesh. Likewise, Castro (2010) obtained 33.04% for 60 mesh for the unpolished Matarredonda variety quinoa flour. The variation in the granulometric analysis could be due to the variety of quinoa and the drying and grinding processes used in the evaluation.

Cerezal Mezquita *et al*. (2011) made the granulometric profile of quinoa flour obtained from the Nestlé Company (Chile). They used a vibrating sieve equipment and observed that 53.91% of the sample was retained in sieves N° 30, N° 60 (250 μm) and N° 80 (180 μm). The remaining 46.09% of flour remained in the sieve end collector.

In this study, higher yields were found in the milling compared to the antecedents published in literature. This situation may be due to the quinoa variety and the grinding equipment.

Conclusions

This study presented information on the conditioning, industrial drying, and milling of quinoa grains of the Hualhuas variety.

The proposed model accurately described the drying kinetics for quinoa grains for all the operating conditions tested. The computational time required to implement the numerical solution was 0.078 s.

The effective diffusivity presented significant differences ($P<0.05$) concerning the drying air temperature. The mass transfer coefficient increased with increasing temperature and velocity of the drying air and was significantly influenced ($P<0.05$) by the drying air velocity. The parameters obtained by the model are suitable for designing industrial equipment or converting a batch process into a continuous process.

From the industrial point of view, the drying treatment at 80°C and 0.7 m s^{-1} required less time and presented higher effective moisture diffusivity and mass transfer coefficients. However, it is necessary to evaluate the costs and final product quality to decide which is the optimum. It is also appropriate to study the bioavailability of the proteins to find out if they are affected by the drying temperature.

Conflict of interest statement

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

Author´s contributions

JL and MFB conducted the research and formal analysis and prepared the initial draft. JL and MFB developed the methodology, provided the study materials, reviewed and edited the manuscript, and supervised the planning of the research activity and execution. MCC reviewed and edited the manuscript, managed and coordinated the planning of the research activity and execution, and acquired financial support for the project. All authors reviewed the final version of the manuscript.

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First record of *Melophagus ovinus* (Linnaeus, 1758) (Diptera: Hippoboscidae) for Nuevo Leon, Mexico

Primer registro de *Melophagus ovinus* (Linnaeus, 1758) (Diptera: Hippoboscidae) para Nuevo León, México

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ABSTRACT

The sheep ked *Melophagus ovinus* (Diptera: Hippoboscidae: Lipopteninae), an ectoparasite of the domestic sheep *Ovis aries* (Artiodactyla: Bovidae: Caprinae), was recorded for the first time in Nuevo Leon, Mexico. This dipteran has a cosmopolitan distribution, likely found wherever its preferred host is present. Records of this parasite in Mexico are scarce and dispersed throughout the literature.

Key words: Hippoboscoidea, Lipopteninae, sheep ked, sheep, invasive species, geographic distribution.

RESUMEN

La falsa garrapata de las ovejas *Melophagus ovinus* (Diptera: Hippoboscidae: Lipopteninae), un ectoparásito de la oveja doméstica *Ovis aries* (Artiodactyla: Bovidae: Caprinae), fue reportada por primera vez en Nuevo León, México. Este díptero tiene una distribución cosmopolita, probablemente se encuentre donde sea que esté presente su huésped preferido. Los registros de este parásito en México son escasos y dispersos en la literatura.

Palabras clave: Hippoboscoidea, Lipopteninae, falsa garrapata de las ovejas, ovejas, especie invasora, distribución geográfica.

Introduction

Hippoboscoidea is a superfamily of blood-feeding parasitic flies with four families: Glossinidae, known as tsetse flies; Hippoboscidae, known as keds or louse flies; and Nycteribiidae and Streblidae, both known as bat flies. With the notable exception of the exclusively Afrotropical tsetse flies, none are known to commonly target humans as their hosts, and so they have not been recorded as vectors of zoonotic diseases. Hippoboscidae parasitizes both avian and mammalian hosts (Reeves & Lloyd, 2019), and is represented in continental North America by three subfamilies: Hippoboscinae (1 genus – parasite of Carnivora); Ornithomyinae (9 genera – parasites of various birds), and Lipopteninae (3 genera – parasites of Artiodactyla) (Maa & Peterson, 1987; Wood, 2010).

The three genera of Lipopteninae found in North America are *Lipoptena* Nitzch, 1818 (3 species – 1 introduced and 2 native parasites of Cervidae), *Melophagus* Latreille, 1802 (1 species – introduced parasite of Bovidae), and *Neolipoptena* Bequaert, 1942 (1 species – native parasite of Cervidae) (Maa & Peterson, 1987). In *Lipoptena* and *Neolipoptena* adults are fully winged, including halteres, but their forewings are shed after settling on the host (Maa & Peterson, 1987; Reeves & Lloyd, 2019). Adult *Melophagus* emerge from their pupal stage completely wingless, lacking even the halteres. *Melophagus ovinus* (Linnaeus, 1758) (Fig. 1A) is the only species of the genus represented in North America; it is a monoxenous parasite of the domestic sheep *Ovis aries* Linnaeus, 1758, with stray records on other species (Maa, 1964; Maa, 1965; Maa, 1969), particularly, domestic goats *Capra hircus* Linnaeus, 1758 (that are raised alongside sheep) (Bequaert, 1942).

This is a commercially important parasite of sheep wherever it is present, and they have the capacity to transmit various pathogens and thus halter the production of wool, meat, and milk (Small, 2005). This manuscript reports *Melophagus ovinus* in Nuevo Leon, Mexico, this is the northernmost record in the country for this insect, increasing its known distribution.

Materials and methods

The adult specimens documented here (Fig. 1A) were collected from the wool of a domestic sheep from the locality of "La Escondida" (24°03'06.8" N, 99°57'30.2" W, 1838

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m a.s.l.) in the municipality of Aramberri, Nuevo León, Mexico. They were identified using the keys found in the works of Maa and Peterson (1987) and Wood (2010): the absence of both wings and halteres identifies this genus and species. The specimens are deposited in the Entomology Laboratory of the Facultad de Ciencias Forestales of the Universidad Autónoma de Nuevo León under the voucher FCF-DIPTE003a–d.

Results and discussion

The four specimens collected (Fig. 1A) corresponded to *Melophagus ovinus* and represent the first records of this economically important parasite from the state of Nuevo Leon (Fig. 1B). Records of *Melophagus ovinus* from Mexico are scarce, since its preferred host species does not thrive in hot or tropical climates; however, it can be found in cooler areas in the highlands of the country (Bequaert, 1942; Maa, 1964; Wood, 2010). Previously, *Melophagus ovinus* had been recorded in the states of Chiapas, Estado de Mexico, Guanajuato, and Hidalgo (Fig. 1B; see Tab. 1 for references). *Melophagus ovinus* causes chronic irritation and dermatitis with associated pruritus in the sheep it infects (Underwood *et al*., 2015), and it is a vector of the protozoan *Trypanosoma melophagium* and the bacteria *Rickettsia melophagi*, both non-pathogenic, as well as viruses of the genus *Orbivirus* that are the causative agents of bluetongue disease (Reeves & Lloyd, 2019) and many other microorganisms (Zhao *et al*., 2018; Werszko *et al*., 2021). Nuevo Leon is not a leading Mexican state for the rearing and breeding of sheep (Hernández-Marín *et al*., 2017), but the activity is present in the state, at a moderate level. Future studies should focus on monitoring the populations dynamics of *Melophagus ovinus* to establish

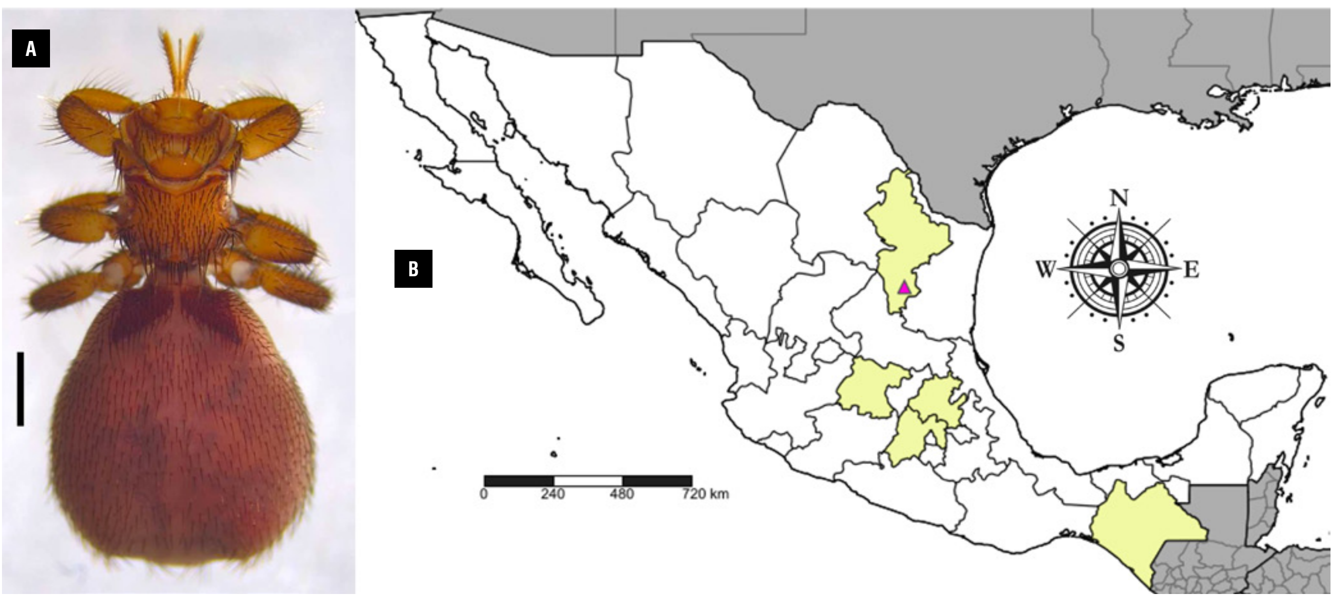

FIGURE 1. Sheep ked *Melophagus ovinus* (Linnaeus, 1758). A) adult male specimen (specimen FCF-DIPTE003a), dorsal view, scale bar = 1 mm; B) map of Mexico showing states from which *Melophagus ovinus* has been recorded (including the new record) in pale yellow; the pink tringle indicates the locality of the new record.

TABLE 1. State records of sheep ked (*Melophagus ovinus*) from Mexico from the available literature, including region and references. All hosts are domestic sheep (*Ovis aries*).

State	Region of Mexico	References
Chiapas	Southern	Perezgrovas Garza and Pedraza Villagómez (2014); Alemán Santillán *et al.* (2001)
Estado de Mexico	Central	Cuéllar Ordaz (2003)
Guanajuato	Central	Bequaert (1942)
Hidalgo	Central	Ayala Castillo (1991)
Nuevo Leon	Northern	Present publication

Material examined: *Melophagus ovinus* (Linnaeus, 1758), 2♂♂ 2♀♀, Mexico, Nuevo Leon, Aramberri, La Encantada, 24°03'06.8" N and 99°57'30.2" W, 1838 m a.s.l., 11-Feb-2023, ex. *Ovis aries* (Bovidae) [DIPTE003a–d].

its prevalence among the sheep to generate management strategies. Another possible research focus is to study the microorganisms present in these parasites as well as those in infected livestock.

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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

MJH thought of the goals and aims of this publication. MdL identified the specimen to the level of genus and species. MdL wrote the article. MJH corrected the initial version. Both authors reviewed the final version of the manuscript.

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SCIENTIFIC NOTE

Calibration of alternative equations to estimate the reference evapotranspiration in Nova Venécia, Espírito Santo, Brazil

Calibración de ecuaciones alternativas para estimar la evapotranspiración de referencia en Nova Venécia, Espírito Santo, Brasil

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ABSTRACT

The estimation of the reference evapotranspiration is fundamental in defining irrigation projects. However, an estimation using the standard equation requires climate variables that are difficult to measure and are not very accessible. Thus, the objective of this study was to calibrate and validate alternative methods to estimate evapotranspiration that use simple variables and to compare performance with the standard Penman-Monteith method for the municipality of Nova Venécia, Espírito Santo, Brazil. For this, a 12-year time series (2008-2019) of meteorological data from the Instituto Nacional de Meteorología was used. The standard FAO-56 Penman–Monteith method was used to evaluate alternative methods: Hargreaves and Samani, Benevides and Lopes, Linacre, Hamon and Camargo. Method performance was analyzed by correlation coefficient, Willmott index, root mean square of normalized error, and performance index. Calibration improved the statistical indices, increasing the performance of the Hargreaves and Samani, Benevides and Lopes, and Linacre methods to "very good" in the rainy season and to "intermediate" in the dry season. They were superior to the Hamon and Camargo methods, which continued to show "tolerable" to "very poor" performance in both periods.

Key words: alternative methods, agricultural meteorology, FAO-56 Penman–Monteith, irrigation.

RESUMEN

La estimación de la evapotranspiración de referencia es fundamental en la definición de proyectos de riego. Sin embargo, una estimación utilizando la ecuación estándar requiere variables climáticas difíciles de medir y poco accesibles. Por lo tanto, el objetivo de este estudio fue calibrar y validar métodos alternativos para estimar la evapotranspiración que utilizan variables simples y comparar el desempeño con el método estándar de Penman-Monteith para el municipio de Nova Venécia, Espírito Santo, Brasil. Para ello se utilizó una serie temporal de 12 años (2008-2019) de datos meteorológicos del Instituto Nacional de Meteorología. Se utilizó el método estándar FAO-56 Penman-Monteith para evaluar métodos alternativos: Hargreaves y Samani, Benevides y Lopes, Linacre, Hamon y Camargo. El desempeño del método se analizó mediante el coeficiente de correlación, el índice de Willmott, la raíz cuadrática media del error normalizado y el índice de desempeño. La calibración mejoró los índices estadísticos, aumentando el desempeño de los métodos de Hargreaves y Samani, Benevides y Lopes, y Linacre a "muy bueno" en época de lluvias y a "intermedio" en época seca. Estos fueron superiores a los métodos de Hamon y Camargo, que continuaron mostrando un desempeño "tolerable" a "muy pobre" en ambos períodos.

Palabras clave: métodos alternativos, meteorología agrícola, FAO-56 Penman–Monteith, irrigación.

Introduction

Coffee cultivation is of significant importance to the world economy, with Brazil as the largest producer and exporter of coffee (*Coffea* spp.), corresponding to 37% of world production, and as the second largest producer of the species *Coffea canephora* (USDA, 2019; Belan *et al*., 2020). In addition, Brazil is also one of the largest producers of black pepper (*Piper nigrum*) (Carneiro *et al*., 2017).

The State of Espírito Santo is currently the second largest coffee producer and the first producer of Conilon coffee, the second producer of papaya, and the largest exporter and second producer of black pepper (Dadalto *et al*., 2016).

The municipality of Nova Venécia is located in the northwest region of the state of Espírito Santo; agriculture is one of the main activities of the region. According to the 2018 Agricultural Census, the municipality was the 9th largest

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producer of Conilon coffee (27,920 t) and the 5th largest producer of black pepper (4,200 t) nationally. One of the main factors contributing to this productive position is the use of irrigation, which according to this census covers approximately 15,000 productive hectares and is present in about 2,500 agricultural establishments (IBGE, 2018).

In irrigated agriculture, the quantification of the water consumption of the crops during development stages allows for the planning, dimensioning, and rational management of the irrigation. One of the techniques that helps to quantify the volume of water needed for irrigation is the determination of crop evapotranspiration (ETc), which reflects the measurement of the total amount of water lost to the atmosphere, resulting from the processes of soil evaporation and plant transpiration.

To determine ETc, it is necessary to estimate the Reference Evapotranspiration (ET_o), which represents a standardized measure of the evapotranspiration rate of a location, considering the local climate variables. Then, ET_o is corrected by the Crop Coefficient (Kc), which is a ratio between ETc and ET_o. The value of Kc varies depending on the crop and its stage of growth.

The United Nations Food and Agriculture Organization (FAO) has defined the Penman-Monteith equation as the standard model, recommending it to estimate and calibrate different ET_o methods (Allen *et al.*, 1998). Studies have shown the efficiency and representativeness of this model to the factors that govern the process of evapotranspiration; however, its great disadvantage is related to the high number of meteorological variables required for its use, since most of the stations do not have enough sensors to collect all the variables, or the quality of the data collected is poor (Palaretti *et al.*, 2014; Carvalho *et al.*, 2015; Tanaka *et al.*, 2016).

Faced with this problem, the use of simpler methods using few meteorological variables to estimate the ET_o has become a viable solution (Fernandes *et al.*, 2012; Sales *et al.*, 2018). In this context, Santana *et al.* (2018) recommend that, before choosing the method to be used, an assessment of the climate adaptability to the region be made comparing its performance to the standard Penman-Monteith FAO model 56. This study is necessary because the methods were generally developed under climatic and crop management conditions different from that in which the model will be employed, making it necessary to calibrate the equations for the region in order to minimize the estimation errors (Pereira *et al.*, 2009; Rigone *et al.*, 2013).

Thus, the aim of this study was to calibrate and validate alternative methods for producers in the region that use only simple variables, comparing their performance with that of the standard method recommended by the FAO-56 Penman-Monteith, for the municipality of Nova Venécia, Espírito Santo State, Brazil.

Materials and methods

In this study, hourly data from the automatic meteorological station of the National Institute of Meteorology (INMET), located in the municipality of Nova Venécia, State of Espírito Santo, Brazil, were used. The station is located at latitude 18º41'43" S, longitude 40º24'27" W and has an altitude of 154 m a.s.l. Nova Venécia is located in the northern region of Espírito Santo (Fig. 1), bounded by latitudes 18º17'58" S and 18º56'48" S, and by longitudes 40º45'30" W and 40º17'46" W, covering a total area of 1,439,571 km².

The northern region of the state is recognized as the main producer of Conilon coffee, standing out in this activity. In addition, this area also stands out for the production of papaya and black pepper.

The climate of Nova Venécia is classified by Köppen and Geiger (1936) and Peel *et al.* (2007) as tropical with dry season, with a temperature range from 11.8 to 18°C during the colder month and 30.7 to 37°C in the warmer month, reaching an annual average of 24°C. The rainy season is between the months of October to February, and the dry season between the months of March to September.

The data used in this study were collected from a meteorological station installed in the region in 2008, with a historical series of 11 years for producers in the region from 2008 to 2019. It is important to emphasize that, although the historical series is relatively short, local studies of this nature play a crucial role in facilitating the suitability of irrigation projects for the region. However, it is essential to use the results of this study with caution, considering the limitations of the historical series.

The meteorological variables used to estimate the ET_o in mm d^{-1} were: maximum (Tx), minimum (Tn) and average (Tm) air temperature in ºC; average dew point temperature (To) in ºC; maximum relative humidity (URx), minimum (URn) and average (URm) humidity of the air at 2 m above ground level in %; global solar radiation (Rg) in MJ m^{-2} d^{-1}; and wind speed at 2 m from ground level (U_2) in m s^{-1}, as shown in Table 1.

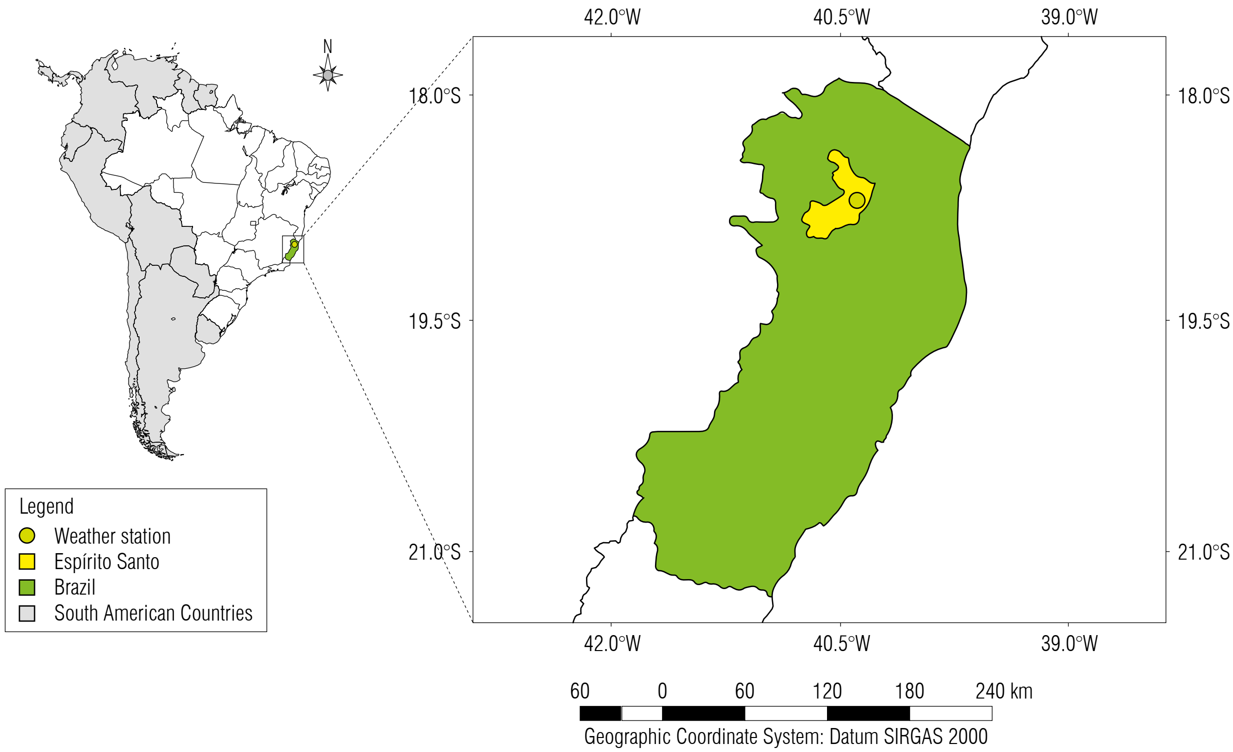

FIGURE 1. Geographical location of the municipality of Nova Venécia in the state of Espírito Santo, Brazil.

TABLE 1. Monthly averages of the climatic data collected by the meteorological station and used by alternative methods to estimate ET_o in Nova Venécia, Espírito Santo, Brazil, between 2008 and 2019.

Month	Rg	Tx	Tn	Tm	To	URx	URn	URm	U_2
January	21.3	32.9	21.3	27.1	17.0	94.1	43.9	69.0	2.1
February	21.8	33.6	21.5	27.6	16.9	94.1	40.4	67.2	1.9
March	18.8	33.2	21.6	27.4	17.2	93.7	43.3	68.5	1.9
April	16.4	31.4	20.4	25.9	17.1	93.7	47.3	70.5	1.8
May	13.7	29.7	18.5	24.1	16.3	93.1	47.6	70.3	1.8
June	11.9	28.4	17.6	23.0	15.8	93.6	48.3	70.9	1.8
July	14.8	27.8	16.7	22.2	15.1	94.0	46.4	70.2	1.9
August	15.8	28.2	16.8	22.5	14.9	93.3	44.4	68.9	2.1
September	17.4	30.0	18.2	24.1	15.2	92.1	41.0	66.6	2.4
October	17.4	30.6	19.7	25.2	16.0	91.75	44.4	68.0	2.5
November	18.1	30.5	20.5	25.5	17.1	93.1	50.4	71.8	2.3
December	20.1	31.8	21.2	26.5	17.5	93.7	48.7	71.2	2.1

Rg - global solar radiation; Tx - maximum air temperature; Tn - minimum air temperature; Tm - average air temperature; To - average dew point temperature; URx - maximum relative humidity; URn - minimum relative humidity; URm - average relative humidity; U_2 - wind speed at 2 m above ground level.

Prior to the estimates, the quality of the data was analyzed in order to eliminate measurement errors, based on the criteria proposed by Sales *et al.* (2018): Tx above 50°C; Tn below 0°C; Tx below Tn for the same day; Rg equal to zero and Rg greater than the extraterrestrial solar radiation. After the analysis, 3865 d remained with consistent data, corresponding to 92% of the data collected by the weather station.

Daily values of ET_o were estimated for the dry and rainy periods, using the alternative methods of (HS) Hargreaves and Samani (1985) (Eq. 1); (BL) García Benevides and López (1970) (Eq. 2); (Li) Linacre (1977) (Eq. 3); (Ho) Hamon (1961) (Eq. 4); (Ca) Camargo (1978) (Eq. 5) and (FAO-56 PM) standard method 56 from FAO Penman-Monteith (Allen *et al.*, 1998) (Eq. 6).

$$ET_o = A\frac{Ra}{2.45}(Tx - Tn)^{0.5}(Tm + B) \quad (1)$$

$$ET_o = A\,10^{(\frac{7.5\,Tm}{237.5+Tm})}(1 - 0.01\,UR) + B\,Tm - C \quad (2)$$

$$ET_o = \frac{A\frac{Tm+0.006\,alt}{100-lat} + B(Tm - To)}{C - Tm} \quad (3)$$

$$ET_o = A\,(\frac{N}{12})^2\frac{B\,exp^{(C\,Tm)}}{100}D \quad (4)$$

$$ET_o = A\frac{Ra}{2.45}Tm \quad (5)$$

$$ET_o = \frac{0.408\Delta(Rn - G) + \gamma(\frac{900}{Tm+273})U_2(e_s - e_a)}{\Delta + \gamma(1 + 0.34U_2)} \quad (6)$$

where:

A, B, C and D - adjusted coefficients;

Ra - solar radiation in absence of atmosphere (MJ m^{-2} d^{-1});

alt - altitude (m);

lat - latitude (degrees);

N- photoperiod (h);

Δ- tangent to the saturation pressure curve of water vapor (kPa °C^{-1});

γ - psychrometric constant (0.0662 kPa °C^{-1});

Rn - radiation balance (MJ m^{-2} d^{-1});

G - soil heat flux density (MJ m^{-2} d^{-1});

e_s - saturation pressure the surface temperature (kPa °C^{-1});

e_a - vapor pressure of the air (kPa °C^{-1}).

The data from the period from July 2008 to December 2015 were used to calibrate the parameters of the equations; the equations from January 2016 to December 2019 were used to validate those parameters.

The parameter calibration was performed by minimizing the square sum of the error obtained by comparing the ET_o estimated by the alternative methods and by the FAO-56 PM standard method, using the Solve activation within the Excel software and the open-source program R Core Team (2020). The performance of the alternative ET_o methods was evaluated by the correlation coefficient (Eq. 7), the root of normalized mean square error (Loague & Green, 1991) (Eq. 8), Willmott's index of agreement (Willmott *et al.*, 1985) (Eq. 9) and confidence coefficient (Camargo & Sentelhas, 1997) (Eq. 10).

$$r = \frac{\sum_{i=1}^{n} \quad (|E_i - \bar{E}|)(|O_i - \bar{O}|)}{\sqrt{\sum_{i=1}^{n} \quad (E_i - \bar{E})^2}\sqrt{\sum_{i=1}^{n} \quad (O_i - \bar{O})^2}} \quad (7)$$

$$RMSE = \sqrt{\sum_{i=1}^{n} \quad \frac{(O_i - E_i)^2}{N}} \quad (8)$$

$$d = 1 - \frac{\sum_{i=1}^{n} \quad (O_i - E_i)^2}{\sum_{i=1}^{n} \quad (|E_i - \bar{O}| + |O_i - \bar{O}|^2)} \quad (9)$$

$$c = r\,d \quad (10)$$

where:

E_i - value obtained by means of the alternative methods (mm d^{-1});

O_i - value estimated through the Penman-Monteith standard method (mm d^{-1});

E - mean of the estimated by means of the alternative methods (mm d^{-1});

O - mean of the estimated by means of the Penman-Monteith standard method (mm d^{-1});

n - number of values;

r - Pearson correlation index;

d - Willmott's index of agreement;

c - confidence coefficients.

The performance of alternative methods was classified based on the variation of the confidence index (c) as: "excellent" (c>0.85); "very good" (c between 0.76 and 0.85); "good" (c between 0.66 and 0.75); "intermediate" (c between 0.61 and 0.65); "tolerable" (c between 0.51 and 0.60); "poor" (c between 0.41 and 0.50) and "very poor" (c<0.40).

Results and discussion

During the period studied, the average annual rainfall was 862.73 mm, with a period of highest concentration between October and March, and a period of drought between April and September (Fig. 2). These results corroborate those reported by Alves *et al.* (2005) who observed a period of higher rainfall in the Southeast region of Brazil, occurring normally between October and March, with approximately 80% of the annual total. At the state level, Uliana *et al.* (2013) identified two distinct periods for Espírito Santo: the first between October and April, which concentrates a large part of the precipitation, and the second between May and September, with a marked decrease in rainfall.

Table 2 shows the descriptive statistics of the weather data collected in the town of Nova Venécia and used in the ET_o calculation. It shows that the dry and rainy periods are well defined with regular temperature and air humidity and with low variation, while data on wind speed and global solar radiation show high variation, similar to what was observed by Gurski *et al.* (2016) in Curitiba, Paraná, Brazil.

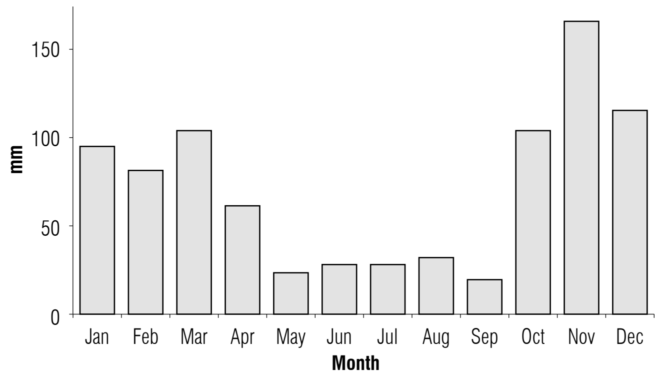

FIGURE 2. Monthly average rainfall in the municipality of Nova Venécia, Espírito Santo, Brazil, between 2008 and 2019.

TABLE 2. Average, standard deviation (SD) and coefficient of variation (CV%) of the meteorological data of the dry and rainy period used in the estimation of ET_0 in the municipality of Nova Venécia, Espírito Santo, Brazil, between 2008 and 2019.

Climatic variable	Dry season			Rainy season		
	Average	SD	CV	Average	SD	CV
Rg	15.04	5.73	38.11	19.74	6.15	31.13
Tx	29.22	2.90	9.93	32.02	3.30	10.40
Tn	17.99	2.14	11.87	20.97	1.48	7.05
Tm	23.60	2.07	8.77	26.50	2.06	7.78
To	15.73	1.78	11.31	16.97	1.82	10.73
URx	93.37	3.45	3.70	93.34	2.60	2.79
URn	45.83	4.18	9.12	45.32	5.31	11.71
URm	69.60	5.77	8.30	69.33	6.90	9.95
U_2	2.00	0.66	32.91	2.18	0.67	30.73

Rg - global solar radiation; Tx - maximum air temperature; Tn - minimum air temperature; Tm - average air temperature; To - average dew point temperature; URx - maximum relative humidity; URn - minimum relative humidity; URm - average relative humidity; U_2 - wind speed at 2 m above ground level.

Oliveira *et al.* (2017), studying the influence of meteorological elements on the reference evapotranspiration estimated by FAO-56 PM, observed that variations greater than 10% cause different behavior in the method estimates due to the high sensitivity of the equation, which was also observed by Lemos *et al.* (2010).

Table 3 shows the values of the original and calibrated coefficients during the dry period and rainy season in the study region. For accuracy purposes, the calibrated coefficients of the alternative methods were presented rounded to 4 decimal places.

TABLE 3. Values of the coefficients of the original and calibrated alternative methods for the municipality of Nova Venécia, Espírito Santo, Brazil, for the period from 2008 to 2015.

Coefficients	HS	Li	BL	Ho	Ca
Original coefficients					
A	0.0023	500.0000	1.2100	0.5500	0.0100
B	17.8000	15.0000	0.2100	4.9300	
C		80.0000	2.3000	0.0620	
D				25.4000	
Calibrated coefficients for the dry period					
A	0.00195	105.0919	1.8959	0.6466	0.0122
B	20.6636	8.9986	0.0276	5.7959	
C		46.3108	0.0000	0.0521	
D				29.8610	
Calibrated coefficients for the rainy period					
A	0.0034	175.2910	2.0580	0.3956	0.0111
B	0.0000	13.2440	0.0428	3.5460	
C		53.0670	0.0000	0.1053	
D				18.2694	

Alternative methods to estimate evapotranspiration: HS - Hargreaves and Samani; Li - Linacre; BL - Benevides and Lopes; Ho - Hamon; Ca - Camargo.

Table 4 presents the average ET_o estimates, the root of normalized mean square error (RMSE), the Willmott's index of agreement (d), the Pearson correlation index (r), the confidence coefficients (c) and performance, obtained from the correlations between ET_o values by the FAO-56 PM method and those obtained by alternative methods during the dry and rainy periods. It shows that the average ET_o of the region is higher during the rainy season, with an average of 4.74 mm d^{-1}, while in the dry period the average is 3.39 mm d^{-1}. This is because global solar radiation and air temperature are the variables with the greatest influence on ET_o calculations (Ismael *et al.*, 2015) since the rainy period in Nova Venécia covers the spring and summer seasons and is characterized by high temperatures and high energy flow from solar radiation, while the dry period spans fall and winter seasons, which present milder conditions.

The calibrated equations presented better statistical parameters than the original equations (Tab. 4). In descending order, the best methods for estimating ET_o were: HS, BL, Li, Ho, and Ca.

The HS, BL and Li methods showed similar performance after calibration. In the rainy season (Tab. 4), the RMSE

TABLE 4. Evaluation and performance of alternative ET_0 estimation methods (mm d^{-1}), relative to standard method, FAO-56 PM before (original) and after the calibration (calibrated) of the parameters for the Municipality of Nova Venécia, Espírito Santo, Brazil, for the period from 2008 to 2015.

	Method	ET_0m	RMSE	r	d	c	Performance
			Rainy season				
Original	FAO-56 PM	4.74	-	-	-	-	-
	HS	4.73	0.75	0.85	0.88	0.75	Good
	BL	5.36	0.99	0.82	0.83	0.68	Good
	Li	5.00	0.84	0.81	0.86	0.70	Good
	Ho	4.00	1.30	0.68	0.57	0.40	Very poor
	Ca	4.31	1.21	0.66	0.52	0.34	Very poor
Calibrated	HS	4.77	0.72	0.85	0.90	0.77	Very good
	BL	4.73	0.73	0.84	0.91	0.76	Very good
	Li	4.73	0.76	0.84	0.90	0.76	Very good
	Ho	4.73	0.96	0.70	0.81	0.57	Tolerable
	Ca	4.78	1.11	0.66	0.59	0.39	Very poor
			Dry season				
Original	FAO-56 PM	3.39	-	-	-	-	-
	HS	3.25	0.65	0.72	0.83	0.60	Tolerable
	BL	4.37	1.03	0.64	0.62	0.40	Poor
	Li	4.00	0.96	0.64	0.71	0.45	Poor
	Ho	2.65	1.07	0.58	0.52	0.30	Very poor
	Ca	2.78	0.97	0.61	0.60	0.37	Very poor
Calibrated	HS	3.39	0.63	0.74	0.85	0.63	Intermediate
	BL	3.38	0.66	0.73	0.83	0.61	Intermediate
	Li	3.38	0.68	0.74	0.84	0.62	Intermediate
	Ho	3.40	0.76	0.60	0.69	0.41	Poor
	Ca	3.39	0.75	0.61	0.74	0.45	Poor

Methods to estimate evapotranspiration: FAO-56 PM - Standard method 56 from FAO Penman-Monteith HS - Hargreaves and Samani; Li - Linacre; BL - Benevides and Lopes; Ho - Hamon; Ca - Camargo; ET_0m - average ETo estimates; RMSE - root of normalized mean square error; r - Pearson correlation index; d - Willmott's index of agreement; c - confidence coefficients.

decreased by 0.03, 0.26, and 0.08 mm d^{-1} and the "c" index increased by 2.66, 11.76, and 8.57% in the respective methods, reclassifying them as "very good".

In the dry period (Tab. 4), the RMSE decreased by 0.02, 0.37, and 0.28 mm d^{-1} and the "c" index increased by 1.61, 52.50, and 37.77%, respectively, reclassifying them as "intermediate". While these methods performed best among those studied, the average error remained elevated, between 0.72<RMSE<0.76 mm d^{-1} during the rainy season and 0.63<RMSE<0.68 mm d^{-1} during the dry period. Sales *et al*. (2018), when calibrating these methods for the city of São Mateus, Espírito Santo State, found RMSE values equal to 24.6, 21.6, and 23.7%, respectively, but with a "d" index greater than 0.80, which indicates discrepant errors in some data pairs and loss of efficiency at some time of the year.

Therefore, the subdivision of the estimated period was recommended. By subdividing the data analysis period into dry and rainy seasons, it was possible to reduce the error; however a larger subdivision is preferred.

For the less precise methods Ho and Ca during the rainy season, RMSE decreased by 0.34 and 0.09 mm d^{-1} and the "c" index increased by 42.50 and 14.70%, reclassifying them as "tolerable" and "very poor", respectively, whereas in the dry period, RMSE decreased by 0.31 and 0.22 mm d^{-1} and the "c" index increased by 36.66 and 21.62%, reclassifying them as "poor". The high estimation error between 0.96<RMSE<1.11 mm d^{-1} during the rainy season and 0.75<RMSE<0.76 mm d^{-1} during the dry season demonstrates the inefficiency of the methods even after calibration.

According to Souza *et al.* (2019), as these methods only use the average air temperature as a variable in their equations, they have a certain dependency resulting in unsatisfactory performance, as observed in studies carried out in different regions of Brazil (Fanaya *et al.*, 2012; Bezerra *et al.*, 2014; Silva *et al.*, 2017).

Figure 3 shows the average monthly evapotranspiration estimated by alternative methods in their original (Fig. 3A) and calibrated (Fig. 3B) equations, compared to the FAO-56 PM. It shows graphically the effect of the RMSE decrease in the behavior of alternative methods over the periods of the year. It evidences the closeness of the calibrated equations to the FAO-56 PM, emphasizing an improvement in performance in the dry and rainy periods.

In addition, all methods presented index (d), (r), and RMSE values close to the series used to calibrate them, with an

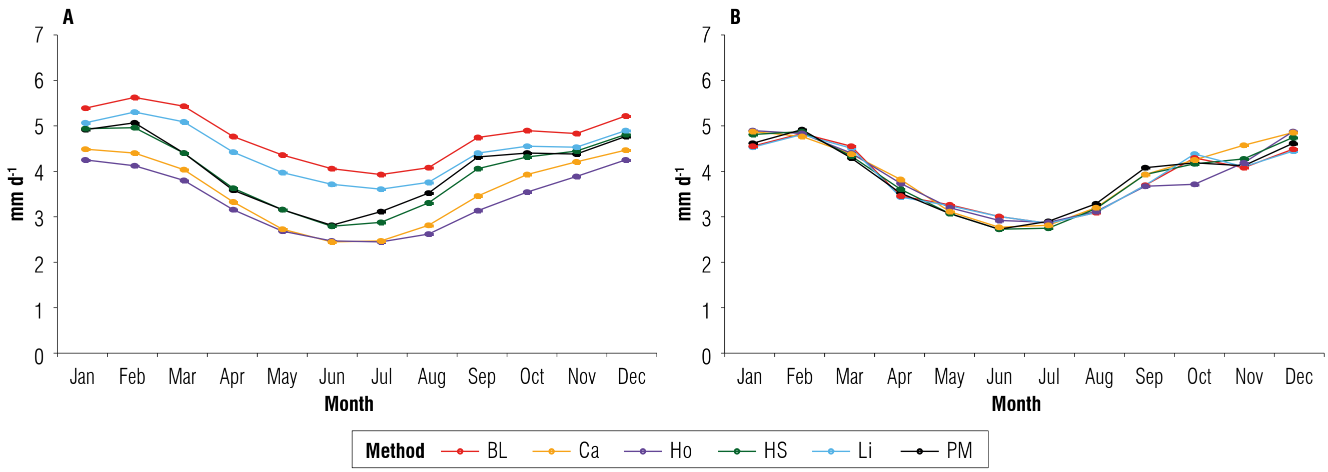

FIGURE 3. Average monthly evapotranspiration estimated by the FAO-56 PM method and by alternative methods with the original (A) and calibrated (B) equations in Nova Venécia, Espírito Santo, Brazil, between 2008 and 2015.

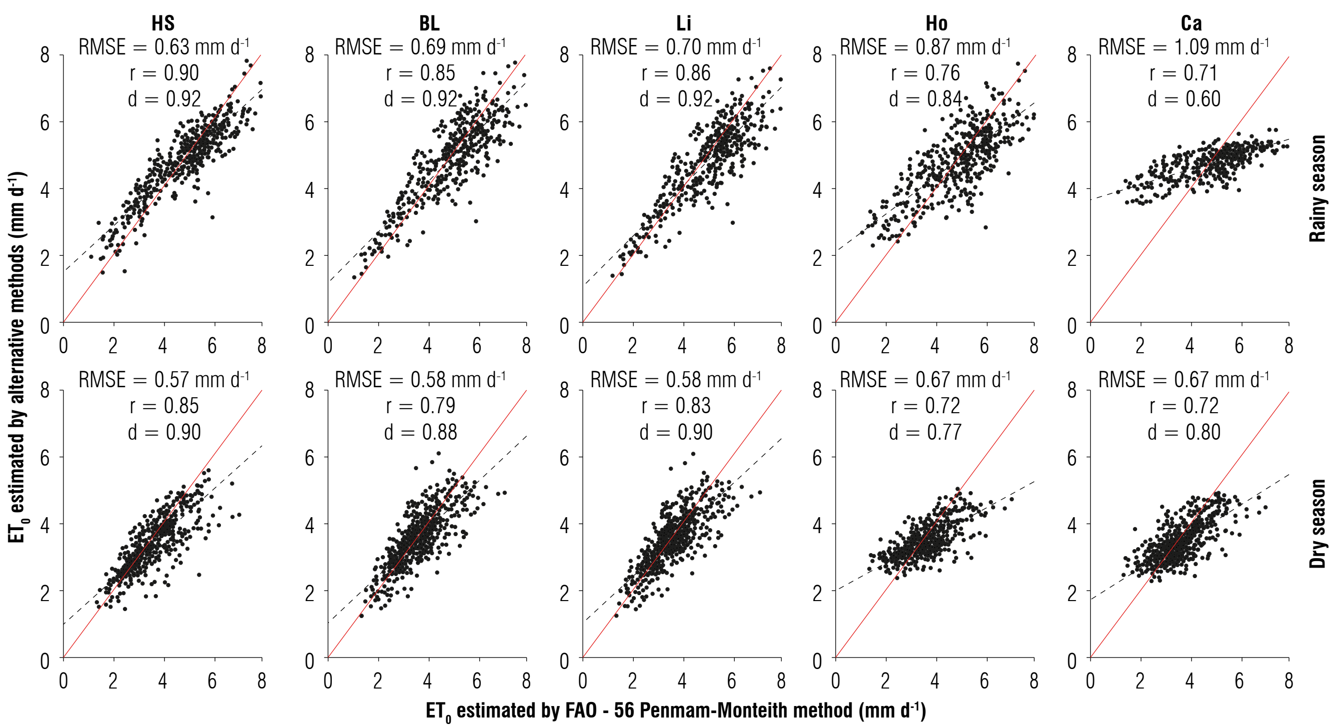

FIGURE 4. Correlations between daily ET_0, estimated by the FAO-56 PM and alternative models (HS, Li, BL, Ho, Ca) for the dry and rainy period of the 2016 to 2019 data series (independent data not used for calibration) in Nova Venécia, Espírito Santo, Brazil. Methods to estimate evapotranspiration: FAO-56 PM - Standard method 56 from FAO Penman-Monteith HS - Hargreaves and Samani; Li - Linacre; BL - Benevides and Lopes; Ho - Hamon; Ca – Camargo. RMSE – root of normalized mean square error. Methods to estimate evapotranspiration: FAO-56 PM - Standard method 56 from FAO Penman-Monteith HS - Hargreaves and Samani; Li - Linacre; BL - Benevides and Lopes; Ho - Hamon; Ca – Camargo.

average variation of 2.19%, 5.12%, and 8.16%, respectively, in the rainy season and 7.27%, 13.57%, and 13.39%, respectively, in the dry season. This confirms the efficiency of the adjustments, making them reliable to use.

The regression analysis of the estimates performed by the HS, BL and Li methods showed dispersion closer to the 1:1 line than those performed by the Ho and Ca methods. This greater homogeneity of data can be verified graphically, by the decreased RMSE values and by the higher r and d indices, revealing the greater accuracy of ET_o estimation in Nova Venécia in both periods of the year.

Conclusions

The HS, BL and Li methods presented the best performances for the region in both the dry and rainy season; the HS method is most interesting because it only requires the use of equipment that measures the air temperature and can be used to replace the standard FAO-56 PM model for estimating irrigation needs in the region.

Alternative methods for estimating ETo, calibrated for the municipality of Nova Venécia in Espirito Santo, Brazil, have a strong potential for use in irrigation projects, since they help in determining the volume of water needed to supply the crop through the use of simple and inexpensive equipment. This, in turn, can help reduce water consumption in agriculture, promote the efficient use of natural resources, as well as enhance the economic exploitation of crops.

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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

GF and ECO carried out the study planning; GF and MSG participated in data collection; GF, RAS and RAS carried out data analysis, production of figures and tables; MSG and EJAB worked on the first draft of the manuscript; RPP, ECO, MCTL participated in the correction and translation. All authors reviewed the final version of the manuscript.

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SCIENTIFIC NOTE

Time of contact of phosphate fertilizer with *Megathyrsus maximus* (cv. Massai) seeds in relation to germination and growth

Tiempo de contacto del fertilizante fosfatado con semillas de *Megathyrsus maximus* (cv. Massai) en relación con la germinación y el crecimiento

Helen Cristina de Arruda Rodrigues[1], Rayanny Rodrigues Machado[1], Rosemary Cordeiro Torres Brito[2], Edson Dias de Oliveira Neto[3*], and Henrique Antunes Souza[4]

ABSTRACT

The aim of this research was to evaluate the effect of the contact time of simple superphosphate fertilizer with Massai grass (*Megathyrsus maximus*) seeds on the physiological quality of the seeds. The experiment was conducted with seeds of Massai grass in two conditions: laboratory and field. The experiment was carried out in Teresina, Piauí, Brazil. The experimental design was completely randomized, with three replicates for both conditions. The treatments consisted of seven contact times: 0, 3, 6, 9, 24, 48, and 72 h. Germination (GP) and emergence (EP) percentages, seedling height (SH), root length (RL), and germination speed index (GSI) were evaluated. The contact time of the fertilizer with the seeds had a significant effect on all variables, except root length and emergence at 7 d. For GP in the second data collection and GSI, there were no differences up to 3 h of contact; however, for GP in the first collection, SH and GSI at 21 d, the contact of seeds with fertilizer for 3 h already had negative effects on these variables, reducing them by 23.33%, 12.13 cm and 14.28% respectively. The longer the contact time between single superphosphate with *M. maximus* (cv. Massai) seeds, the lower the germination and vigor values.

Key words: seedling emergence, physiological quality, forage, seed germination.

RESUMEN

El objetivo de esta investigación fue evaluar el efecto del tiempo de contacto del fertilizante superfosfato simple con semillas de pasto Massai (*Megathyrsus maximus*) sobre la calidad fisiológica de las semillas. El experimento se realizó con semillas de pasto Massai en dos condiciones: laboratorio y campo. El experimento se llevó a cabo en Teresina, Piauí, Brasil. El diseño experimental fue completamente al azar, con tres repeticiones para ambas condiciones. Los tratamientos consistieron en siete tiempos de contacto: 0, 3, 6, 9, 24, 48 y 72 h. Se evaluaron porcentajes de germinación (PG) y emergencia (PE), altura de plántula (AP), longitud de raíz (LR) e índice de velocidad de germinación (IVG). El tiempo de contacto del fertilizante con las semillas tuvo un efecto significativo en todas las variables, excepto en la longitud de las raíces y la emergencia a los 7 d. Para PG en la segunda toma de datos e IVG no hubo diferencias hasta las 3 h de contacto; sin embargo, para PG en la primera colecta, SH e IVG a los 21 d, el contacto de las semillas con el fertilizante durante 3 h tuvo efectos negativos sobre estas variables, reduciéndolas en 23.33%, 12.13 cm y 14.28% respectivamente. A mayor tiempo de contacto entre el superfosfato simple y las semillas de *M. maximus* (cv. Massai), menores valores de germinación y vigor.

Palabras clave: emergencia de plántulas, calidad fisiológica, forraje, germinación de semillas.

Introduction

The use of fertilizers when sowing forage seeds requires care, as the sowing time is very variable and interruption of sowing due to damage to agricultural implements, rain or other problems can increase the contact time of the seeds with the fertilizer (Almeida *et al*., 2017).

Sowing efficiency depends on the type of fertilizer and the contact time between the fertilizer and the seeds (Lima *et al.,* 2010). Sowing must consider that fertilizer can harm seed germination and seedling emergence, mainly due to saline effect of fertilizers (Pereira *et al.,* 2018).

Some production systems, such as integrated systems, suggest the use of forage together with annual crops, such as in maize-grass intercropping, where sowing of forage seeds is generally carried out together with fertilizers in the feed-box of a seed drill (Almeida *et al.,* 2017; Choudhary *et al*., 2018).

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The choice of forage species in the implementation of a pasture reform and adequate recommendation of mineral nutrients requires consideration by growers. In this sense, forage plants of the genus *Megathyrsus maximus* have drawn interest among researchers and growers of Brazil, due to their high productivity and wide adaptability to edaphic and climatic conditions of the country, akin to those of their center of origin (Pereira *et al.,* 2021).

Some practices employed in intensive production systems are the application of fertilizers at pre-sowing. In this context, mainly in the Northeastern Cerrado, where planting windows are short, there is a need to optimize planting because growers often apply only phosphorus fertilizer at sowing (Donagemma *et al.,* 2016).

Considering the requirements when mixing fertilizers and forage species in integrated systems and the growing use of species of the genus *Megathyrsus*, the aim of the present study was to evaluate the effect of the contact time of single superphosphate (SSP) fertilizer on the physiological quality of Massai grass seeds.

Materials and methods

The experiment was carried out at the State University of Piauí, located in Teresina, Piauí state, Brazil (5°04'34.4" S, 42°49'36.9" W) (Fig. 1), under both controlled laboratory conditions and in the field under full sun exposure.

The experimental design was completely randomized, with three replicates (20 g of seed in each). The treatments consisted of seven contact times between seeds and granulated phosphate fertilizer (single superphosphate, 18% P_2O_5): 0, 3, 6, 9, 24, 48, and 72 h. The design was the same for both trials (laboratory and full sun field exposure). The seeds of Massai grass (*M. maximus*) were in contact with SSP according to the mentioned periods, with a fertilizer rate equivalent to 20 kg ha^{-1} of P_2O_5 and a sowing rate of 5 kg ha^{-1}.

The mixture of fertilizer and seeds was kept in a closed plastic package of 0.5 dm^3, consisting of 20 g of forage seeds and 80 g of fertilizer. Homogenization (mixture of fertilizer with seeds) was performed according to each treatment; the control received no contact. The seeds had

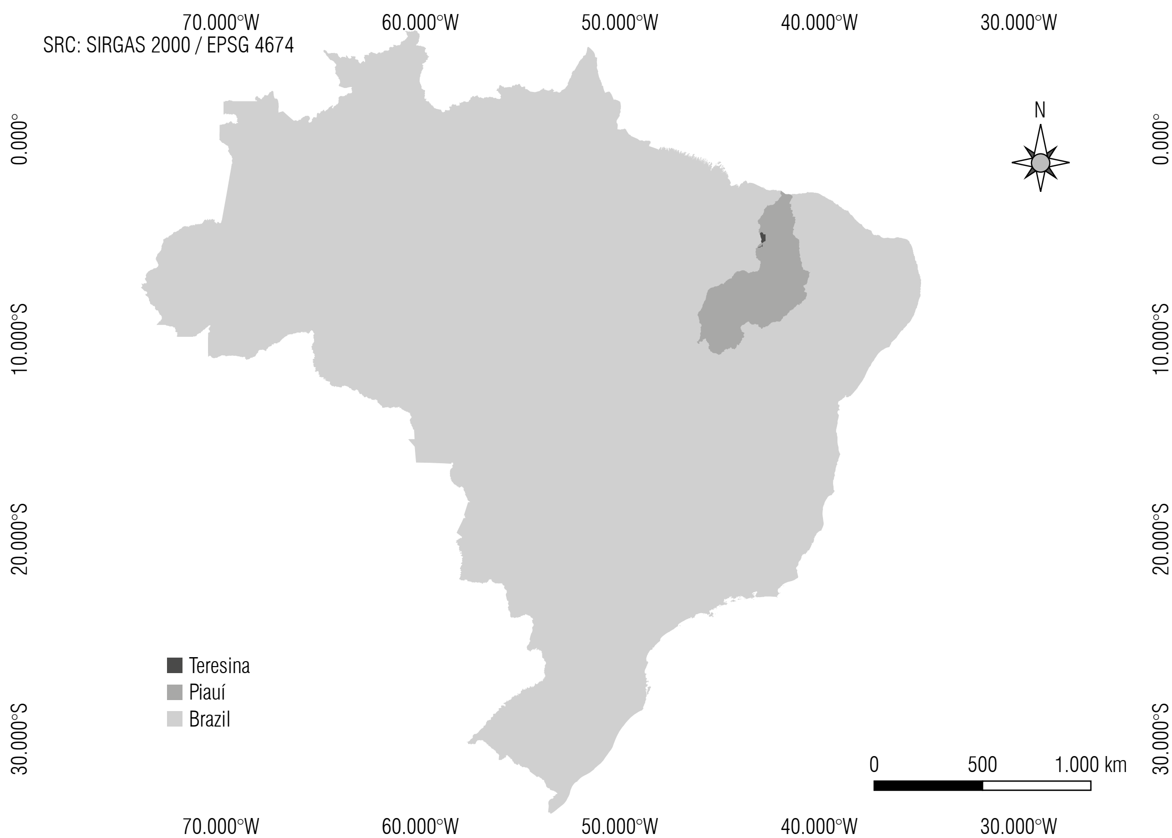

FIGURE 1. Map of Brazil highlighting the state of Piauí and the town of Teresina.

a percentage Pure Life Seed (PLS) of 80% according to information contained on the label of the purchased batch.

After the pre-established contact times (3, 6, 9, 24, 48, or 72 h) of the Massai grass seeds with the phosphate fertilizer, tweezers were used to place 50 seeds on paper for seed germination (paper towel – "germitest"), in triplicate.

The seeds were initially moistened with 12 ml of distilled water, using 2.5 times the mass of the dry paper soaked in water, maintaining the necessary moisture throughout the trial. The papers were rolled and placed in a BOD (biochemical oxygen demand) incubator - Novatécnica: Equipamentos para laboratório®, Brazil - for 8 h at 35°C in light and for the remaining 16 h at 25°C in the dark, according to Santos *et al*. (2013).

Germination was assessed at 7 and 21 d and the seedlings considered normal were computed according to the criteria established in the Rules for Seed Analysis (MAPA, 2009). The length of normal seedlings from the germination test was measured using a millimeter ruler from the apex of the primary root to the apex of the aerial part (MAPA, 2009).

Furthermore, an analogous method was carried out in soil-filled pots by planting 50 seeds in triplicates of each treatment and leaving them in full sun conditions (November to December of 2016, without rain in the period, and average temperature in 28°C). Soil moisture was kept constant, and the duration of the test was the same as the laboratory test, evaluating the emergence at 7 and 21 d. The seeds were incorporated into the soil at a depth of 1 cm. The soil had the following chemical characteristics: pH (water), 7.7; P (Mehlich 1) 12 mg dm^{-3}; K, 0.51 $cmol_c\ dm^{-3}$; H+Al,1.5 $cmol_c\ dm^{-3}$; OM, 1.1 g dm^3, and sandy texture (9% clay).

In the full sun trial, the germination speed index (GSI) was also evaluated, using Equation 1 (Maguire, 1962):

$$GSI = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \dots + \frac{G_n}{N_n} \quad (1)$$

where: G_1, G_2, G_n = number of seedlings germinated at the first, second, until the last count and N_1, N_2, N_n = number of days from the first, second, until the last count.

Assumptions of normality (Shapiro-Wilk test) and homogeneity of variances (Bartlett test) were tested to determine if the data were suitable for analysis of variance using the F test; when significant, the Scott-Knott mean test (5%) was applied and polynomial regression analysis was performed with the aid of the SISVAR software (Ferreira, 2011).

Results and discussion

Table 1 presents the average values of PG, growth, and seedling emergence of Massai grass as a function of contact times with SSP, which were significant for germination at the first and second counts (measurement days), shoot height of seedlings, emergence at 21 d, and GSI. For the second germination count (21 d) and GSI, no differences were found for up to 3 h of contact; however, for the first germination count (7 d), shoot height and emergence at 21 d, the contact of seeds with fertilizer for 3 h already promoted negative effects on these variables of 23.33, 12.13, and 14.28%, respectively.

TABLE 1. Average values of seed germination of Massai grass at 7 and 21 d, length of radicle and shoot, and seedling emergence percentage at 7 and 21 d as a function of the seed contact time with single superphosphate.

Times h	Germination		Radicle length	Shoot height	Emergence		GSI
	7 d	21 d			7 d	21 d	
	%		cm		%		
0	30[a]	51[a]	2.71	1.73a	44	77[a]	49[a]
3	23[b]	51[a]	2.96	1.52[b]	37	71[b]	42[a]
6	20[c]	41[b]	2.95	1.36[c]	33	67[c]	37[b]
9	26[b]	40[b]	2.97	1.36[c]	37	64[c]	35[b]
24	23[b]	40[b]	3.23	1.34[c]	35	63[c]	35[b]
48	17[c]	37[b]	3.03	1.33[c]	21	63[c]	28[b]
72	15[c]	36[b]	3.02	1.15[d]	38	64[c]	34[b]
Test F	**	**	ns	**	ns	**	**
CV (%)	11.5	13.7	11.2	6.9	21.8	4.6	11.7

[ns], ** - Non significant and significant at 5% probability. [1]Means followed by the same letter in the column do not differ according to the Scott-Knott test (5%). GSI - germination speed index.

Germination rates at 7 d (Fig. 2) fitted best to a decreasing linear model (P<0.05). However, for germination at 21 d (Fig. 3), shoot height (Fig. 4), seedling emergence at 21 d (Fig. 5) and GSI (Fig. 6), they were fitted to a decreasing exponential model, with stabilization of the reduction of these variables after 24 h of contact of seeds with the phosphate fertilizer. A similar result has already been verified in other studies evaluating contact times between fertilizers and forage seeds (Maciel *et al.,* 2019; Ferreira *et al.,* 2020).

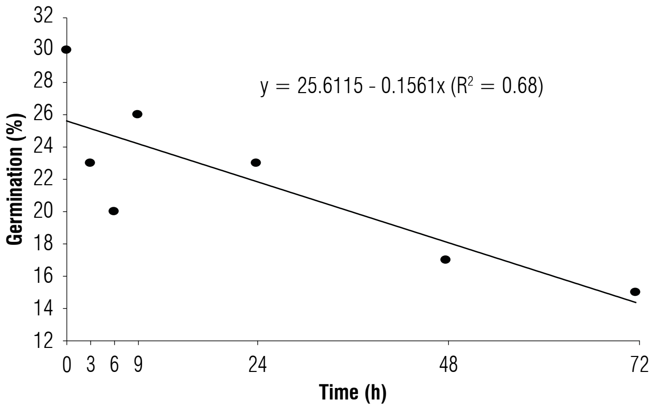

FIGURE 2. *Megathyrsus maximus* Masai grass seed germination at 7 d as a function of contact times with single superphosphate.

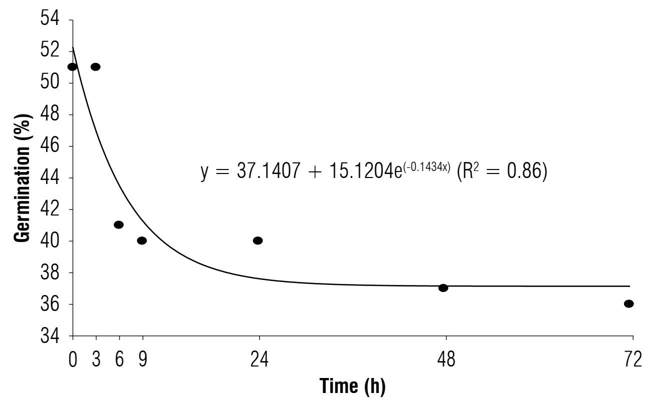

FIGURE 3. *Megathyrsus maximus* Masai grass seed germination at 21 d as a function of contact times with single superphosphate.

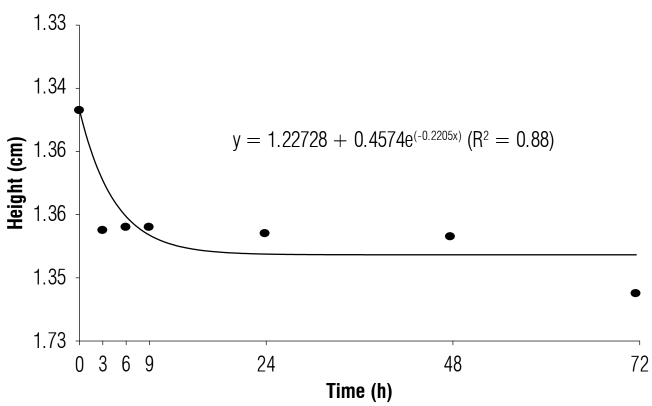

FIGURE 4. Shoot height of *Megathyrsus maximus* 'Massai' grass seedlings as a function of contact times with single superphosphate.

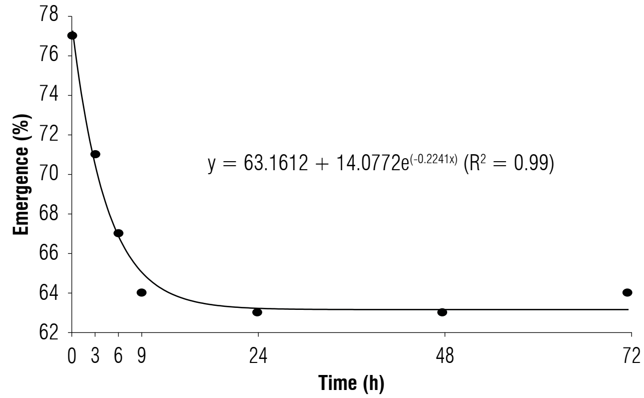

FIGURE 5. Seedling emergence at 21 d of *Megathyrsus maximus* 'Massai' grass as a function of contact times of seeds with single superphosphate.

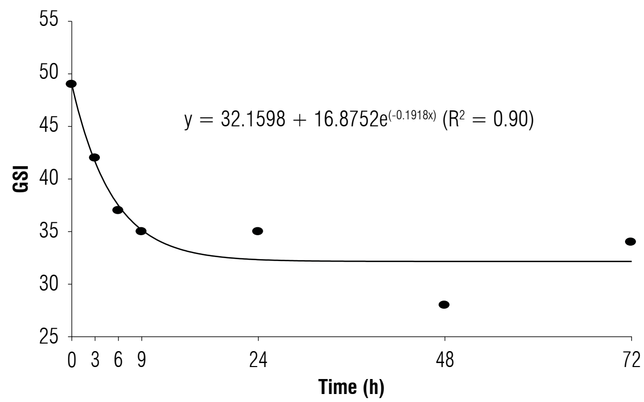

FIGURE 6. Germination speed index (GSI) of *Megathyrsus maximus* 'Massai' grass as a function of contact times of seeds with single superphosphate.

The stabilization could be due to the saline effect of the fertilizer. From 0 to 9 h of ion-seed contact, seeds had not yet reached their maximum osmotic potential (Ψs), which only occurs within 24 to 72 h, possibly allowing the fertilizer to break the cell cytoplasmatic membrane, consequently reducing seed germination and seedling development.

However, other studies mention that phosphate fertilizers are obtained by processing the phosphate rock using sulfuric and phosphoric acids (Cheremisina *et al.*, 2019; Sari *et al.*, 2020; Samrane & Bouhaouss, 2022). Therefore, some processing residues could negatively affect the germination and vigor of the seeds, and such negative effect can be accentuated with the contact time with fertilizer (Soratto *et al.,* 2003).

When evaluating the development of the plant, a small growth in height of the seedlings was observed when they remained in contact with the fertilizer for a longer period; thus, possible deleterious effects could be linked not only to seed germination and vigor. The decrease in values obtained in the SH variable is due to the loss of water and nutrients present in the seed in mixture of fertilizer, probably due to its salinity. With little water inside the cells, cell turgidity does not occur, which together with calcium, phosphorus and nitrogen, is responsible for cell division and consequently the increase in SH. This result has also been observed for another source of phosphorus (triple superphosphate) mixed with seeds (Lima *et al*., 2009).

Another point to be highlighted in the present work is that both trials, full sun field exposure and laboratory, yielded results with the same trend, with a decrease in germination and emergence as the contact times increased.

Studies on the effects of contact time between fertilizers and seeds evaluated millet (Soratto *et al.,* 2003), *Brachiaria brizantha* (Lima *et al.,* 2009; Lima *et al.,* 2010), and *Brachiaria ruziziensis* (Dan *et al.,* 2011). All studies reported trends close to those of the present study. Therefore, regardless of the species, contact between fertilizers and forage seeds is deleterious if it remains for long periods.

Single superphosphate (SSP) and monoammonium phosphate (MAP), another source of phosphorus, were kept in contact of with seeds of *Brachiaria brizantha* cv. Marandu. Considering an acceptable germination rate of 60%, the seeds can be kept in contact with SSP and MAP at a rate of 80 kg P_2O_5 ha^{-1} for 71.2 and 16.2 h, respectively (Peres *et al.,* 2012).

The loss of physiological quality of *M. maximus* cv. Massai seeds was, probably, due to the imbalance in osmotic potential of the seeds, which may be due to the cationic characteristic of the phosphate fertilizer, which prevented water uptake by the seeds (Chien *et al.,* 2011). Due to the ionic strength of several salts, the fertilizer can be in contact with the developing plant tissues, potentially causing phytotoxicity (Pereira *et al.,* 2012).

Conclusion

The longer the contact time between single superphosphate with *M. maximus* seeds (cv. Massai), the lower the germination and vigor values.

The mixture of seeds with single superphosphate for the renewal or planting of pastures can be recommended as long as the seed sowing is immediate.

Conflict of interest statement

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

Author's contributions

HCAR formulated the overarching research goals and aims. RRM carried out activities to annotate scrub data and maintain research data for initial use and later re-use. RCTB applied statistical, mathematical, computational. EDON and HAS carried out the critical review. All authors reviewed the final version of the manuscript.

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