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EVALUADORES

El Comité Editorial dentro de sus políticas, envía los artículos a especialistas, con el fin de que sean revisados. Sus observaciones en adición a las que hacen los editores, contribuyen a la obtención de una publicación de reconocida calidad en el ámbito de las Ciencias Agrarias. Sus nombres son mencionados como una expresión de agradecimiento.

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EDITORIAL

El aumento de las inundaciones generado por el cambio climático afectará nuestros cultivos

En las primeras publicaciones parciales del Sexto Informe del Grupo Intergubernamental de Expertos sobre el Cambio Climático (IPCC) de agosto de 2021 se advierte sobre la situación climática que se está agravando. Por las recientes inundaciones en varios países del mundo y los conocidos episodios de lluvias torrenciales en Colombia en los últimos 15 años, el cambio climático tiene mucho que ver con este fenómeno que se manifiesta en eventos extremos, como es el cambio en el patrón de lluvias, el aumento de la temperatura y sequías prolongadas que influyen en la seguridad alimentaria. El anegamiento afecta la agricultura reduciendo la calidad de los suelos y la productividad de muchos cultivos.

Las predicciones en el cambio de patrón e intensidad de las lluvias pronostican inundaciones en varias partes del mundo, para el caso de los Andes especialmente en la parte norte y con más frecuencia en la altitud, mientras en los terrenos bajos y en el sur de Sudamérica se reducirán. Sumando a esto, se estima que el 13% de las tierras de América Latina se caracterizan por un drenaje deficiente debido a su fisiografía que propicia la inundación. El mayor peligro lo tienen las plantaciones cercanas a los ríos que no sólo dependen del cambio climático sino también de la ocurrencia de factores ambientales como lluvias intensas, tormentas, desbordamiento de ríos y riego excesivo. De todas maneras, los incidentes por anegamientos e inundaciones han aumentado en su frecuencia y son impredecibles en todo el mundo, sobre todo por precipitaciones erráticas y no estacionales. Las épocas pluviosas en suelos con drenajes deficientes producen condiciones anaeróbicas que son perjudiciales para las raíces de las plantas.

El oxígeno en terrenos inundados disminuye porque la difusión de gases en el agua es 10.000 veces más lenta en comparación al suelo bien aireado, lo que genera una crisis energética para los tejidos de las raíces por el ambiente anóxico, ocasionando hasta la muerte de la planta. Además, la deficiencia de O_2 en el suelo perjudica las comunidades microbianas y reduce numerosos nutrientes oxidados (NO^{3-}, Fe^{3+}, SO_4^{2-}) generando niveles altos de compuestos reducidos (Mn^{2+}, Fe^{2+}, NH^{4+}, H_2S) y compuestos orgánicos que pueden ser tóxicos para las plantas.
En la planta, además del efecto sobre la absorción de nutrientes y agua por la carencia de energía, el impacto más grave lo provoca sobre la fotosíntesis debido a la reducida conductancia estomática y el cierre de estomas, así como el menor crecimiento de las hojas, clorosis, quemazón y finalmente la caída foliar. Las condiciones de exceso de humedad en el suelo favorecen la incidencia de patógenos, como por ejemplo, la *Phytophthora* spp. en aguacate, papaya y piña; *Fusarium* spp. en uchuva y banano. También es para tener en cuenta que una elevada temperatura del suelo y/o agua y una radiación solar alta durante el anegamiento aumenta su efecto adverso sobre las plantas.

Hay diferentes grados de tolerancia que las plantas presentan a las condiciones hipóxicas del suelo, siendo por ejemplo, las guayabas y cocos tolerantes, mientras el aguacate, la papaya y las pasifloráceas no lo son.

Varias especies de plantas lograron desarrollar adaptaciones de tipo morfológico, fisiológico y bioquímico a las condiciones de anegamiento. Las más efectivas son el desarrollo de un aerénquima, bien conocida en el cultivo de arroz de inundación, que son espacios intercelulares interconectados promoviendo el intercambio gaseoso entre la parte aérea y las raíces de la planta. Otra adaptación es la formación de raíces adventicias como se observó en la Sabana de Bogotá para tomate chonto o la inducción de lenticelas hipertróficas para la absorción de oxígeno desde

el agua, como las que desarrollan algunas variedades de mango. Otras plantas reaccionan con la epinastia foliar o el desprendimiento de las hojas para disminuir la transpiración, observado en uchuva.

El agricultor puede mejorar la resistencia de las plantas a esta adversidad como es injertar las variedades sobre patrones más tolerantes en el caso de los frutales. Igualmente, la micorrización ha mostrado buenos resultados mejorando la retención foliar y la absorción de nutrientes en plantas anegadas. También, la aplicación de nutrientes y fitohormonas vía foliar prolonga la tolerancia de los cultivos a las condiciones de anegamiento. En general, la siembra de cultivos no tolerantes a la hipoxia de raíces cerca de ríos, cuerpos de agua y sitios bajos en los valles debe evitarse, contando más bien con un drenaje funcional o arados con subsoladores profundos en terrenos bien nivelados. Otra posibilidad es la siembra de las plantas sobre caballones o instalar zanjas profundas entre las filas de las plantas (como por ejemplo en papaya o banano) en sitios propensos a las inundaciones.

Los eventos extremos a causa del anegamiento de terrenos agrícolas van a seguir aumentando, también en sitios en los cuales antes no se esperaba por lo cual un enfoque multifacético es necesario incluyendo la creación de variedades y patrones tolerantes, estudios de la fisiología de la planta anegada y las medidas de manejo apropiadas para enfrentar este riesgo climático.

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P.S. El autor de este editorial publicará un artículo de revisión extenso sobre el tema del anegamiento en uno de los próximos números de esta revista.

Research article

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Grafting effect on photosynthetic activity and yield of tomato under a plastic house in Colombia

UNIVERSIDAD NACIONAL DE COLOMBIA

Efecto del portainjerto sobre la actividad fotosintética y el rendimiento del tomate cultivado bajo cubierta en Colombia

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ABSTRACT

Grafting is an effective approach to improve tomato yield and for tolerance to various abiotic and biotic stresses. This technique consists of using a vigorous or resistant plant (rootstock) to replace the root system of a genotype of economic interest (scion) but susceptible to one or more stress factors. The present work aimed to evaluate the physiological and productive response of a commercial tomato scion grafted on different rootstocks in Colombia's high-Andean region. For this purpose, a tomato cv. Libertador was grafted on two commercial ("Olimpo" and "Armada") tomato rootstocks in a randomized complete block experimental design. Four scion×rootstock combinations were evaluated by vigor rootstock, resistant rootstock, self-grafting, and non-grafted plants. Net photosynthesis, transpiration rate, stomatal conductance, water use efficiency, and radiation use efficiency were evaluated during six phenological stages (701, 704, 706, 708, 710, and 712), according to the BBCH scale; while the leaf area index and quantum yield were analyzed in five phenological stages (except 706). The highest values of photosynthesis, stomatal conductance, water and radiation use efficiency were registered in the initial phase of the production stage (701), which tended to decline at the end of the life cycle (712). Transpiration rate was similar throughout the growth cycle. Nevertheless, vigor rootstock presented the lowest photosynthesis rate; it was superior in terms of leaf area index, leaves dry matter, and tomato yield. The quantum yield values of the photosystem II did not indicate photochemical injuries in any of the scion×rootstock combinations. The higher tomato yield was reached in vigor rootstock and was associated with a more significant accumulation of dry matter in the leaf and higher leaf area index.

Keywords:
Fruit yield
Photosynthesis
Quantum yield
Scion-rootstock interaction

RESUMEN

La injertación se considera una herramienta eficaz para contrarrestar múltiples factores bióticos y abióticos que limitan la producción del tomate. Esta técnica consiste en utilizar una planta vigorosao resistente (portainjerto) para reemplazar el sistema radical de un genotipo de interés económico (vástago) pero susceptible a uno o más factores de estrés. Este trabajo tuvo como objetivo evaluar la respuesta fisiológica y productiva de un vástago comercial de tomate injertado en diferentes patrones bajo condiciones de la región altoandina de Colombia. Para este propósito, el cultivar de tomate Libertador fue injertado sobre dos patrones comerciales de tomate ("Olimpo" y "Armada") en un diseño experimental de bloques completos al azar con cuatro repeticiones. Se evaluaron cuatro combinaciones de copa-portainjerto: portainjerto de vigor, portainjerto resistente, auto injertación y plantas no injertadas. La fotosíntesis neta, la tasa de transpiración, la conductancia estomática, el uso eficiente del agua y el uso eficiente de la radiación se evaluaron durante seis etapas fenológicas (701, 704, 706, 708, 710 y 712), según la escala BBCH, mientras que el índice de área foliar y el rendimiento cuántico fueron analizados en cinco etapas fenológicas (excepto 706). Los valores más altos de fotosíntesis neta, conductancia estomática, uso eficiente del agua y uso eficiente de la radiación se registraron en la fase inicial de la etapa de producción (701), con una reducción al final del ciclo de vida del tomate (712). Aunque el tratamiento vigor presentó la menorfotosíntesis neta, este fue superior en términos de índice de área foliar, materia seca de hojas y rendimiento de frutos de tomate. Los valores del rendimiento cuántico del fotosistema II no indicaron lesiones fotoquímicas en ninguna de las combinaciones de injerto y portainjerto. El mayor rendimiento de frutos se alcanzó con el uso de un portainjerto de vigor y se asoció con una acumulación más significativa de materia seca en la hoja y un mayor índice de área foliar.

Palabras clave:
Rendimiento de fruta
Fotosíntesis
Rendimiento cuántico
Interacción patrón-vástago

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Tomato (*Solanum lycopersicum* L.) is the most important fresh and processed vegetable globally (it is also considered as a fruit), and after potatoes, it is the most consumed vegetable depicting 16% of the total consumption (Heuvelink, 2018). In 2019, China (1'086,771 ha; 62.86 million t), India (781,000 ha; 19.0 million t), Turkey (181,488 ha; 12.84 million t), and United States (110,760 ha; 10.85 million t) were the major producers and have remained for several years as the world leaders in tomato cultivation (FAOSTAT, 2019). The world average tomato productivity was about 3.7 kg m^{-2}; nonetheless, the Netherlands stands out as the country with the highest yield (50.7 kg m^{-2}), far from countries considered as main producers, such as China (5.78 kg m^{-2}), India (2.43 kg m^{-2}), Turkey (7.07 kg m^{-2}) and United States (9.8 kg m^{-2}) (FAOSTAT, 2019). In Colombia (18,608 ha; 0.82 million t) the average tomato yield in open-field cultivation is 2.86 kg m^{-2}, while in greenhouse conditions reaches values of 8.3 kg m^{-2} (Agronet, 2019). However, despite the huge global area cultivated with tomato, the use of grafted plants represents only 0.81% (600 ha) in the United States and a maximum of 1% in China (10,000 ha). By contrast, in countries such as Vietnam, Korea, Japan, France, and the Netherlands, the grafted tomato crop represents between 25 and 75% of the total area (Singh *et al*., 2017; Singh *et al*., 2020). According to Lee *et al*. (2010), the use of grafted plants is a strategy that has been increasing in parallel with the growth of the tomato crop under protected conditions. In its beginnings, the use of grafted materials was done for the prevention of biotic limitations, but nowadays, grafting is considered an effective strategy to improve tolerance of plants to abiotic stresses (Ashok and Sanket, 2017; Gaion *et al*., 2018; Meimandi and Kappel, 2020; Reddy, 2016; Sen *et al*., 2018; Singh *et al*., 2019), such as soil salinization, extreme temperatures and humidity, high or low radiation, water stresses, heavy metals and organic pollutant (Nordey *et al*., 2020; Rouphael *et al*., 2017; Singh *et al*., 2020; Xie *et al*., 2020; Zhang *et al*., 2019). Regarding the use of tomato grafted plants, various studies have shown increases in yield compared to non-grafted plants (Grieneisen *et al*., 2018; Khah *et al*., 2006; Reddy, 2016; Turhan *et al*., 2011; Zeist *et al*., 2017); nonetheless, Bhatt *et al*. (2015) found no differences between grafted and non-grafted plants, and even Goto *et al*. (2013) reported that the use of graft plants has a negative effect on tomato crop yield.

Various studies have been carried out to determine the effect of this relationship on the grafted plants growth, development, biomass production, and photosynthetic activity (Martínez-Ballesta *et al*., 2010). Bhatt *et al*. (2015) observed an increase in photosynthetic activity (23%) in young tomato grafted plants (60 to 70 days after transplanting - *dat*) relating to self-grafted and non-grafted plants. In comparison, He *et al*. (2009) indicated that no significant differences in photosynthetic activity were found for the same combination of plants, with values between 23 and 25 µmol CO_2 m^{-2} s^{-1}. Similar results were obtained by Zhang and Guo (2019), who determined that photosynthesis in tomato scion grafted to a potato rootstock was similar to tomato seed plants (14.4 to 16.4 µmol CO_2 m^{-2} s^{-1}). On the other hand, few studies have been carried out in adult plants (>70 *dat*). Fullana-Pericàs *et al*. (2018) measured gas exchange (100 *dat*) in tomato plants grafted in "Maxifort" commercial rootstock showed significantly higher net photosynthesis (33 µmol CO_2 m^{-2} s^{-1}) than non-grafted (25 µmol CO_2 m^{-2} s^{-1}) and grafted on 'Beaufort' rootstock (27 µmol CO_2 m^{-2} s^{-1}). Nonetheless, there is no consensus about the benefits of grafting tomato, despite the variety of research carried out worldwide; the results have not been conclusive, ranging from a positive physiology effect and production parameters to adverse outcomes, for that reason, "the use of grafting affects the physiology of the tomato plant and increase fruit yield becoming a good strategy for the Colombian tomato producer" was stated as hypotheses; keeping this in view, the present work aimed to evaluate the physiological response of a commercial tomato scion, grafted on different rootstocks in conditions of the high-Andean region in Colombia.

MATERIALS AND METHODS

Location: the present experiment was carried out under plastic house conditions, at the municipality of El Santuario, Antioquia, Colombia (6°6'55.8"N and 75°13'10.15"W, an altitude of 2,251 m), which is localized in the high-Andean region. A sandy loam-clay-sandy textural class soil was used in the experiment, pH (5.0), EC (0.06 $dS \cdot m^{-1}$), soil organic matter (5.8%), phosphorus (66 $mg \cdot kg^{-1}$ soil), sulfur (53.2 $mg \cdot kg^{-1}$ soil), Ca (10.6 cmolc kg^{-1}), Mg (3.0 $cmolc \cdot kg^{-1}$), K (2.47 cmolc kg^{-1}), ECEC (16.5 cmolc kg^{-1}), Fe (74 mg kg^{-1}), Mn (9 mg kg^{-1}), Cu (9 mg kg^{-1}), Zn (5 mg kg^{-1}) and B (0.2 mg kg^{-1}). During

the tomato growth period (April 29 to October 25, 2019), with a portable thermohygrometer (Extech RHT20), the climatic variables recorded inside the plastic house were minimum, mean, and maximum temperature (°C) and relative humidity (%).

Experimental design: a randomized complete block experimental design was used, with four (4) replication and four (4) treatments. The treatments consisted of a single commercial tomato scion grafted on different rootstocks combination: vigor rootstock (VR), resistant rootstock (RR), self-grafting plants (SELF), and plants no grafted (Seedlings).

Plant material: the genotype (*S. lycopresicum* L.) used as a scion was a tomato cv. Libertador, and as a rootstock, two commercial materials were used: "Olimpo" as vigorous rootstock, and "Armada" as resistant rootstock to *Ralstonia solanacearum* and *Fusarium oxysporum* f. sp. *radicis-lycopersici*. The grafting method applied was the tongue approach grafting, described by Lee *et al*. (2010). The Seedlings and grafted plants were transplanted to the plastic house on April 29, 2019, each of them, with the third leaf on the main shoot folded, corresponding to 103 stages according to BBCH scale proposed by Feller *et al*. (1997). The field planting distance was 1.1 m between rows and 0.45 m between plants, and as part of the management, the first lateral shoot was allowed to grow below the first inflorescence to have two stems per plant, for a density of 20,200 plants ha^{-1} and 40,400 stems ha^{-1}. The growth of the plants was allowed until the ninth fruit cluster's emission on the main stem and seventh fruit cluster on the lateral stem, for a total of 16 fruit clusters emitted throughout the life cycle.

Gas exchange parameters: according to the BBCH scale in the 701 (first fruit cluster), 704 (fourth fruit cluster), 706 (sixth fruit cluster), 708 (eighth fruit cluster), 710 (tenth fruit cluster), and 712 (twelfth fruit cluster) phenological stages, with the portable infrared gas analyzer LCi - ADC (Bioscience, UK), provided by an external halogen lamp ADC (Bioscience, UK) gas-exchange measurements were done within the youngest completely expanded leaf closes to each fruit cluster, under a saturating photosynthetic photon flux density (*PPFD*) of 1,100 µmol m^{-2} s^{-1}. Net photosynthesis (*A*, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) and transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}) were measured between 9:00 am and 11:00 am. The water-use efficiency (*WUE*) was calculated as the ratio between the net CO_2 assimilation rate (*A*) and the transpiration rate (µmol CO_2 mmol H_2O^{-1}), and radiation use efficiency (*RUE*) as the ratio *A*/*PPFD* (µmol CO_2·µmol $photon^{-1}$).

Chlorophyll fluorescence: it was measured in the same leaf used to determine the gas exchange parameters at the same phenological stages (except 706). Measurements were performed with a portable fluorimeter Opti-Sciences (Opti-Sciences Inc., Tyngsboro, MA, EEUU). The measurements were taken on dark-adapted leaves for 30 min before each reading began, and daily measurements of quantum yield of PSII- *Qy* (*Fv*/*Fm*) were made.

Leaf area index (LAI): it was measured in the same tomato phenological stages used to determine the chlorophyll fluorescence parameters through a 'SunScan' Canopy Analysis System System SS1 (Delta-T, UK).

Dry matter and tomato yield: at the end of the crop cycle, the remaining leaves of each plant were harvested, and the dry matter was determined. For this, the fresh biomass was dried in an oven (Memmert UL 80) at 60 °C, until a constant weight was reached. Harvest index (HI) was determined by the proportion of dry biomass of tomato in relation to total dry biomass. Tomato yield was estimated as the sum of the total weight of 16 fruit clusters harvested per plant (kg $plant^{-1}$). The harvest started at 89 *dat* (07/21/2019) and ended at 188 *dat* (10/28/2019).

Statistical analysis: one-way ANOVA analyzed the variances among different treatments, and their multiple comparisons were analyzed by Tukey's HSD (Honestly significant difference) test, $P<0.05$. When the data did not meet the assumptions of normality, additivity and homogeneity of variance, the Kruskal-Wallis test, and the mean comparison (LSD, $P<0.05$) were applied using the R project "agricolae" package.

RESULTS AND DISCUSSION

The mean temperature into plastic house fluctuated between 20 and 25 °C, within the optimal range (18 to 25 °C) to develop the crop (Camejo *et al*., 2005; Heuvelink, 2018). On the other hand, 30 to 40 °C was the maximum temperature reached, and the minimum temperature

was between 10 to 15 °C (Figure 1). Regarding relative humidity, mean daily values between 70 and 90% were recorded, which, as indicated by Jaramillo-Noreña *et al*. (2012), would be above the optimal mean daily values from 50 to 65% of this climatic variable for the crop (Figure 1).

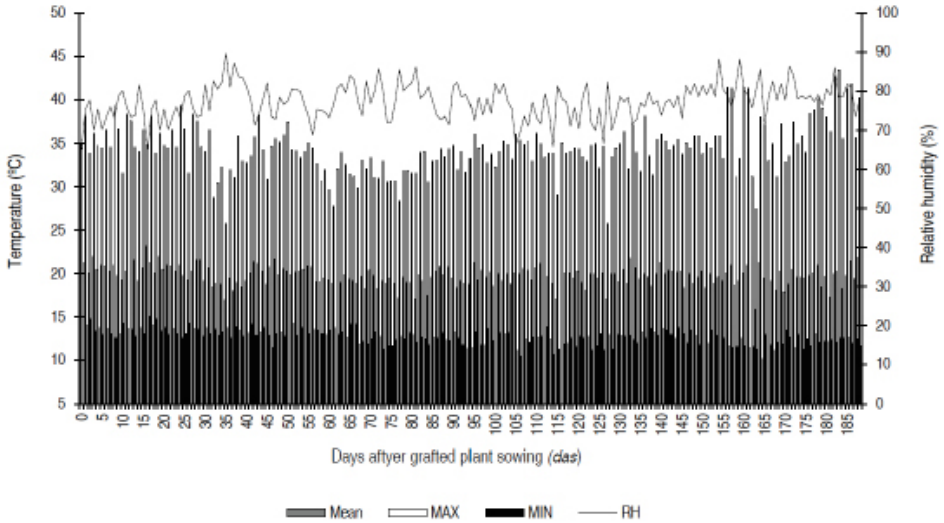

Figure 1. Daily mean (Mean), maximum (MAX) and minimum (MIN) temperature and relative humidity (RH) into plastic house between April 23 and October 25, 2019.

Gas exchange: the highest photosynthesis rate (*A*) was recorded in the initial stages of the production phase (701), reaching values of 26 to 32 µmol CO_2 m^{-2} s^{-1}, about the final phase (712), where photosynthetic activity declined on average by 73% (Table 1). At the initial stages (plants 37 *dat*) of development (701), no significant differences were found between the treatments due to scion×rootstock combinations; nevertheless, in more advanced stages of development 704 (fourth fruit cluster), the use of resistant rootstocks (RR) and self-graft (SELF) reached the highest *A* values, which showed significant differences respect to the vigor rootstock (VR) and non-grafted plants (Seedling) (Table 1).

Large differences between the initial phenological stages (701) and the other ones may indicate that the tomato in its initial stages becomes a strong source because this part of the cycle begins processes of floral differentiation followed by fruit formation; likewise, the emission of lateral branches occurs and the leaf area increases, thus, the photosynthetic activity decreases.

The *A* values for young plants (701 - 37 *dat*) are considered high, since the values reported by Bhatt *et al*. (2015) for scion×rootstock combinations do not exceed a fixation rate of 20 µmol CO_2 m^{-2} s^{-1} and, even according to Camejo *et al*. (2005) and Ogweno *et al*. (2008) not grafted plants (60 growing days) reached 10 µmol CO_2 m^{-2} s^{-1} of *A* at constant *PPFD* of 350 µmol photons m^{-2} s^{-1} and 20 µmol CO_2 m^{-2} s^{-1} to 800 µmol photons m^{-2} s^{-1} of radiation. On the other hand, Khan *et al*. (2019) in non-grafted plants determined an increase in photosynthetic activity with age, *A* values of 8 and 13 µmol CO_2 m^{-2} s^{-1}, with a *PPFD* of 800 µmol photons m^{-2} s^{-1}, were observed for plants with 60 and 90 *dat* respectively; which was different from that observed in the present study, where plants in advanced phenological states, recorded less than 10 µmol CO_2 m^{-2} s^{-1} for *A* values.

Both plants, at the beginning and end of development, non-statistical differences were observed in transpiration rate (*E*), with an average of 7 mmol H_2O m^{-2} s^{-1}, except to phenological stage 708, where the VR and RR treatments showed an increase 78% of *E* compared to self-grafted and non-grafted plants of the same age (Table 1).

Similar to photosynthesis, stomatal conductance (g_s) was higher in both grafted and non-grafted plants, at

Table 1. Evaluated parameters among different scion×rootstock combinations in different tomato phenological states according to the BBCH scale *(*701, 704, 706, 708, 710 and 712).

Parameter	Scion×rootstock	Phenological stage *(*BBCH)					
		701	704	706	708	710	712
A (µmol CO_2 m^{-2} s^{-1})	VR	26.13±4.28 a	5.70±1.03 b	7.65±1.13 b	4.82±2.83 b	6.54±0.43 ab	7.58±1.69 b
	RR	31.90±4.01 a	6.92±1.95 ab	9.38±1.83 a	7.68±2.34 a	6.77±2.46 ab	11.35±5.53 a
	SELF	28.37±3.08 a	7.91±2.33 a	10.73±2.60 a	5.74±3.11 ab	8.94±3.54 a	10.88±4.98 a
	Seedling	25.96±3.92 a	7.21±2.41 a	7.79±3.09 a	1.94±1.30 c	5.45±3.02 b	9.40±1.27 a
E (mmol H_2O m^{-2} s^{-1})	VR	6.58±1.02 b	6.23±0.90 a	7.06±0.69 a	10.12±1.52 a	3.57±0.18 c	8.25±2.39 a
	RR	7.97±0.91 a	6.38±1.35 a	7.12±0.87 a	10.39±1,67 a	6.72±3.18 ab	7.50±1.49 a
	SELF	8.31±2.19 a	6.52±0,89 a	7.32±2.17 a	6.89±2.17 b	6.25±0.90 ab	7.98±2.12 a
	Seedling	7.41±0.91 ab	6.33±0.95 a	6.94±0,52 a	4.65±0.98 c	5.63±3.59 b	7.88±2.54 a
g_s (mol H_2O m^{-2} s^{-1})	VR	0.54±0.34 ab	0.26±0.03 c	0.34±0.04 a	0.29±0.07 a	0.09±0.01 b	0.26±0.09 a
	RR	0.54±0.34 ab	0.27±0.06 ab	0.33±0,02 a	0.29±0,08 a	0.19±0,11 a	0.21±0.06 a
	SELF	0.44±0.30 b	0.30±0.02 a	0.35±0.03 a	0.17±0.08 b	0.17±0.04 a	0.26±0.10 a
	Seedling	0.55±0.33 a	0.27±0.04 bc	0.32±0.06 a	0.09±0.02 c	0.17±0,13 a	0.24±0.11 a
WUE (µmol CO_2 $mmol^{-1}$ H_2O)	VR	3.82±1.33 a	0.94±0.25 a	1.10±0.22 b	0.46±0.22 b	1.83±0.10 a	0.98±0.32 b
	RR	4.12±1.46 a	1.10±0.24 a	1.32±0.25 ab	0.73±0.14 a	1.27±0.72 b	1.65±0.89 a
	SELF	3.66±1.11 a	1.27±0.55 a	1.47±0.29 a	0.82±0.39 a	1.37±0.74 b	1.40±0.57 a
	Seedling	3.61±1.03 a	1.19±0.52 a	1.14±0.50 b	0.38±0.21 b	1.10±0.36 b	1.31±0.43 a
RUE (µmol CO_2 $µmol^{-1}$ photon)	VR	0.024±0.011 a	0.005±0.001 b	0.007±0.001 b	0.004±0.003 b	0.002±0.000 a	0.007±0.002 b
	RR	0.029±0.008 a	0.006±0.002 ab	0.009±0.002 a	0.007±0.002 a	0.002±0.001 b	0.010±0.005 a
	SELF	0.026±0.003 a	0.007±0.002 a	0.010±0.002 a	0.005±0.003 ab	0.002±0.001 b	0.010±0.005 a
	Seedling	0.024±0.004 a	0.007±0.002 a	0.007±0.003 b	0.002±0.001 c	0.002±0.000 b	0.009±0.001 a

Vigor rootstock (VR), resistance rootstock (RR), self-grafting (SELF) and plants no grafted (Seedlings). Data are means±s.d. *(*n=4). Different letters denote significant differences among combinations (scion×rootstock) within each phenological state, according to LDS test *(P*<0.05).

the beginning (701) of the productive stage, compared to those in more advanced production states (710), where g_s declined on average by 53% (Table 1). In the 701 phenological stage, significant differences were observed with a reduction of g_s by 19%, in self-graft plats, concerning the other two combinations of scion×rootstock (VR and RR) and plants from seed (Seedling) (Table 1). Although the higher g_s (0.54 mol H_2O m^{-2} s^{-1}) was achieved at the start of production (701) in the VR, RR, and SELF-treatments, these observed values are lower than those reported in other works, where g_s varied between 0.6 a 0.8 mol H_2O m^{-2} s^{-1} (Camejo *et al*., 2005; Khan *et al*., 2019; Ogweno *et al*., 2008). On the other hand, higher g_s values were reported by Bhatt *et al*. (2015) in young tomato plants grafted, self-grafted, and non-grafted, where stomatal conductance between 0.2 and 0.25 mol H_2O m^{-2} s^{-1} was reached.

All scion×rootstock combinations make young tomato plants more efficient, *WUE* and *RUE* showed values of 4 µmol CO_2 $mmol^{-1}$ H_2O and 0.025 mmol CO_2 $µmol^{-1}$ photons. Nevertheless, a decrease of *WUE* (70%) and *RUE* (77%) was observed at the final phenological stages (Table 1). In general, no relevant result for *WUE* and *RUE* was observed for any scion×rootstock combination through different phenological stages. Bhatt *et al*. (2015) showed *WUE* values for young grafted and non-grafted tomato plants of 3.2 to 3.4 µmol CO_2 $mmol^{-1}$ H_2O, and for plants in the productive stage of 1.1 µmol CO_2 $mmol^{-1}$

H_2O, comparable to those reported by Khan *et al.* (2019). According to some authors, the *RUE* for tomato varies from 0.0141 to 0.0286 mmol CO_2·µmol^{-1} photons, being similar to the efficiency between tomato plants in the initial and final stages of the productive cycle (Bhatt *et al.*, 2015; Camejo *et al.*, 2005; Khan *et al.*, 2019; Ogweno *et al.*, 2008).

Chlorophyll fluorescence: the quantum yield (*Qy*) of photosystem II (*PSII*) did not differ significantly through the different phenological stages (701 to 712) of the tomato according to BBCH scale, for scion×rootstock combinations evaluated (Figure 2). The *Qy* values varied from 0.74 to 0.79, according to various authors, these values are considered as normal in healthy plants and without stress, which depending on the genotype, can vary between 0.69 and 0.80 (Bhatt *et al.*, 2015; Goto *et al.*, 2013; Zhou *et al.*, 2018).

Calatayud *et al.* (2013) considered *Qy* as a practical and direct parameter to determine incompatibility between rootstocks and scion. On the other hand, Martínez-Ballesta *et al.* (2010) affirm that the incompatibility of the graft can become evident in the early stages, when vascular connections are formed, or also, in the fruiting stage, when the plant has a high demand for water and nutrients. Nevertheless, in this study, as shown in Figure 2, the *Qy* of *PSII* was always in values that indicated physiological health and the absence of post-graft stress.

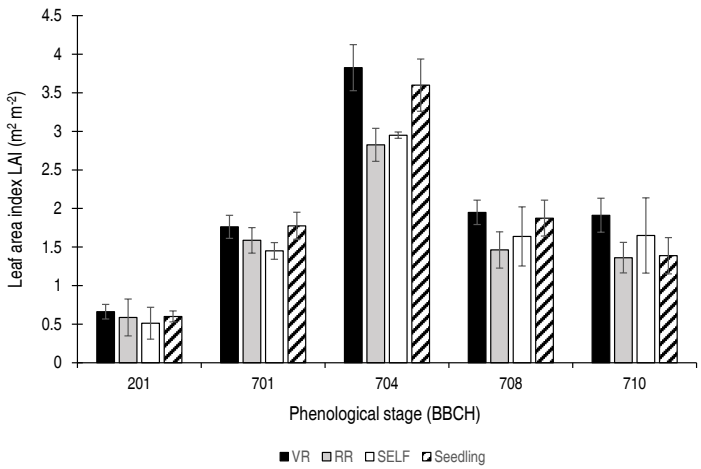

Figure 2. Quantum yield *(Qy)* among the different scion×rootstock combinations: vigor rootstock *(*VR), resistance rootstock *(*RR). Self-grafting *(*SELF) and plants no grafted (Seedlings), in different tomato phenological states (701, 704, 708, 710 and 712) according to the BBCH scale. Data are means±s.d. (n=4) and denote significant differences among combinations within each phenological state, according to LDS test (*P*<0.05).

Leaf area index: when tomato plants reached their highest leaf development (704), significant differences were found between scion×rootstock combinations. The highest *LAI* was obtained by VR (3.8) compared to RR (2.8) and SELF (2.95), but similar to Seedling (3.6), indicating that the rootstock can modify the physiological components of yield in tomato, such as *LAI* (Figure 3). According to Heuvelink (2018), the optimal *LAI* for tomato plants is 4 to 5, values that were achieved for VR treatment at 704 phenological stage (3.8). From this moment of cultivation, it was necessary to carry out pruning to form the plant architecture and eliminate lateral shoots and diseased branches and leaves, thus, from this phenological stage (704), was evident an *LAI* reduction, with values remaining between 1.5 to 2.0, without significant differences between scion×rootstock combinations.

Dry matter: the scion×rootstock combination that generated the greatest accumulation of dry biomass in leaves, fruits, and the whole tomato plant was the VR, differing significantly from the other combinations. VR accumulated 44% more leaf biomass and 28% more dry biomass in the entire plant, which can be related positively to the highest *LAI* reached at 704 phenological stage by RV (Figure 3, Table 2). However, although authors such as Nilsen *et al.* (2014) affirmed that the callus formed by

the scion×rootstock union can reduce not only the flow of water to the shoots but also the leaf area and limits the transport of photoassimilates to the roots; this results in a slower growth of grafted plants than the non-grafted ones. In this study, the results did not show a negative effect leaf development by the scion×rootstock combinations evaluated (Figure 3, Table 2), the use of a rootstock with vigor characteristics (VR) represented a significant gain in the total dry matter of leaves and fruits in relation to the plants grafted in resistant rootstock (RR), self-grafted (SELF) and plants from seed (Figure 3); similar to that exposed by Calatayud *et al*. (2013) who indicate that leaf weight and leaf area are directly correlated, the higher the weight, the greater the leaf area.

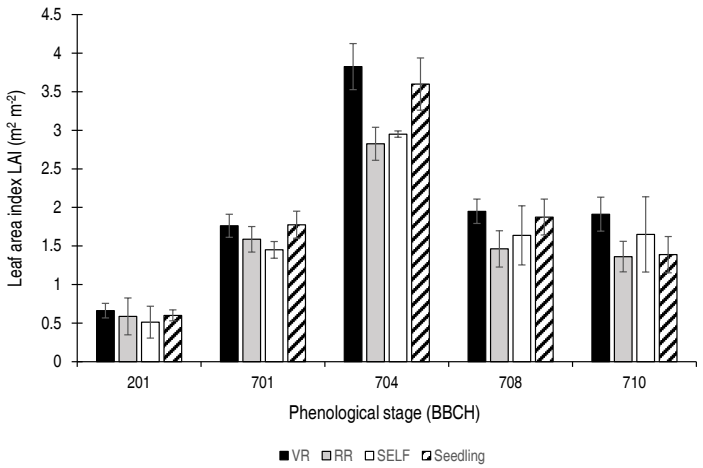

Figure 3. Leaf area index (*LAI*) among the different scion×rootstock combinations: vigor rootstock *(*VR), resistance rootstock *(*RR). Self-grafting *(*SELF) and plants no grafted (Seedlings), in different tomato phenological states *(*701, 704, 708, 710 and 712) according to the BBCH scale. Data are means±s.d. (n=4) and denote significant differences among combinations within each phenological stage, according to Honestly Significant Difference - HSD test *(P* <0.05).

An interesting relationship between the *LAI* and leaf dry matter values is proposed in the plants grafted on VR rootstock. The higher *LAI* observed on VR plants at 704 before pruning allows a higher photosynthesis in the whole canopy. In this sense, increases in photosynthesis and carbohydrate export from leaves to fruits should increase tomato fruit growth at the end of the cycle shown for VR (Lemoine *et al*., 2013).

Table 2. Dry matter, harvest index (HI) and tomato yield among the different scion×rootstock combinations in different tomato phenological states (701, 704, 706, 708, 710 and 712) according to the BBCH scale.

Dry matter	Leaf	Fruit	Whole Plant	HI	Yield
		g			kg plant^{-1}
P value	0.004213	0.000142	0.001473	0.859806	0.00321
VR	134.7±26.25 a	595.46±7.09 a	893.39±35.73 a	0.667±0.026 a	10.71±1.29 a
RR	91.8±8.45 b	452.70±38.04 b	682.82±15.70 b	0.662±0.008 a	8.31±1.14 b
SELF	91.2±10.06 b	478.54±42.59 b	707.48±11.75 b	0.675±0.027 a	8.77±1.06 b
Seedling	93.7±9.80 b	468.67±28.03 b	702.67±12.78 b	0.667±0.004 a	8.88±1.07 b

Data are means ± s.d. *(*n=4). Vigor rootstock (VR), resistance rootstock (RR). Self-grafting (SELF) and plants no grafted (Seedlings). Letters denote significant differences among combinations within each phenological state, according to Honestly Significant Difference - HSD test (*P* <0.05).

Tomato fruit yield and harvest index: regarding the HI, there was no significant difference; that is, the proportion of biomass distributed towards tomato fruits was not affected by the scion×rootstock combination. However, a significant effect on tomato fruit yield was observed. VR was the scion×rootstock combination that showed the highest yield (kg tomato plant^{-1}), which differed significantly by 22% more in yield, concerning RR, SELF, and Seedling treatments did not present statistical differences among them (Table 2). These results are opposite to the VR combinations but similar to RR and SELF combinations reported by Goto *et al*. (2013). These authors determined that grafting decreased the dry matter in leaves, the total yield of fruits compared to non-grafted plants. The grafting practice by itself did not increase the yields, as was seen in the self-grafting treatment, which did not have significant difference with the non-grafted plants (Table 2). This result is consistent with that reported by Grieneisen *et al*. (2018), who did not find significant differences in tomato yield between self-grafted and non-grafted plants, unlike the increase in tomato yield (37%) obtained when hetero grafted plants were used (rootstocks genetically different).

Nevertheless, according to Grieneisen *et al*. (2018), grafting as an agronomic strategy will not always be associated with a gain in yield; in some cases, the rootstock confers other characteristics, such as resistance to biotic stress and tolerance to abiotic stresses, without a significant increase in the scion fruit yield. To sum up, the yield parameter is the most important when deciding which scion × combination to choose. The other parameters will help to understand what makes one treatment the same or different from another.

CONCLUSION

Net photosynthesis, stomatal conductance, efficient use of water, and efficient use of radiation were higher in the initial stages of the productive stage. The quantum efficiency of the PSII did not show evidence of any stress due to grafting, since the values were those characteristics of plants with adequate functioning. Physiological parameters of the commercial scion when grafted on a vigorous rootstock were lower than those of the other scion×rootstock combinations; however, the yield, dry matter and *LAI* had an opposite behavior. Despite vigor rootstock presented the lowest values of *A*, it was the one that showed the greatest leaf, fruit, and whole plant dry matter accumulation, and the highest *LAI*, resulting in higher production of tomato fruit.

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Studies on the nature of relationships between grain yield and yield-related traits in durum wheat (*Triticum durum* Desf.) populations

Estudios sobre la naturaleza de las relaciones entre rendimiento de grano y rasgos relacionados con el rendimiento en poblaciones de trigo duro (*Triticum durum* Desf.)

https://doi.org/ 10.15446/rfnam.v74n3.92488

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ABSTRACT

This experiment was conducted at the Field Crops Institute, Agricultural Experimental Station of Setif (ITGC-AES), Eastern semi-arid areas of Algeria, during two successive cropping seasons, 2010/11 and 2011/12. The aim of the study was to evaluate the association of yield and yield-related traits and determine the direct and indirect effects of yield-related traits on grain yield. The plant materials consisted of 330 F_3 and 174 F_4 durum wheat lines along with their four parents and one control cultivar, which were evaluated under rainfed conditions in a semi-arid region. Data on nine agronomic traits were recorded. Sufficient genetic variability was observed among wheat traits as indicated by the minimum and maximum mean values and confirmed by the phenotypic and genotypic coefficients of variation that took intermediate and high estimates for most of the traits evaluated both in F_3 and F_4 generations. A high heritability (>60%) was observed for almost all the traits studied indicating the involvement of the additive action of genes in their genetic determinism. Results of stepwise regression and path analysis showed that biological yield, harvest index and number of spikes were the most determinant components of grain yield, exhibiting high positive direct effects (0.697, 0.683 and 0.293 in F_3 vs 0.695, 0.205 and 0.560 in F_4, respectively) coupled with positive and significant correlations (r=0.696*, r=0.778* and r=0.127* in F_3 vs r=0.686*, r=0.628* and r=0.491* in F_4, respectively) with this trait. These three yield-contributing traits can be considered as suitable indirect selection criteria to improve grain yield in the subsequent generation of the wheat breeding program.

Keywords:
Correlation
Durum wheat
Selection
Stepwise regression
Variability

RESUMEN

Este experimento se llevó a cabo en el Instituto Cultivos de Campo, Estación Experimental Agrícola de Setif (ITGC-AES), áreas semiáridas del este de Argelia durante dos temporadas de cultivo sucesivas, 2010/11 y 2011/12. El objetivo de este estudio fue evaluar la asociación de rendimiento y rasgos relacionados con el rendimiento y determinar los efectos directos e indirectos de los rasgos relacionados con el rendimiento de grano. El material vegetal consistió en líneas de trigo duro 330 F_3 y 174 F_4 junto con sus cuatro padres y un cultivar testigo que se evaluaron en condiciones de secano en una región semiárida. Se registraron datos sobre nueve características agronómicas. Se observó suficiente variabilidad genética entre los rasgos del trigo según lo indicado por los valores medios mínimo y máximo y confirmado por los coeficientes de variación fenotípicos y genotípicos que tomaron estimaciones intermedias y altas para la mayoría de los rasgos evaluados tanto en las generaciones F_3 como F_4. Se observó una alta heredabilidad (> 60%) para casi todos los rasgos estudiados, lo que indica la participación de la acción aditiva de los genes en su determinismo genético. Los resultados de la regresión escalonada y el análisis de ruta mostraron que el rendimiento biológico, el índice de cosecha y el número de espigas revelaron efectos directos positivos elevados junto con correlaciones positivas y significativas con el rendimiento de grano, exhibiendo grandes efectos positivos directos (0.697, 0.683 y 0.293 en F3 vs 0.695, 0.205 y 0.560 en F4, respectivamente) acoplados con correlaciones positivas y significativas (r=0.696*, r=0.778* y r=0.127* en F3 vs r=0.686*, r=0.628* y r=0.491* en F4, respectivamente) con este rasgo.. Estos tres rasgos que contribuyen al rendimiento se consideran como los mejores criterios de selección indirecta para mejorar el rendimiento de grano en la generación posterior de este programa de mejoramiento de trigo.

Palabras clave:
Correlación
Trigo duro
Selección
Regression escalonada
Variabilidad

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Durum wheat (*Triticum durum* Desf.) is the most important staple crop in Algeria. Annually, it is cultivated over 1.2 million ha with an average production of 2.2 million t in the last decade (MADRP-DSASI, 2017). It is mainly grown under rainfed conditions where its productivity is profoundly affected by abiotic stresses. In national wheat breeding programs, improving yield taking into account adaptation to environmental variation is a primary aim after the foreseeable effects of climate change, which will accentuate the action of abiotic stresses in the conditions of southern Mediterranean countries (Annicchiarico *et al.,* 2005; Rabti *et al.,* 2020; Xynias *et al.,* 2020). Under these limiting conditions of growth, where water scarcity is highly frequent, it is necessary to select adapted plant material that possesses high-yield qualities. In this context, several researchers such as Slafer *et al*. (2005), Oulmi *et al*. (2017) and Fellahi *et al.* (2020) suggested to look for genotypic variation, including the response of genotypes to abiotic such as, water deficit and end-of-season heat stress. Although genetic improvement has been responsible for 50% of yield increase under relatively less favorable conditions (Reynolds and Tuberosa, 2008), adaptation appears as a necessary characteristic to stabilize the crop production (Fellahi *et al.,* 2018; Sallam *et al.,* 2019). In this context, duration of the vegetative growth cycle, plant height and above-ground biomass have proven their significant direct effects on the yield potential achievement.

Breeding cereals for yield potential via the classical approach is based on crosses between complementary parental lines and the follow-up of hundreds or even thousands of segregant derived lines, to identify the most suitable for specific environments (Martin and Geraldi, 2002). This approach resulted in improved yield performance, particularly in favorable environments, using the grain yield as a direct selection criterion. However, it is time consuming and expensive in addition to the complexity of the genetic system that controls grain yield. Breeders are actually looking for other selection assistance methods more effective and easier to handle. Indeed, it is very interesting that the indirect selection method rapidly and efficiently identifies the best genotypes after the screening a sufficiently large number of segregating lines (Fellahi *et al.,* 2018; 2020). Applying the morphological and/or physiological traits as selection criteria is an interesting approach that attracts the attention of breeders and physiologists (Bennett *et al.,* 2011; Mühleisen *et al.,* 2013; Ben-Amar *et al.,* 2020). Limitation of indirect selection lies by the fact that the relationship between these morpho-physiological characteristics and yield is sometimes weak, complex and depends on the genetic background and the environment (Oulmi *et al.,* 2014; Haddad *et al.,* 2016). The existence of sufficient variability of physiological responses of the plant to abiotic stresses is necessary for the breeder to make any progress in improving tolerance. The main goals of the study were to study the phenotypic variability within F_3 and F_4 filial generations, analyze the association between grain yield and yield-related traits, and to identify traits that have the most direct and indirect effects on grain yield. These traits will be used as criteria of selection that can lead to the improvement of durum wheat yield under water-limited conditions.

MATERIALS AND METHODS

This study was conducted at the Agricultural Experimental Station of the Technical Field Crops Institute (ITGC-AES) in Setif (Eastern Algeria) 36°15'N, 5°87'E at 1081 masl, during two successive cropping seasons, 2010/11 and 2011/12. It focused on evaluation of F_3 and F_4 populations of durum wheat generated from three crosses made between Ofanto, Mohammed Ben Bachir (MBB), Waha and Mrb_5 varieties.

The plant material consisted of the four parents, F_3 and F_4 breeding lines and a control cultivar Boussalem, which were planted in November, each in two rows of 5 m long, 0.2 m apart. The plant material was set up in an augmented design, parents and control were replicated four times while the 330 F_3 and 174 F_4 breeding lines were not replicated. The seeding rate was 200 seeds m^{-2}. All cultural practices (soil management, fertilization, … etc.) followed for the durum wheat growing, from sowing to harvest, were those practiced by the ITGC-AES as described by Chennafi *et al.* (2011a).

The measurements were made on the duration of the vegetative phase (DVP, days), plant height (PHT, cm), above-ground biomass (BIO, g m^{-2}), number of spikes (NS, m^{-2}), number of grains (NG, m^{-2}) and grain yield (GY, g m^{-2}).

The straw yield (Str.Y, g m^{-2}) was determined by the difference between the BIO and GY. Harvest index (HI, %) was estimated as the ratio of GY and BIO. The economic yield (Econ.Y, m^{-2}) was calculated according to Annicchiarico *et al.* (2005) by using the equation (1):

$$\text{Econ. Y} = \text{GY} + 0.3\ \text{Str. Y} \qquad (1)$$

Where: GY is the grain yield and Str.Y is the straw yield. The economic assessment was simply expressed in terms of grain-equivalent value (Annicchiarico and Pecetti, 2003).

The measured variables were analyzed using descriptive statistics to obtain means, extreme values, variances and frequencies. The relationships between measured variables were studied by analyzing the phenotypic correlation coefficients. The variables that determine GY and BIO were derived by stepway regression and path analysis (Fellahi *et al.,* 2013a). The coefficients of phenotypic (CV_p) and genotypic (CV_g) variation were calculated by using the equations (2) and (3) proposed by Acquaah (2007):

$$CV_p(\%) = 100\ (\sqrt{\sigma^2_p}\ /\ \bar{Y}) \qquad (2)$$

$$CV_g(\%) = 100\ (\sqrt{\sigma^2_p}\ /\ \bar{Y}) \qquad (3)$$

Where: σ^2_p and σ^2_g are the phenotypic and genotypic variances, respectively. σ^2_p was calculated based on the phenotypic values of the traits measured in the F_3 and F_4 lines and σ^2_g was calculated as the difference σ^2_p - σ^2_e in which σ^2_e was obtained from the values of the traits measured in the replicated parents and control cultivar. $\bar{Y}$ is the mean of the measured trait.

Broad-sense heritability (h^2_{bs}) is calculated according to the equation (4) by Acquaah (2007).

$$h^2_{bs}(\%) = 100(\sigma^2_g\ /\ \sigma^2_p) \qquad (4)$$

Where: σ^2_g and σ^2_p are, respectively, the genotypic and phenotypic variances.

Descriptive statistical analyzes were done by using CropStat 7.2.3 software (IRRI, 2009), PAST a Paleontological statistics software package (Hammer *et al.,* 2001) was used to estimate the correlation coefficients, while LazStats (Miller, 2013) was employed to run the path analysis and stepwise regression. The least significant difference was calculated at 5% level ($Lsd_{5\%}$) based on the residual variance for all the variables measured in the parental lines that are repeated.

RESULTS AND DISCUSSION

Variability and heritability of the traits of the F_3 and F_4 generations

The means, minimum and maximum values, genotypic and environmental variances, broad-sense heritability, phenotypic and genotypic correlation coefficients of the measured variables are given in Table 1. For BIO and Str.Y produced at maturity, the mean values of the F_3 generation ranged widely from minima of 202.0 g m^{-2} and 108.0 g m^{-2} to maxima of 860.0 g m^{-2} and 608.9 g m^{-2}, around general mean estimates of 398.6 g m^{-2} and 265.4 g m^{-2}, respectively. This information showed that there was sufficient genetic variability to justify selection for improvement in the durum wheat genotypes studied. Candidate lines for selection with high biomass and straw are located in the right fraction of the distribution curves of BIO and Str.Y. Fellahi *et al.* (2013a) and Hannachi *et al.* (2013) also reported that considerable progress in wheat breeding program could be achieved by exploiting these traits in semi-arid environment. In this research study, lines selected within wheat populations could induce a significant genetic gain since this selection concerns individuals that perform better phenotypically (and therefore, genetically) than the rest of the F_3 lines (Fellahi *et al.,* 2020). In F_4 generation, the characteristic values of BIO and Str.Y produced at maturity took relatively lower values than those recorded in F_3 generation ranging from minima of 159.7 g m^{-2}, 97 g m^{-2}, up to maxima of 521.7 g m^{-2} and 328.7 g m^{-2} with overall means of 319.0 g m^{-2}, 203.3 g m^{-2}, respectively. Compared to F_3 breeding lines, means of F_4 generation were reduced by 20.0 and 23.4% for BIO and Str.Y, respectively. Similarly, the minimum values were reduced by 20.9 and 10.2%, while the maximum values were reduced by 39.3 and 46.0% in the same order. The decrease of phenotypic variability of BIO and Str.Y in F_4 could be explained by the fact that the F_3 generation was subjected to a visual selection that resulted in the elimination of undesirable individuals considering some important traits such as diseases, excessive height,

dwarfism, lardivity and threshing. Other studies have reported that segregation of breeding generations may fluctuate in performance from year to year (Ahmad *et al.,* 2018). According to Brown and Caligari (2008), environmental variation is always unpredictable and the highest yielding progeny lines derived from F_2 and F_3 generations may at the some point fail to produce the highest yielding segregants.

Table 1. Variables and traits measured in F_3 (n=330) and F_4 (n=174).

Parameters	Gen	Mean	Min	Max	σ^2_e	σ^2_g	h^2_{bs}	CV_p	CV_g	$Lsd_{(5\%)}$
BIO (g m^{-2})	F_3	398.6	202.0	860.0	3030.4	12131.3	80.0	30.9	27.6	38.9
	F_4	319.0	159.7	521.7	2417.3	3799.5	61.1	24.7	15.4	92.6
Str.Y (g m^{-2})	F_3	265.4	108.0	608.9	3104.7	5441.4	63.7	34.8	27.8	39.4
	F_4	203.3	97.0	328.7	747.7	1975.3	72.5	25.7	13.4	51.5
PHT (cm)	F_3	90.1	56.0	133.0	13.9	304.2	95.6	19.8	19.4	2.6
	F_4	93.8	64.0	127.5	4.2	208.4	98.0	15.5	2.2	3.9
DVP (days)	F_3	130.3	128.0	135.0	0.3	1.7	86.8	1.1	1.0	0.36
	F_4	115.1	110.0	122.0	0.6	7.5	92.9	2.5	0.7	1.4
NS m^{-2}	F_3	99.7	49.0	219.0	239.0	721.5	75.1	31.1	26.9	10.9
	F_4	98.9	48.0	190.0	271.0	504.9	65.1	28.2	16.6	31.0
NG m^{-2}	F_3	3489.4	1649.4	8371.0	246659.2	1035674.6	80.8	32.5	29.2	351.2
	F_4	2495.8	961.6	4647.8	171901.5	339972.2	66.4	28.7	16.6	780.6
GY (g m^{-2})	F_3	133.2	61.0	260.2	279.5	1268.0	81.9	29.5	26.7	11.8
	F_4	115.7	43.0	214.8	520.1	589.7	53.1	28.8	19.7	42.9
Econ.Y (g m^{-2})	F_3	212.8	111.6	433.8	452.8	3384.3	88.2	29.1	27.3	15.0
	F_4	176.7	85.7	298.5	668.7	1401.4	67.7	25.7	14.6	48.7
HI (%)	F_3	34.1	18.0	49.3	9.6	22.8	70.3	16.7	14.0	2.2
	F_4	36.2	19.8	57.7	4.9	22.5	82.0	14.5	6.1	4.2

BIO=above-ground biomass, Str.Y=straw yield, PHT=plant height, NS=number of spikes m^{-2}, NG=number of grains m^{-2}, GY=grain yield, Econ. Y=economic yield, HI=harvest index, DVP=duration of the vegetative growth phase.

PHT in F_3 populations ranged from 56.0 to 133.0 cm, with a general mean of 90.1 cm. Close values in F_4 were found, varying from 64.0 to 93.8 cm with a mean of 127.5 cm. A 7 days range (128.0 to 135.0 days) of the duration of the vegetative growth phase was observed in the F_3 population with a general mean of 130.3 days. This amplitude suggests the possibility of removing part of the plant cycle of the crop subjected to the terminal drought and heat stress. In such a situation, the elimination of subsequent breeding lines on the basis of their DVP estimates during the early segregating generations before selection for yield performance is justified as indicated by Mekhlouf *et al.* (2006) and Mansouri *et al.* (2018). In F_4, the duration of the vegetative growth phase varied from 110.0 to 122.0 days with a general mean of 115.1 days. DVP distribution values of F_4 had a greater amplitude than that observed in F_3 populations, indicating that the selection pressure applied in F_3 did not seem to affect the variability of this trait, as for BIO and Str.Y. Compared to F_3 generation, the F_4 generation showed a substantial change in the mean position of this characteristic suggesting a shortening of the duration of this phase compared to that of the F_3 generation. This acceleration of development rate which induced a reduction in the DVP of 15 days between F_3 and F_4 generations might suggest more intense effect of drought, and especially heat stress during the second year of the experiment. Also, the reduced number of breeding lines in F_4 compared to F_3 generation due to selection pressure might reflect on the average of DVP since late lines were discarded. It is well known in the literature that early headed wheat genotypes under

rainfed south Mediterranean environment are more productive and early generation selection based on DVP as an indirect selection criterion to improve GY is commonly used by wheat breeders (Haddad *et al.,* 2021). Under the same environmental conditions of the present study, Rabti *et al.* (2020) evaluated 58 durum wheat genotypes grown in Algeria and noted that recent varieties produced a higher yield 7.05 days earlier, on average, than landraces. The variation in NS per square meter is rather wide, ranging from 49.0 to 219.0 spikes m^{-2}, with a general mean of 99.7 spikes m^{-2} in F_3 generation. These values remain much lower than those usually observed in the region where this experiment was conducted. Values of this characteristic varied in the F_4 generation from 48.0 to 190.0 spikes m^{-2}, with an overall mean of 98.9 spikes m^{-2}. NG per unit area varied from 1649.4 to 8371.0 grains m^{-2} in F_3 and from 961.6 to 4647.8 grains m^{-2} in F_4 with an average estimated of 3489.4 and 2495.8 grains m^{-2}, respectively. The same pattern was observed for GY in which lower performances were recorded for F_4 when compared to F_3 populations (115.7 vs. 133.2 g m^{-2}). GY ranged between 61.0 and 260.2 g m^{-2} in F_3 and between 43.0 and 214.8 g m^{-2} in F_4 filial generation. These results suggest that the environment was less favorable to the expression of this characteristic for F_4 than for F_3. Amein and Atta (2016) also revealed that the magnitude of phenotypic and genotypic variances was decreased through generations (F_2, F_3 and F_4) when analyzing the variability and relative response to selection in bread wheat crossing over three seasons. Ahmad *et al.* (2018) also found that segregants lost their superiority in F_4 generation. According to Mather and Jinks (1971), superiority of F_2 and F_3 segregants are mainly due to additive × additive and dominant × dominant interactions. Bernardo (2003) stated that early generation selection in different self-pollinated crops, including small grains, is sometimes effective and sometimes ineffective. This selection approach is expected to be effective partly because these species have only low levels of dominance gene action. In the current study, the decreasing trend of the generations of selfed means suggests that the genes are preponderantly dominant or epistatic (Salmi *et al.,* 2019). According to Brown and Caligari (2008), genotype × environment interaction also affects the segregating performance throughout the breeding stages due to uncontrollable environmental conditions from one year to next year. The Econ.Y ranged, for F_3 populations, from a minimum of 111.6 to a maximum of 433.8 g m^{-2}, with a general mean of 212.8 g m^{-2}. Values of this trait were lower in F_4 generation varying from 85.7 to 298.5 g m^{-2} with an average of 176.7 g m^{-2}. HI was, on average, higher for F_4 as compared to F_3 populations (34.1% vs. 36.2%). The range varied from 18.0 to 49.3 for F_3 and from 19.8 to 57.5% for F_4 generation. In autogamous species as wheat, breeders often discard inferior segregants in an early selfing generation so that more resources can be devoted to further testing and selection of the most promising lines (Bernardo, 2003). These results revealed that the selection applied in F_3, in which elimination of low performance segregants were carried out, reduced the range of variability for Econ.Y and HI but increased, on average, HI mean value of the F_4 populations. Donmez *et al.* (2001) indicated that the improvement in the yield of wheat varieties released from 1873 to 1995 was associated with increase in harvest index and biomass. Likewise, Haddad *et al.* (2021) investigated the performance of a set of 16 durum wheat varieties, released during the past 67-years, under rainfed conditions of the eastern high plateaus of Algeria and concluded that high yielding varieties headed early, exhibiting high spike weight, number of spikes, number of kernels m^{-2} as well as increased Econ.Y.

This hypothesis of variability is supported by the values of the phenotypic (CV_p) and genotypic (CV_g) coefficients of variability. In F_3, a high CVp was observed along with high CVg estimates for BIO, Str.Y, NS, NG, Econ.Y and GY and at a lesser degree PHT. These findings suggest the presence of great variability for these traits, which implies that genotype contributed more than the environment in their expression and selection based on phenotypic values is feasible. Similar finding was obtained by Mansouri *et al.* (2018) and Salmi *et al.* (2019). Intermediate values for HI comprised between 10.0 and 20.0%, and low values for the duration of the vegetative growth phase were also recorded. In F_4, the CV_p estimates were high (above 20%) for almost all the traits except for PHT and DVP. CV_g were medium for BIO, Str.Y, NS, NG, Econ.Y and GY and HI. The lowest CV_g were recorded for PHT and DVP, indicating the difficulty of improvement these traits through selection. The rather large difference between the CV_p and CV_g values for some traits is due to the greater contribution of

the environmental variance to the phenotypic variability. The above statement is fully supported by Gerema (2020) who observed moderate and low CV_p and CV_g for plant height and days to maturity, respectively. The CV_p and CV_g values observed were much higher in F_3 when compared to their respective estimates in F_4 generation, except for CV_p and DVP. This result proves that the pedigree selection applied on F_3 generation negatively impacted the variability on F_4. Practically, the increase in homozygosity in advanced generations results in a decrease in the observed variability. Because the CV_p is a combination of additive and environmental variances, any increase observed in CV_p value in next generation may be due to environmental factors, not strictly due to additive or dominant gene action (Amein and Atta 2016; Ahmad *et al.,* 2018).

The broad-sense heritability in F_3 ranged from 63.7% for Str.Y to 95.6% for PHT. These values, calculated in a single generation, were quite high, suggesting that these parameters were less affected by the environmental factors and/or under the control of additive genetic effects where an early selection in F_3 should lead to a rapid genetic improvement of the plant material. In F_4, broad-sense heritability estimates were high (>60%) for all traits, except for GY. The difference in heritability values observed between F_3 and those of F_4 could be attributed to the influence of environment on the expression of traits in both populations with a better contribution of the genotype to the phenotype expression within each generation. Wiggins (2012) attributed the different estimates of heritability between generations to the large genotype × environment interaction and to differences in the way the equations calculated heritability.

Correlations of the F_3 and F_4 generation traits

The phenotypic correlation coefficients between the variables measured in the F_3 and F_4 generations are given in Table 2. Regarding F_3 generation, the BIO significantly correlated to Str.Y (r=0.973), NS (r=0.829), NG (r=0.755), GY (r=0.843) and Econ.Y (r=0.971). However, no significant correlations of BIO with the DVP were indicated. These results suggest that selection of BIO should be effective and lead to appreciable improvements, in the positive sense, in at least five traits (Str.Y, NS, NG, GY and Econ.Y). This selection, based on BIO, is expected to result in a lesser improvement in PHT and HI.

Table 2. Phenotypic correlation coefficients (only significant correlations at 5% probability level are displayed) between F_3 generation (n=330, below the diagonal) and the F_4 generation (n=174, above the diagonal).

Parameters	BIO	Str.Y	PHT	NS m^{-2}	NG m^{-2}	GY	Econ.Y	HI	DVP
BIO		0.952		0.680	0.857	0.876	0.969		
Str.Y	0.973		0.187	0.627	0.679	0.686	0.846	-0.273	
PHT	0.333	0.373							0.290
NS m^{-2}	0.829	0.774			0.675	0.628	0.676		-0.204
NG m^{-2}	0.755	0.619		0.826		0.964	0.940	0.457	
GY	0.843	0.697	0.166	0.777	0.910		0.968	0.491	
Econ.Y	0.971	0.890	0.272	0.840	0.855	0.947		0.266	
HI	-0.402	-0.590	-0.367	-0.195	0.144	0.127	-0.183		
DVP					-0.101	-0.180	-0.116	-0.213	

BIO=above-ground biomass, Str.Y=straw yield, PHT=plant height, NS=number of spikes m^{-2}, NG=number of grains m^{-2}, GY=grain yield, Econ.Y=economic yield, HI=harvest index, DVP=duration of the vegetative growth phase.

Str.Y exhibited significant correlations with PHT (r=0.373), NS (r=0.774), NG (r=0.619), GY (r=0.697) and Econ.Y (r=0.890). Straw-based selection induced indirect improvement in PHT, NS, NG, Econ.Y and GY and decreased in HI. GY had fairly strong correlations with the BIO (r=0.843), NG (r=0.910) and Econ.Y (r=0.968). Its correlations with Str.Y produced (r=0.697) and NS (r=0.777) were less strong. However, it had weak associations with PHT (r=0.166), HI (r=0.127) and DVP (r=-0.180). The analysis of these correlations indicates

that GY-based selection leads to improvements in BIO, Econ.Y, and NG. It is well known in the literature that GY is polygenic complex trait, its measurement is subject to errors that makes the direct selection on the basis of this character less effective due also to the presence of genotype × environment interactions, which leads to a change in the ranking order of genotype performances from one environment to another and from generation to generation (Meziani *et al.,* 2011; Bendjamaa *et al.,* 2014; Haddad *et al.,* 2016; Fellahi *et al.,* 2018, Mansouri *et al.,* 2018; Fellahi *et al.,* 2020; Rabti *et al.,* 2020).

The Econ.Y showed quite strong correlations with the BIO (r=0.971), Str.Y (r=0.890), NS (r=0.840), NG (r=0.855) and GY (r=0.947). Correlations with PHT (r=0.272), HI (r=-0.183) and DVP(r=-0.160) were rather low. Str.Y is strongly influenced by the environmental variations and selection on the basis of this trait is less efficient. Therefore, it can only be used as an indirect selection criterion if its correlation with GY is high (Joshi *et al.,* 2019). Under the conditions in which the experience was carried out, the cereal-livestock farming system is largely adopted; thus, varieties with high Str.Y without penalty on GY are sought (Annicchiarico *et al.,* 2005; Chennafi *et al.,* 2011b; Benider *et al.,* 2017). This is not always the case under constraining conditions, such as those that characterize the eastern high plateaus of Algeria where water stress causes variation in the decrease of BIO and/or HI (Haddad *et al.,* 2016; Rabti *et al.,* 2020). NS was significantly correlated with BIO (r=-0.829), Str.Y (r=-0.774), NG (r=0.826), GY (r=0.777) and Econ.Y (r=0.840). The correlation of this trait with HI (r=-0.195) was rather negative and low. The measurement of NS is relatively less laborious and time consuming than those of the variables discussed above. As a visual selection criterion, NS is widely used by experienced wheat breeders in the field to rank segregating populations. Fellahi *et al.* (2015) illustrated that any increase in NS improved both BIO and GY. HI had positive associations with NG (r=0.144) and GY (r=0.127). Correlations with the other traits, including BIO, Str.Y, PHT and NS, Econ.Y were negatives. These results indicate that, within the F_3 generation, HI-based selection significantly improved the HI itself and NG in a short genetic background. NG, GY and Econ.Y, in addition to the high correlations between them (r=0.910, r=0.855, r=0.947), they also exhibited very high relationships with BIO (r=0.755, r=0.843, r=0.971), Str.Y (r=0.619, r=0.697, r=0.890) and NS (r=0.826, r=0.777, r=0.840). These results indicate that BIO, Str.Y and NS positively influenced both grain yield and Econ.Y as well as NG produced per unit area. It was observed that DVP had the least influence on the other traits measured, probably because this characteristic had low genetic variability within the plant material studied as previously indicated by the CV_p and CV_g. Overall, the analysis of the correlations between the measured variables of the F_3 generation suggests that the traits influencing GY, NG and Econ.Y were BIO and NS. These characters are taken into consideration when screening this generation to improve the traits of interest, either individually or as a combination of characters as an index. Mekhlouf and Bouzerzour (2005) analyzed the efficiency of direct and indirect early selection based on grain yield-related traits in two durum wheat populations. According to their findings, the multitraits selection based on BIO and HI was as efficient as direct selection based on GY itself. They concluded also that indirect selection based on BIO and NS was more efficient than indirect selection based on NG.

The phenotypic correlation coefficients in F_4 confirmed what it was discussed in F_3. The six traits. BIO, Str.Y, NS, NG, GY and Econ.Y had very high correlations between them. Relationships of HI, DVP and PHT with the other traits were weak or insignificant. The findings of this study are in line with the work of researchers previously reported. Terrile *et al.* (2017) and Boussakouran *et al.* (2021) pointed out that the best GY in semi-arid areas are the result of the genetic ability to produce more spikes per unit area associated with good spikes fertility. Slafer *et al.* (2005) as well as Fellahi *et al.* (2017) mentioned that the contribution to NS was more pronounced than that from NG which is formed in a more favorable period. In contrast, Bányai *et al.* (2020) reported that mean grain weight played an important role in determining GY in semi-arid environments. Bensemane *et al.* (2011) and Meziani *et al.* (2011) reported that the improvement of NS was a cause of the increase in GY of new varieties, as the changes in this plant material for NG were due more to NS produced. Of the nine variables measured in this study, only BIO and GY are of great interest in selection for the targeted region.

Direct and indirect effects intra generation

Determinants of grain yield in F_3. The multiple regressions including five traits significantly explained the variation of the GY of the F_3 generation GY with a coefficient of determination of 96% (Table 3). The analysis of the partial regression coefficients indicated that among the five traits included in the model, the contribution of DVP was not significant (P>0.05) to the explanation of GY variation of the F_3 generation as indicated by the partial regression coefficient (b=0.149) of DVP on GY (Table 3). On the other hand, the retained model showed the significant contribution of the three yield related components, namely Str.Y, PHT and HI on the GY.

Table 3. Regression of grain yield on the relevant variables of the F_3 generation (n=330).

Source of variation	df	Sum of squares	Mean square	F.ratio	Prob.>F.ratio
Regression	7	488710.51	69815.78	1101.89	0.000
Residual	322	20407.14	63.37		
Total	329	509117.65			
r	**R^2**	**F.ratio**	**Prob.>F.ratio**	**DF1**	**DF2**
0.98	0.96	1101.89	0	7	322
Traits	**β**	**b**	**SE_b**	**t**	**Prob.>t**
BIO	0.697	0.169	0.023	7.397	0.000
PHT	0.034	0.075	0.032	2.341	0.020
NS m^{-2}	0.683	0.867	0.058	14.858	0.000
HI	0.293	2.027	0.265	7.637	0.000
DVP	0.005	0.149	0.333	0.447	0.655
Constant=-268.220					

BIO=above-ground biomass, PHT=plant height, NS=number of spikes m^{-2}, HI=harvest index, DVP=duration of the vegetative growth phase.

The direct and indirect effects of the determinant variables of GY in the F_3 generation are given in Table 4. The results indicate that the most important direct effects come from the BIO produced (0.697), followed by NS (0.683) and HI (0.293). BIO, in addition to its important direct effect, acted indirectly through NS (0.528) and HI (-0.173). These results are in agreement with the findings of Hannachi *et al.* (2013) and Mekaoussi *et al.* (2021) who pointed out that BIO, HI, spike fertility and NS are the most yield determinants traits in the wheat breeding program in eastern semi-arid areas of Algeria. PHT acted indirectly via Str.Y (0.148) and HI (-0.108). In addition to its positive direct effect, the number of spikes acted indirectly via BIO (0.307). Harvest had negative indirect effects on grain yield via BIO (-0.234) and NS (-0.133) even though its direct effect on grain yield was positive. These results indicate that, apart from the DVP, which does not seem to have an effect on the expression of GY of the F_3 generation, PHT, Str.Y and HI played an important role, directly and/or indirectly, in grain yield determination. Mekaoussi *et al.* (2021) found positive direct effect of HI on GY and negative indirect effects through BIO and NS. The same authors also showed that PHT exhibited sizeable indirect effects, positive via NS and negative via NG. In a previous study by Fellahi *et al.* (2013a), it was demonstrated that the highest positive indirect effects on yield were observed for Str.Y followed by NS per plant and thousand kernel weight (TKW) via BIO.

GY appears to be more complex and as the result of direct and indirect effects of several traits including NS, PHT, HI and Str.Y. These results suggest that indirect single-trait selection to improve yield may not be effective, as well as direct selection, because of the large number of variables that determine this trait. Selection-based index appears to be more effective. Indeed, according to Menad *et al.* (2011), the selection of GY is effective only if the environmental conditions that allowed the achievement

Table 4. Direct (diagonal) and indirect effects of the determinants of grain yield of the F_3 generation (n=330).

Variables	BIO	PHT	NS m^{-2}	HI	DVP	$r_{i/GY}$
BIO	**0.697**	0.013	0.528	-0.173	0.000	0.696
PHT	0.148	0.034	0.054	-0.108	0.000	0.167
NS m^{-2}	0.307	0.003	**0.683**	-0.057	0.000	0.778
HI	-0.234	-0.012	-0.133	**0.293**	-0.001	0.127
DVP	-0.001	-0.003	-0.060	-0.062	0.005	-0.181

BIO=above-ground biomass, PHT=plant height, NS=number of spikes m^{-2}, HI=harvest index, DVP=duration of the vegetative growth phase. $r_{i/GY}$=correlation coefficient of grain yield (GY) with the other measured traits. Significant direct effects are indicated in bold.

of a given GY, are repeated regularly. In this context, Baye *et al.* (2020) showed that the direct effects of yield components on GY are positive. This indicates that if the means of the components not taken as selection criteria are kept constant, the yield can be improved by increasing the component used as a selection criterion. However, according to Benmahammed *et al.* (2010), it is practically difficult to control the variation of the components not taken into account in the selection process, following the presence of the genotype × environment interaction. Indeed, according to Fellahi *et al.* (2018), the selection-based index appears, theoretically and practically, more efficient, given that it offers the possibility of evaluating the role of characters, individually or combined to each other, in randomly matched genetic backgrounds. Fellahi *et al.* (2013b) reported that the different methods used (correlations, step way regression, path analysis, selection index and principal component analysis) to identify selection criteria, indicate that NS, NG and TKW as determinants of GY. This finding is consistent with the results of this study.

Determinants of grain yield in F_4. The multiple regressions including five traits significantly explained the variation of GY of the F_4 generation with a coefficient of determination of 99% (Table 5). The analysis of the partial regression coefficient indicated that among the five variables included in this model, PHT did not contribute significantly to the modification of grain yield variation. The partial regression coefficient did not significantly differ from zero (Table 5). The imported model showed that BIO, DVP, NS and HI significantly affected the GY formation (Table 5).

Table 5. Regression of grain yield on relevant F_4 generation variables (n=174).

Source of variation	df	SS	MS	F.ratio	Prob.>F.ratio
Regression	7	186004.61	26572.08	895.88	0.000
Residual	165	4894.06	29.66		
Total	172	190898.67			
R	**R^2**	**F.ratio**	**Prob.>F.ratio**	**df1**	**df2**
0.987	0.974	895.88	0.000	7	165
Traits	**β**	**b**	**SE_b**	**t**	**Prob.>t**
BIO	0.695	0.443	0.032	13.96	0.000
PHT	-0.027	-0.062	0.033	-1.898	0.059
NS m^{-2}	0.205	0.246	0.063	3.900	0.000
HI	0.560	3.562	0.265	13.427	0.000
DVP	-0.042	-0.495	0.165	-2.991	0.003
Constant = 126.849					

BIO=above-ground biomass, PHT=plant height, NS=number of spikes m^{-2}, HI=harvest index, DVP=duration of the vegetative growth phase.

The direct and indirect effects of the F_4 generation variables are given in Table 6. The highest direct effects on GY were obtained from the BIO (0.695), HI (0.560) and NS (0.205). BIO also affected GY via NS (0.129) and HI (-0.153). NS acted indirectly via BIO (0.436). HI (-0.190) and PHT (0.130) also contributed indirectly via BIO on yield formation with negative and positive indirect effects, respectively.

Table 6. Direct (diagonal) and indirect effects of the determinants of grain yield of the F_4 generation (n=174).

Variables	BIO	PHT	NS m^{-2}	HI	DVP	$r_{i/GY}$
BIO	**0.695**	-0.005	0.129	-0.153	0.002	0.686
PHT	0.130	-0.027	-0.038	-0.072	-0.012	0.032
NS m^{-2}	0.436	0.005	**0.205**	0.049	0.009	0.628
HI	-0.190	0.003	0.018	**0.560**	-0.002	0.491
DVP	-0.033	-0.008	-0.042	0.027	-0.042	-0.057

BIO=above-ground biomass, PHT=plant height, NS=number of spikes m^{-2}, HI=harvest index, DVP=duration of the vegetative growth phase. Significant direct effects at 5% probability level are indicated in bold. $r_{i/GY}$=correlation coefficient of grain yield (GY) with the other measured traits.

As in F_3, the results suggest that in F_4, indirect single-trait selection to improve yield may not be effective due to the large number of variables determining this trait. Index selection is likely to be more effective. When using path analysis, Fellahi *et al.* (2013a) attributed an important role to BIO and HI as indirect criteria for improving GY of an incomplete diallel of bread wheat. These authors reported direct effect values of 1.051 and 0.364 for these two variables, respectively. Hannachi *et al.* (2013) reported in a half diallel cross of durum wheat that GY was significantly and positively related to BIO, Str.Y and HI. The stepwise regression analysis filtered only BIO and HI as determinants of GY. Mecha *et al.* (2017) reported positive and significant direct effects of BIO (1.14) and HI (0.780) on GY. These authors suggested taking into account the variation of these variables during selection to improve the yield of bread wheat. The results of this study are also consistent with those of Dabi *et al.* (2016) who reported significant and positive direct effects of BIO and HI on wheat GY. These authors recommended that the constitution of genetic backgrounds, the choice of parents to be crossed and selection method to increase the yield must be based on these two characteristics.

CONCLUSIONS

Selection can only be effective when significant genetic variability exists in breeding nurseries. In this study, sufficient genetic variability was observed for most of measured variables as indicated by the phenotypic and genotypic coefficients of variability that were found to be high in magnitude both in F_3 and F_4 generations. These results demonstrated the existence of candidate lines for selection, considering both desired senses of selection (increase or decrease of the traits of interest). Grain yield showed significant and positive correlations with all the traits measured except PHT and HI in F_4. Moreover, these results showed that BIO, HI and NS had the highest direct effects associated with significant and positive correlations with GY. These did not change significantly their effects over generations. The true relationship between these traits and GY suggests that selection based on high BIO, NS and HI together is recommended as selection method for further GY improvement in future generations of this breeding program.

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Effect of organic and chemical fertilizers on the growth and production of soybean (*Glycine max*) in dry land

UNIVERSIDAD NACIONAL DE COLOMBIA

Estudio de la aplicación de fertilizantes orgánicos y químicos en la producción de soya (*Glycine max*) en suelo seco

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ABSTRACT

Soybean is known for its high protein content, which is the reason why it is widely used as one of the main food sources for humans and animals. In order to optimize soybean growth, farmers tend to add excessive dosage of chemical fertilizer to this crop. Furthermore, a continuous chemical fertilizer application without organic fertilizer addition may cause a rapid depletion of nutrients in the soil. This study aimed to evaluate the effectiveness of organic fertilizer treatment to reduce the amount of urea as chemical fertilizer needed in soybean cultivation. A complete randomized design was conducted using 21 treatments of organic and chemical fertilizer in triplicate with a 4x3 m plot size. Analysis of variance was carried out to compare the means of measurement data and Duncan multiple range test (DMRT 5%) was applied. The treatment 2,000 kg ha^{-1} compost + 50 kg ha^{-1} urea ($O_2K_2A_1$) resulted the highest dry yield in soybean and had significant differences with urea-only treatment. A mixture of chemical and organic fertilizers had no significant result over the yield compared to the use of chemical fertilizer only. Compost application of 1,000-2,000 kg ha^{-1} with urea 50-100 kg ha^{-1} ($O_2K_2A_1$ and $O_2K_1A_2$) showed an increase in seed yield of 35-38 % with a profit reaching 333-340 USD ha^{-1} compared to standard treatment using urea 50 kg ha^{-1} + SP-36 50 kg ha^{-1} + 50 KCl kg ha^{-1} ($O_0K_0A_1$).

Keywords:
Compost
Depletion
Manure
Nutrients
Yield

RESUMEN

La soya es conocida por su alto contenido en proteínas, por lo que es ampliamente usada como una de las principales fuentes de alimento para los seres humanos y animales. Para optimizar el crecimiento de la soja, los agricultores tienden a agregar una dosis excesiva de fertilizante químico a este cultivo. Además, una aplicación continua de fertilizantes químicos sin la adición de fertilizantes orgánicos puede causar un rápido agotamiento de los nutrientes en el suelo. Este estudio tuvo como objetivo evaluar la efectividad del tratamiento con fertilizantes orgánicos para reducir la cantidad de urea como fertilizante químico necesario en dicho cultivo. Se realizó una combinación de 21 tratamientos de fertilizante orgánico y químico, con diseño completamente aleatorio. Se llevó a cabo un análisis de varianza para comparar las medias de los datos de medición y se aplicó con la prueba de rango múltiple de Duncan (DMRT 5%). Los resultados mostraron que el tratamiento con 2.000 kg ha^{-1} de compost + 50 kg ha^{-1} urea ($O_2K_2A_1$) obtuvo el mayor rendimiento (en peso seco) de soya y mostró diferencias significativas con la aplicación de urea a dosis similares que fueron suministradas sin compost. Una mezcla de fertilizantes químicos y orgánicos no tuvo un resultado significativo sobre el rendimiento en comparación con el uso de fertilizantes químicos solamente. La aplicación de 1.000-2.000 kg ha^{-1} compost + 50-100 kg ha^{-1} urea ($O_2K_2A_1$) y ($O_2K_1A_2$) obtuvo un aumento en el rendimiento de semillas de 35-38% con una ganancia de USD 333-340 ha^{-1} en comparación con la fertilización estándar de 50 kg ha^{-1} urea + 50 SP-36 kg ha^{-1} + 50 KCl kg ha^{-1} ($O_0K_0A_1$).

Palabras clave:
Compost
Agotamiento
Estiércol
Nutrientes
Rendimiento

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Soybean (*Glycine max* (L.)) is one of the agricultural commodities with important value to humans. Soybean is known for its high protein content, making this commodity widely utilized as a main food source for humans and husbandry as well as oil producer (Capriotti *et al*., 2014; Pagano and Miransari, 2016). In order to optimize its growth, fertilization plays an important role to improve soybean productivity, especially in marginal soils. Chemical fertilizers can affect the microflora of the soil by modifying the chemical composition and physical character of the soil (Cwalina-Ambroziak and Bowszys, 2009; Klein *et al*., 2011; Wei *et al*., 2014; Wei *et al*., 2012). Naturally, the need of nitrogen (N)for legumes such as soybean has been partially met through the symbiosis of *Rhizobium* bacteria and root nodule, which absorbs nitrogen from the air. However, farmers tend to add chemical fertilizer excessively during soybean cultivation based on assumption that it may increase the yield (Abbasi *et al*., 2010). On the other hand, soybean can only absorb 35 to 70% of the entire nitrogen fertilizer applied. It means that chemical fertilizer would leave residues and contaminate the soil, deteriorating the soil quality. Therefore, organic fertilizer should be used to improve land productivity (Masaka *et al*., 2014), since this kind of fertilizer could be a better choice to mitigate the negative effects of chemical fertilizers by slowing soil degradation. According to some studies, organic fertilizers have a significant impact on soil nutrient availability, aggregate formation, and soil bacterial communities (Chai *et al*., 2019; Qaswar *et al*., 2020; Ye *et al*., 2019). Several researchers found that a high amount of chemical fertilizer would decrease root nodule formation and N-fixation from the air (Hinson, 1975; Saito *et al*., 2014; Dong *et al*., 2016). Furthermore, an overuse application of chemical fertilizers leads to a loss of soil fertility, bacterial diversity (Dinesh *et al*., 2010) and soil structure (Melero *et al*., 2011).

Several papers showed that treatments using organic fertilizer could solve this problem. Organic fertilizers play significant roles by fulfilling plants nutritional needs as well as improve the physical, chemical and biological properties in the soil. Organic fertilizers have a variety of beneficial effects on crop management, as they can be used as a source of various macro- and micro-nutrients for plants (Ahmad *et al*., 2019, Khan *et al*., 2020, Wajid *et al*., 2020). Organic substances might undergo decomposition by itself, it was considered to be a relatively slow process; therefore, human intervention was needed by adding compost decomposer. After such intervention, decomposition process would run faster and producing a better compost quality. In this context, this study aimed to evaluate the effectiveness of organic fertilizer treatment to reduce the amount of urea as chemical fertilizer needed in soybean cultivation.

MATERIALS AND METHODS

The research was conducted in wet season of 2018 in Bunbarat Village, Rubaru District, Sumenep Regency of Indonesia. Soil condition was relatively infertile; it was sandy in texture and contain C-organic matters. Content of P_2O_5 and K in the soil was very low (Table 1).

Table 1. Soil analysis before organic fertilization experiments were performed.

Analysis	Result	Interpretation
Soil Textures		
Sand (%)	82	-
Dust (%)	6	-
Clay (%)	12	-
Class	-	Sandy loam
pH: H_2O	5.9	A bit sour
C-organic (%)	0.7	Very low
N-Total (%)	0.06	Very low
Ratio C/N	11.67	-

Continuation Table 1

Analysis	Result	Interpretation
P-Olsen (ppm)	39	Very low
K (cmol(+) kg^{-1})	0.14	Low
Na (cmol(+) kg^{-1})	0.02	Very low
Ca (cmol(+) kg^{-1})	3.78	Low
Mg (cmol(+) kg^{-1})	0.66	Low
KTK (cmol(+) kg^{-1})	6.68	Low

Organic fertilizer was made from cow manure with liquid decomposer, urea, SP-36, and KCl. Using a complete randomized design, combination of 21 treatments were repeated 3 times with 4x3 m plot size. Analysis of variance was carried out to compare the means of measurement data and then a Duncan Real Difference Test (DMRT 5%) was performed (Gomez and Gomez, 1984). Organic and chemical fertilizer combination of the 21 treatments is described in Table 2.

Table 2. Combination of Organic and Chemical Fertilizer treatments.

Number	Code	Organic fertilizer	Organic fertilizer dosage (kg ha^{-1})	Urea fertilizer dosage (kg ha^{-1})
1	$O_0K_0A_0$	Without	0	0
2	$O_0K_0A_1$	Without	0	50
3	$O_0K_0A_2$	Without	0	100
4	$O_1K_1A_0$	Cow manure	1,000	0
5	$O_1K_1A_1$	Cow manure	1,000	50
6	$O_1K_1A_2$	Cow manure	1,000	100
7	$O_1K_2A_0$	Cow manure	2,000	0
8	$O_1K_2A_1$	Cow manure	2,000	50
9	$O_1K_2A_2$	Cow manure	2,000	100
10	$O_1K_3A_0$	Cow manure	3,000	0
11	$O_1K_3A_1$	Cow manure	3,000	50
12	$O_1K_3A_2$	Cow manure	3,000	100
13	$O_2K_1A_0$	Compost decomposer	1,000	0
14	$O_2K_1A_1$	Compost decomposer	1,000	50
15	$O_2K_1A_2$	Compost decomposer	1,000	100
16	$O_2K_2A_0$	Compost decomposer	2,000	0
17	$O_2K_2A_1$	Compost decomposer	2,000	50
18	$O_2K_2A_2$	Compost decomposer	2,000	100
19	$O_2K_3A_0$	Compost decomposer	3,000	0
20	$O_2K_3A_1$	Compost decomposer	3,000	50
21	$O_2K_3A_2$	Compost decomposer	3,000	100

O: kind of organic fertilizer (cow manure=1; compost decomposer=2); K: dose of organic fertilizer; A: urea fertilizer treatment.

Cow manure and compost decomposer used in this research were made from cow dung. An application of additional decomposer was done to produce the compost decomposer which is not applied on cow manure fertilizer. This decomposer contains many kinds of fungi and bacteria which are important to accelerate the decomposition process (Table 3).

Argomulyo soybean [(*Glycine max* (L) Merr var. Argomulyo] was cultivated with a distance of 40x15 cm, 2 grains per hole. Organic fertilizer was added during tillage while urea (according to treatment), SP-36 and KCl were provided after 7-10 dap (days after planting). Soil nutrients, plant height, number of branches, number of nodes, number of pods and seeds per plant, number of active root nodules, number of inactive root nodules, dry weight of roots (oven), weight of 100 grains and yield (kg ha^{-1}), also, financial analysis of soybean were quantitative parameters analyzed in a pre-experimental phase. Observations on these parameters and weight of dry roots were carried out using destructive method for plants at 60 dap. Active root nodule observations were recorded if the inner slice of the root nodule was dark red, while inactive root nodule was white to reddish white in color, which means that the soybeans have generated a symbiosis with *Rhizobium* by forming the root nodules.

Table 3. Analysis of the decomposer composition.

Parameter	Result	Agriculture Ministry of Indonesia Standard
Bacteria		
Bacillus sp	2.4×10^{12}	$> 10^7$ CFU mL^{-1}
Pseudomonas sp.	2.9×10^{11}	$> 10^7$ CFU mL^{-1}
Fungi		
Trichoderma sp.	4.1×10^7	$> 10^7$ CFU g^{-1} sample dry weight
Aspergillus sp.	2.7×10^7	$> 10^7$ CFU g^{-1} sample dry weight
Pathogenicity	Negative	Negative
Functional:		
Bacteria		
a. N-fixate	2.4×10^{12}	Positive
b. P- Solvent	2.9×10^{11}	Positive
Fungi		
a. N-fixate	-	10^3 CFU g^{-1}
b. P- Solvent	2.7×10^7	10^3 CFU g^{-1}
Contaminant:		
E. coli	3.0×10^7	Max 10^3 MPN g^{-1} or MPN mL^{-1}
Salmonella sp.	0	Max 10^3 MPN g^{-1} or MPN mL^{-1}
pH	5.5 – 7.41	5.0-8.0

CFU: colony forming unit; MPN: most probable number

RESULTS AND DISCUSSION

Soybean growth and yield

The growth of soybean was influenced by its genetic factors and environment. A mixture of chemical and organic fertilizer has a significant result on the plant height, number of branches, number of nodes, number of active and inactive root nodules and dry weight of roots and root nodules. Plant height and number of branches between 30 and 60 dap showed a noticeable difference according to the fertilization treatment, while differences in the number of plant nodes only was noticeable at 30 dap (Table 4).

Table 4. Effect of organic fertilizer on plant height, number of branches and soybean nodes in Rubaru District, Sumenep Regency, wet season 2018.

No	Treatment	Plant height (cm)*		Number of branches per plant*		Number of nodes per plant*	
		30 dap	60 dap	30 dap	60 dap	30 dap	60 dap**
1	$O_0K_0A_0$	30.00 a	58.47 a	0.13 a	1.00 cdef	7.60 a	12.67
2	$O_0K_0A_1$	39.87 defg	72.47 def	0.47 bcd	0.93 cde	8.53 ab	12.8
3	$O_0K_0A_2$	41.87 fg	73.40 def	1.07 hij	1.27 fghi	8.47 ab	13.47
4	$O_1K_1A_0$	40.67 defg	69.80 cde	0.73 defg	1.13 cdefg	8.47 ab	13.27
5	$O_1K_1A_1$	36.87 bcdefg	68.07 bcd	1.20 ij	1.40 ghij	8.00 ab	12.87
6	$O_1K_1A_2$	37.67 bcdefg	71.13 cdef	0.47 bcd	1.53 hij	8.20 ab	13.6
7	$O_1K_2A_0$	32.67 ab	65.13 bc	0.87 fgh	0.93 cde	8.27 ab	13.07
8	$O_1K_2A_1$	38.47 cdefg	68.13 bcde	0.40 bc	0.80 c	8.40 ab	12.6
9	$O_1K_2A_2$	35.33 abcd	76.80 f	0.33 ab	1.60 ij	8.40 ab	13.73
10	$O_1K_3A_0$	33.53 abc	64.87 bc	1.00 ghi	1.13 cdefg	7.87 ab	13.13
11	$O_1K_3A_1$	34.93 abcd	70.93 cdef	0.80 efgh	0.93 cde	8.47 ab	13.67
12	$O_1K_3A_2$	41.13 efg	72.60 def	1.33 j	1.33 fghi	8.87 ab	12.8
13	$O_2K_1A_0$	39.27 cdefg	69.40 cde	0.53 bcd	0.87 cd	8.20 ab	12.73
14	$O_2K_1A_1$	40.67 efg	67.80 bcd	0.80 efgh	1.00 cdef	8.60 ab	13
15	$O_2K_1A_2$	38.67 cdefg	72.07 def	0.53	1.40 ghij	8.47 ab	13.33
16	$O_2K_2A_0$	38.53 cdefg	62.00 ab	0.67 efgh	1.33 fghi	7.80 a	13.47
17	$O_2K_2A_1$	38.73 cdefg	68.80 cde	0.93 bcde	1.07 cdefg	7.93 ab	12.93
18	$O_2K_2A_2$	42.60 g	75.00 ef	1.20 ab	1.20 defg	9.13 b	13.07
19	$O_2K_3A_0$	35.73 bcde	71.13 cdef	0.47 ghi	1.67 j	8.53 ab	13.67
20	$O_2K_3A_1$	36.67 bcdef	67.67 bcd	0.73 efgh	1.07 cdefg	7.73 a	13.33
21	$O_2K_3A_2$	37.33 bcdefg	67.80 cde	0.67 efgh	1.47 fghi	8.13 ab	13.4
CV (%)		7.69	5	20.16	14.61	7.88	5.03
DMRT value		5.72	5.7	0.26	0.33	1.52	1.08

* Different letters in the same column represent results with statistical difference, according to the DMRT test at $P<0.05$.
** No significant statistical differences

Treatment ($O_2K_2A_2$) using 2,000 kg ha^{-1} compost with urea 100 kg ha^{-1} and treatment ($O_1K_2A_2$) using 2,000 kg ha^{-1} cow manure with 100 kg ha^{-1} urea at 30 and 60 dap showed the highest plant height for each period, respectively. Meanwhile, the largest number of branches was found in treatment ($O_1K_3A_2$) using 3,000 kg ha^{-1} cow manure with 100 kg urea ha^{-1}. At the age of 60 dap, the largest number of branches was obtained using 3,000 kg ha^{-1} compost without urea ($O_2K_3A_0$) treatment, but it did not show real difference with treatment of 1,000-2,000 kg ha^{-1} ($O_1K_1A_1$ and $O_1K_1A_2$) manure or compost 1,000 kg ha^{-1} with 100 kg of urea ha^{-1} ($O_2K_1A_2$). The differences in number of nodules were noticed significantly at 30 dap and treatment using 2,000 kg ha^{-1} compost + 100 kg of urea ha^{-1} showed the highest number of nodules ($O_2K_2A_2$). Results of this study

demonstrated that the addition of organic and chemical fertilizer showed higher yield in soybean compared to treatment using only chemical fertilizer (Qaswar, 2020). Some research studies showed that the application of cow manure compost would increase both soil fertility and crop yield (Hernandez, 2014; Liu *et al*., 2018). Application of organic and inorganic fertilizer has showed effect for sustainability in soil and may positively affect soybean production (Choudhary *et al*., 2021).

The highest number of active root nodules (8.50 nodule) was found in treatment ($O_2K_3A_1$) 3,000 kg ha^{-1} compost with 50 kg of urea ha^{-1} (Table 5). On the other hand, the most inactive root nodule (15 nodules) was found in treatment ($O_1K_2A_1$) 2,000 kg ha^{-1} manure + 50 kg of urea ha^{-1}.

Table 5. Effect of organic fertilizer on the number of active and inactive root nodule per plant and dry weight of roots per soybean plant in Rubaru District, Sumenep Regency, wet Season 2018.

No	Treatment	Number of active nodules per plant*	Number of inactive nodules per plant*	Root dry weight per plant (g)*
1	$O_0K_0A_0$	5.17 f	-	6.97 fghi
2	$O_0K_0A_1$	2.50 c	3.33 def	6.57 efgh
3	$O_0K_0A_2$	2.33 c	5.00 gh	7.63 hij
4	$O_1K_1A_0$	8.17 f	4.33 fg	7.23 ghi
5	$O_1K_1A_1$	7.67 gh	3.83 ef	7.35 ghij
6	$O_1K_1A_2$	6.83 g	1.83 bc	7.20 ghi
7	$O_1K_2A_0$	3.67 d	5.50 h	5.90 ef
8	$O_1K_2A_1$	0.83 a	15.00 k	8.33 a
9	$O_1K_2A_2$	3.50 d	5.17 gh	7.26 ghij
10	$O_1K_3A_0$	8.17 h	0.20 a	6.70 fghi
11	$O_1K_3A_1$	2.17 bc	1.50 bc	6.31 efg
12	$O_1K_3A_2$	4.83 ef	4.33 fg	6.81 fghi
13	$O_2K_1A_0$	5.17 f	10.67 j	10.21 k
14	$O_2K_1A_1$	1.33 gh	3.00 de	5.55 e
15	$O_2K_1A_2$	5.33 f	0.30 a	7.32 ghij
16	$O_2K_2A_0$	5.00 f	3.17 de	6.96 fghi
17	$O_2K_2A_1$	5.17 f	2.33 cd	6.77 fghi
18	$O_2K_2A_2$	2.50 c	3.00 de	6.49 fghi
19	$O_2K_3A_0$	3.67 d	1.00 ab	7.84 ij
20	$O_2K_3A_1$	8.50 h	3.67 ef	6.62 efgh
21	$O_2K_3A_2$	4.00 cd	1.00 ab	6.01 ef
CV (%)		11.75	14.54	8.14
DMRT value		0.98	1.25	0.93

* Different letters in the same column represent results with statistical difference, according to the DMRT test at $P<0.05$.

The highest dry weight of roots per plant (10.21 g) was found in treatment ($O_2K_1A_0$), which used 1,000 kg ha^{-1} compost without urea fertilizer. In accordance with this result, it was confirmed that organic management has a significant impact in rhizobia genotype presentation in that area. Rhizobia numbers was increased in term of diversity in this kind of field, since organic fertilizer is a good medium for the development of the rhizobia genotype so that it has an effect on N-fixation from air (Grossman *et al*., 2011).

Soybean yield was determined by the development of the plant from the beginning of sowing to the harvest period,

where the role of fertilization was of great importance. Application of different organic and chemical fertilizers would affect one or all of its yield components, namely: number of pods per plant, number of seeds per plant, weight of 100 dried seeds and yield of dried seeds. Number of pods per plant, number of seeds per plant, weight of 100 seeds and yield of dried seeds showed a significant difference during treatment using organic fertilizers and chemical fertilizers (Table 6).

Table 6. Effect of organic fertilizer on the number of pods, number of grains per plant, weight of 100 seeds and yield of soybean dried seeds in Rubaru District, Sumenep Regency, wet season 2018.

No	Treatment	Number of pods per plant*	Number of grains per plant*	Dry weight of 100 grains of soybean seeds* (g)	Dry yield* (t ha^{-1})
1	$O_0K_0A_0$	15.60 a	46.80 a	14.76 abcd	1.52 a
2	$O_0K_0A_1$	17.20 ab	51.60 ab	14.69 abcd	2.05 cde
3	$O_0K_0A_2$	20.40 cd	61.20 de	14.79 abcd	2.29 cdefg
4	$O_1K_1A_0$	16.60 ab	49.80 ab	15.07 bcdef	1.91 bcd
5	$O_1K_1A_1$	19.67 cd	59.00 de	15.51 cdefg	2.44 fghij
6	$O_1K_1A_2$	20.07 cd	60.20 de	15.10 cdefg	2.48 ghijk
7	$O_1K_2A_0$	16.47 a	49.40 a	14.91 abcde	2.08 cdef
8	$O_1K_2A_1$	16.60 a	49.80 a	13.92 ab	1.83 n abcd
9	$O_1K_2A_2$	24.47 e	73.40 e	13.85 a	2.28 efghi
10	$O_1K_3A_0$	21.07 d	63.20 d	16.11 fg	2.14 cdefg
11	$O_1K_3A_1$	24.73 e	74.20 e	15.71 defg	1.79 abc
12	$O_1K_3A_2$	24.27 e	72.80 e	15.12 bcdef	2.55 hijk
13	$O_2K_1A_0$	18.60 bc	55.80 bc	15.05 bcdef	1.62 ab
14	$O_2K_1A_1$	20.60 d	61.80 e	14.57 abcd	2.09 cdef
15	$O_2K_1A_2$	26.93 f	80.80 f	14.59 abcd	2.77 jk
16	$O_2K_2A_0$	20.87 d	62.60 e	16.05 efg	2.60 ijk
17	$O_2K_2A_1$	20.27 cd	60.80 de	16.37 g	2.82 k
18	$O_2K_2A_2$	20.93 d	62.80 e	14.45 abc	2.38 efghi
19	$O_2K_3A_0$	20.33 cd	61.00 de	14.70 abcd	2.20 defgh
20	$O_2K_3A_1$	19.80 cd	59.40 de	16.10 efg	1.84 abcd
21	$O_2K_3A_2$	27.00 f	81.00 f	15.15 cdef	2.62 ghijk
	CV (%)	4.98	4.98	3.96	8.87
	DMRT value	1.99	6.3	1.19	0.38

*Different letters in the same column represent results with statistical difference, according to the DMRT test at $P<0.05$.

The highest number of pods per plant (26.93-27.00 pods) was found in treatments ($O_2K_1A_2$ and $O_2K_3A_2$) 1,000 kg ha^{-1} and 3,000 kg ha^{-1} compost + 100 kg of urea ha^{-1}. Similarly, the largest number of seeds per plant was found in treatments 1,000kg ha^{-1} and treatment 3,000 kg ha^{-1} compost plus 100 kg of urea ha^{-1} ($O_2K_1A_2$ and $O_2K_3A_2$) with 80.80 seeds and 81.00 seeds, respectively.

The highest score in weight of 100 dried seeds was found in treatment 2,000 kg ha^{-1} compost + 50 kg of urea ha^{-1} ($O_2K_2A_1$), but it did not differ significantly with treatment 2,000 kg ha^{-1} compost without urea ($O_2K_2A_0$), or with in treatment 1,000 kg ha^{-1} compost and 3,000 kg ha^{-1} manure + urea 0-50 kg ha^{-1} ($O_1K_1A_1$, $O_1K_3A_0$, and $O_1K_3A_1$) and in treatment 3,000 kg ha^{-1} compost decomposer with 50 kg urea ha^{-1} ($O_2K_3A_1$).

Dry seed (water content 11%) yield found in treatment 2,000 kg ha^{-1} compost plus 50 kg of urea ha^{-1} ($O_2K_2A_1$) was 2,820 kg ha^{-1}, but it did not change manifestly from treatment 1,000 kg ha^{-1} compost + 100 kg of urea ha^{-1} ($O_2K_1A_2$) and treatment 2,000 kg ha^{-1} compost without

urea ($O_2K_2A_0$) as well as treatment using 1,000 ha^{-1} compost and 3,000 kg ha^{-1} manure + 100 kg of urea ha^{-1} ($O_1K_1A_2$ and $O_1K_3A_2$).

Compost decomposer has been shown to be able to increase soybean crop. And interestingly, in this study, crop yield using treatment 2000 kg ha^{-1} organic fertilizer and 50 kg ha^{-1} chemical fertilizer combined with compost decomposer showed the highest results of all treatments considering the dry yield. As shown in laboratory analysis results, compost decomposer contained beneficial fungi and bacteria for plants (Table 3). Thus, microorganisms within the compost decomposer would be able to increase the positive effect of fertilizers. Inoculation of *Bacillus sp* and *Trichoderma asperellum* plays important role in supporting plant growth through phosphate solubilization process and auxin and hydrolytic enzymes synthesis (Moreira *et al*., 2021).

Other parameters observed in this study, such as number of pods and number of seeds per plant also correlated with production yield. In line with prior study, higher results in dry weight of 100 grain soybeans indicate larger grain size which would contribute to higher production yield (Soverda, 2009). This study also showed that the number of active nodules did not significantly affect the production yield and this was contrary to a previous study suggesting that total active nodules in soybean was closely related to the *Rhizobium* activities in the root to bind N and increase soybean growth and production yield (Birnadi, 2014). The root nodule was a very complex organ from the nodule formation itself and in the transport between membranes that occurs in it (Oldroyd and Downie, 2008; van Hameren *et al*., 2013). Although studies regarding root nodules have shown some progress, more studies were necessary to comprehend processes that occurred within this organ (Oldroyd and Dixon, 2014; Sulieman *et al*., 2014).

The highest productivity value (per hectare) was obtained using 1,000 kg ha^{-1} compost decomposer and 50 kg ha^{-1} chemical fertilizer followed by the treatment of 2,000 kg ha^{-1} combination of compost decomposer and organic fertilizers along with 50 kg ha^{-1} chemical fertilizer (Table 7). The relationship between productivity and cost (fertilizer) showed the highest results using 2,000 kg ha^{-1} combination of compost decomposer and organic fertilizers along with 50 kg ha^{-1} chemical fertilizer followed by 1,000 kg ha^{-1} compost decomposer and 50 kg ha^{-1} chemical fertilizer. Moreover, comparison concerning the amount of energy consumed between each system should be considered. A study conducted in the province of Jilin, China showed that production cost in conventional systems was 33% lower and its net income was 25% higher compared to organic systems (Zhang *et al*., 2015). Deficient energy use in organic farming is due to the use of fuel and plastic mulch, which result in a lower production yield (Lee and Choe, 2019).

Agricultural Analysis

Result of soybean analysis of this study was based on the increase of production cost after applying urea fertilization and organic fertilizer and also based on the seed yield as a result of fertilization treatment at the study site in Rubaru-Sumenep. Table 7 showed financial analysis of soybean at the study site.

Table 7. Financial analysis of organic fertilizer delivery of soybeans, Rubaru District, Sumenep Regency, Rainy season 2018.

Treatment	Fertilizer cost N+organic (USD ha^{-1})	Δ Fertilizer cost compared with standard N fertilizer (USD ha^{-1})	Grain Productivity (t ha^{-1})	Production value (USD ha^{-1})	Δ Productivity compared with fertilizer cost (USD ha^{-1})	Δ Productivity value (USD ha^{-1})
$O_0K_0A_0$	-	-6.42	1.52	813.06	-283.5	-277.08
$O_0K_0A_1$	6.42	-	2.05	1,096.56	-	-
$O_0K_0A_2$	12.48	6.42	2.29	1,224.94	128.38	121.96
$O_1K_1A_0$	35.66	29.24	1.91	1,021.67	74.89	-104.13
$O_1K_1A_1$	42.08	35.66	2.44	1,305.17	208.61	172.95
$O_1K_1A_2$	48.50	42.08	2.48	1,326.57	230.01	187.93

Continuation Table 7

Treatment	Fertilizer cost N+organic (USD ha^{-1})	Δ Fertilizer cost compared with standard N fertilizer (USD ha^{-1})	Grain Productivity (t ha^{-1})	Production value (USD ha^{-1})	Δ Productivity compared with fertilizer cost (USD ha^{-1})	Δ Productivity value (USD ha^{-1})
$O_1K_2A_0$	71.32	64.90	2.08	1,112.61	16.05	-48.85
$O_1K_2A_1$	77.74	71.32	1.83	978.88	-117.68	-189
$O_1K_2A_2$	84.16	77.74	2.28	1,219.59	123.03	45.29
$O_1K_3A_0$	106.98	100.56	2.14	1,144.70	48.14	-52.42
$O_1K_3A_1$	113.4	106.98	1.79	957.48	-139.08	-246.06
$O_1K_3A_2$	119.82	113.40	2.55	1,364.01	267.45	154.05
$O_2K_1A_0$	39.23	32.81	1.62	866.55	-230.01	-262.82
$O_2K_1A_1$	45.65	39.23	2.09	1,117.96	21.40	-17.83
$O_2K_1A_2$	52.06	45.65	2.77	1,481.69	385.13	339.49
$O_2K_2A_0$	78.45	72.03	2.60	1,390.76	294.20	222.16
$O_2K_2A_1$	84.87	78.45	2.82	1,508.44	411.88	333.43
$O_2K_2A_2$	91.29	84.87	2.38	1,273.08	176.52	91.65
$O_2K_3A_0$	117.68	111.26	2.20	1,176.80	80.24	-31.02
$O_2K_3A_1$	124.10	117.68	1.84	962.83	-112.33	-230.01
$O_2K_3A_2$	130.52	124.10	2.62	1,401.46	304.90	180.8

Description: Urea Price: USD. 0.13 kg^{-1}; manure=0.036 USD kg^{-1}; compost: 0.039 USD kg^{-1}; Consumption seed yield: USD. 0.53 kg^{-1}

A combination of 2,000 kg ha^{-1} compost and urea 50 kg ha^{-1} ($O_2K_2A_1$) resulted an additional yield about 770 kg ha^{-1} compared to application of 50 kg urea only. Thus, farmers could obtain additional gross profit up to 411.88- or 333.43 USD ha^{-1} for net profit. This profit number could be obtained by $O_2K_1A_2$ treatment.

CONCLUSIONS

Fertilization significantly increases the dry yield in comparison to the control treatment. This current study demonstrated the beneficial impact of chemical and organic fertilizer on promoting soybean yield. A mixture of chemical and organic fertilizers has higher result over the yield compared to the use of chemical fertilizer only. The application of 1,000-2,000 kg ha^{-1} compost with urea 50-100 kg ha^{-1} ($O_2K_2A_1$ and $O_2K_1A_2$) showed an increase in 35.12-37.56% seed yield with profit reaching USD 333-340 per hectare compared to standard treatment. These results suggest that combining chemical and organic fertilizers has the potential to sustain high grain yield mitigating the environmental impact caused by the overuse of chemical fertilizers.

Furthermore, 1-2 t of compost treatment is recommended particularly for soil with low organic content or soil that has never been planted with soybean. In the future, studies regarding the effect of organic fertilizers in soybean cultivation might be done using rice fields or inundated soils and also using soil with medium to high organic content. Besides, comprehensive farming analysis for production cost components including price of labor and pesticides should be done to obtain more accurate economic estimation.

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Estimation of leaf nitrogen content from non-destructive methods in *Eucalyptus tereticornis* and *Eucalyptus saligna* plantations

Estimación del contenido de nitrógeno foliar por métodos no destructivos en plantaciones de *Eucalyptus tereticornis* y *Eucalyptus saligna*

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ABSTRACT

The determination of leaf nitrogen content (LNC) by indirect methods is essential for silvicultural management of forest crops. The application of photography or rapid measurement equipment, such as chlorophyll index (soil-plant analysis development-SPAD), is increasingly used due to its low-cost, ease of estimation and accuracy. Therefore, the aim of this study was to estimate foliar nitrogen content from nondestructive methods in plantations of *Eucalyptus tereticornis* and *Eucalyptus saligna* using three urea treatments (120 kg N ha^{-1}, 240 kg N ha^{-1} and a control treatment without urea). For each treatment, 10 trees were selected, including four for the validation of the equations. The LNC was directly evaluated for color with the CIEL*a*b* model, photographic measurement with the RGB model, SPAD measurement and destructive estimation of nitrogen in leaves. The results showed negative relationships with the L* (luminosity) and b* (trend from yellow to green) indices, while the a* (red to green trend) index was discarded, with SPAD positive relationships were found with LNC and RGB space. In the R and B indices, the greatest negative relationships were found. It was determined that the multivariate equation $Y=a+b_1x_1+b_2x_2+\ldots+b_nx_n$ can be used for this type of study. It was also determined that the LNC=0.389+0.026SPAD model was the optimum for *E. tereticornis* and the LNC=3.826- 0.001R- 0.10B equation was the optimum for *E. saligna*.

Keywords:
CIEL*a*b*
Digital color photography
Leaf color
RGB
SPAD

RESUMEN

La determinación del contenido de nitrógeno foliar (LNC) por métodos indirectos es esencial para el manejo silvícola de cultivos forestales. La aplicación de fotografía o equipos de medición rápida, como el índice de clorofila (SPAD), se utiliza cada vez más debido a su bajo costo, facilidad de estimación y precisión. Por tanto, el objetivo de este estudio consistió en estimar el LNC a partir de métodos no destructivos en plantaciones de *Eucalyptus tereticornis* y *Eucalyptus saligna* utilizando tres tratamientos de urea (120 kg N ha^{-1}, 240 kg N ha^{-1} y un tratamiento testigo sin urea). Para cada tratamiento, se seleccionaron 10 árboles, incluidos cuatro utilizados para la validación de las ecuaciones. El LNC se evaluó directamente en cuanto a color con el modelo CIEL*a*b*, medición fotográfica con el modelo RGB, medición SPAD y estimación destructiva de nitrógeno en hojas. Los resultados mostraron relaciones negativas con los índices L* (luminosidad) y b* (tendencia de amarillo a verde), mientras que se descartó el índice a* (tendencia de rojo a verde), encontrándose SPAD relaciones positivas con el espacio LNC y RGB. En los índices R y B, mostraron las mayores relaciones negativas. Se determinó que la ecuación multivariada $Y=a+b_1x_1+b_2x_2+\ldots+b_nx_n$ se puede utilizar para este tipo de estudio. También se determinó que el modelo LNC=0,389+0,026SPAD fue el óptimo para *E. tereticornis* y la ecuación LNC=3,826-0,001R-0,10B fue la óptima para *E. saligna*.

Palabras clave:
CIEL*a*b*
Fotografía digital en color
Color de hoja
RGB
SPAD

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Nitrogen (N) is a primary element for the development of plants (Aldea *et al*., 2006); it is the largest component of chlorophyll, amino acids and proteins that are directly related to leaf greenness (León-Sánchez *et al*., 2016; Valverde *et al*., 2021) and plant growth (Malavolta, 2006). Deficiencies associated with N influence leaf size reduction, also, chlorosis occurs due to the inability to synthesize chlorophyll, which generates yellowing in young leaves, and therefore, a decrease in growth (accumulation of biomass) which, in severe cases, can induce physiological plant stress (Alchanatis *et al*., 2005; Berntsen *et al*., 2006). Studies developed by Djumaeva *et al*. (2012) mention that N is a fundamental nutrient for tree productivity, thus, it should be considered in the evaluation of the soil at the time of establishing a plantation, as well as in fertilization programs.

Given the importance of N in developing productive forest crops, it is necessary to implement nutrient evaluation methodologies. There are two groups of tests, destructive and nondestructive, applied to leaf cover (Wang *et al*., 2014). The destructive test consists of collecting leaves and, through elemental analysis developed in the laboratory, the leaf nitrogen content is determined (LNC), which is an accurate system, but it is a time consuming and expensive process and can cause damage to the plant by reducing the foliar area (Silla *et al*., 2010; Rotbart *et al.,* 2013).

Alternatively, nondestructive methods are based on the reflectance spectrum of the leaf, which is related to the concentration of N (Dana and Ivo, 2008); among the most common methods, measurement with multispectral sensors (Weber *et al*., 2006), hyperspectral sensors (Tao *et al*., 2014), chlorophyll concentration sensors (Chang and Robison, 2003) and commercial photographic cameras (Nieto *et al*., 2017). The last two are the most widely implemented due to their economical accessibility and rapid and nondestructive evaluation of the plant (Djumaeva *et al*., 2012; Tao *et al*., 2014). The SPAD meter (Minolta Camera Co.) is an instrument that allows to measure the chlorophyll concentration and indirectly the N concentration, using a 6 mm^2 optical sensor that is fast, nondestructive and straightforward to use, but with limitations in terms of leaf sampling (it must be measured several times) demanding equations of correction that allow doing the real determination of nitrogen concentration (Limantara *et al*., 2015).

The implementation of photographic cameras can measure spectral information in the spectral bands of the visible spectrum (Ali *et al.,* 2013; Wang *et al*., 2014). Currently on the market, there are cameras with high resolution (higher than 10 megapixels) with sensors capable of collecting a large amount of crop information (Kipp *et al*., 2014). Aspects such as canopy structure, leaf area, leaf orientation and biomass accumulation can be easily collected with a color image (Netto *et al*., 2002) and processed by software such as R, MATLAB® or Image J (Riccardi *et al*., 2014). Previous studies on agricultural and shrub species have shown that it is possible to develop N prediction models by extracting color information from leaves by combining photography and greenness extraction in RGB and CIEL*a*b* formats (Conesa *et al*., 2019).

Studies developed by Hu *et al*. (2010) allowed the creation of the Triangular Greening Index (TGI), which permits the nitrogen potential to be projected from the red, green and blue bands available in a digital photograph with high sensitivity. Pagola *et al*. (2009) developed a set of color equations from photographic greenness through the CIEL*a*b* system with shrub species, being the b* parameter, the factor related to N. In all these cases, the luminosity and shooting conditions are relevant, which can generate variations in the green color and thus, in the projections of N (Riccardi *et al*., 2014).

In the case of forest species, the study of N relationship models with indirect methods is very limited. Coste *et al*. (2010), with SPAD values, allowing the creation of linear and exponential projection equations of N with ratios greater then 0.80. On the other hand, Barrantes *et al*. (2018) with tree forest species developed a SPAD index of prediction of N with an indirect method, which allowed to determine prediction ranges from color. In the specific case of the CIEL*a*b* color values relationship with leaf N concentrations, no studies were found for tree species, being a significant information gap. In this context, this study aimed to estimate the leaf nitrogen content from nondestructive methods in *Eucalyptus tereticornis* and *Eucalyptus saligna* plantations.

MATERIALS AND METHODS

Experimental site

The study was carried out in a plantation of *E. tereticornis* and *E. saligna* located in Cartago, Costa Rica (9°50'57.91"N; 83°54'37.27" W) at an altitude of 1,392 m.The average annual temperature of the site is 24 °C and the annual rainfall is 2,100 mm, distributed over seven months of rain (IMN, 2020).The site presented a flat topography, with a slope of less than 10°, with a clay loam soil, with a pH 5.2, 0.93 g kg^{-1} of nitrogen content and 0.56 g kg^{-1} of potassium in the first 15 cm of the soil. The climate of the site was classified according to Köppen-Geiger as a subtropical high mountain oceanic climate (Köppen, 1990).

The plantation was 26 months old, with a sowing spacing of 1.0x1.0 m (10000 tree ha^{-1}). the crop was characterized by having minimal silvicultural management due to its objective for the generation of biomass for energy purposes. A total of 30 individuals per species were selected (20 trees were used for model generation and 10 trees were used for validation, they were not mixed to generate the models) with a homogeneous diameter and leaf area index (Table 1). Three groups were randomized to apply nitrogen treatments 120 kg N ha^{-1} (N1) and 240 kg N ha^{-1} N2 and a control treatment (no nitrogen was applied). The application was performed in May 2020, when the rainy season started in the study area. For the research, the evaluations were developed for four months after the application of the nitrogen treatments (the application of the different doses of nitrogen was developed only to guarantee a wide range of nitrogen concentrations in the leaves, which facilitated the development of equations).

Table 1. General characteristics of the study plantations in Cartago, Costa Rica.

Attribute	Species	
	E. tereticornis	***E. saligna***
dbh (cm)	5.8±2.9	5.1±1.8
Height (m)	7.2±1.8	6.6±1.2
Canopy lenght (m)	6.5±0.7	5.9±0.5

dbh: diameter at breast height (1.3 m). LAI: leaf area index. mean±standard deviation.

Nondestructive sampling of N

According to Valverde and Arias (2018), for each individual, five leaves were selected from the middle part of the canopy, as these are not very old or immature leaves, and are not affected by pathogens or deformities. The selected leaves were evaluated with three nondestructive methods: (i) chlorophyll meter (SPAD), (ii) indirect measurement of color with a photographic camera and (iii) direct measurement with a colorimeter.

The relative chlorophyll content value was made using a SPAD-502 (Konica Minolta®) that has an effective area of evaluation of 6 mm^2. For each leaf analyzed, three measurements were taken to generate a mean value; the measurement points were located at the ends and at the midpoint of each leaf according to Valverde and Arias (2018).

Color measurement was carried out by analysis of photographs. Therefore, a Sony camera model Alpha 7 ILCE7K/B was implemented, with a photography dimension of 4000×6000 pixels (24 megapixels resolution) with an interchangeable lens of FE 3.5-5.6 / 28-70. It was implemented with an aperture of f/5.6, ISO 300, with a white balance of 4900k, with automatic posture, and flashed off. The camera was placed on a tripod at 40 cm above the samples, which were placed on a matte black background. The luminosity of the site was 1000 lux and it was photographed after the nitrogen potential Subsequently, the images were processed by means of the Adobe Photoshop CC2020 software, the eyedropper tool was implemented, and a sample of 25 individual points of the leaf was analyzed to determine an average value of the color. The generated color values were handled by the RGB color space (R=Red, G=Green, and B=Blue), with values between 0 to 255.

Direct color measurement was performed with a standardized CIE chromatography NIX Pro spectrophotometer. The color

was determined in the 400 to 700 nm range with a 10 mm diameter measurement port. For the reflection of the specular component (SCI mode) was included an angle of 10°, which is typical for the surface of leaves (D65/10); and with a D65 (corresponding to daylight at 6500 K). Selected color space was CIEL*a*b*, which generated three parameters to explain the color that consisted of L* (luminosity) that ranges from 0 to 100, where 0 is black and 100 is white; a* (color trend from red to green), positive values tend to red and negative values to green and b* (color trend from yellow to blue), positive values tend to yellow and negative values to blue.

Destructive sampling of N

The same leaves that were used in the nondestructive analysis were used for destructive sampling. Each leaf was dried at 60 °C for 72 h according to Valverde *et al.* (2017). Subsequently, it was grounded until the size of the particles was 225 to 250 µm. The material was stored for 72 h in an air-conditioned area (22 °C and 50% relative humidity). The moisture content of the material was 12%, then 0.5 mg of this material was analyzed in an elemental analyzer model Vario Micro Cube to estimate the LNC.

Statistical analysis

First, the Anderson-Darling test (normality test) and the Breush-Pagan test (homoscedasticity test of the variance) were performed on each database obtained from the leaves to determine that there was a parametric behavior in the data; afterward, a descriptive statistical analysis was carried out. The characterization of the different variables was analyzed. To determine the relationships between the LNC and the values of the color spaces under study and SPAD values, simple relationships were performed, in which each color index and SPAD were placed on the X-axis and LNC on the Y-axis, later analysis of Pearson's correlation where the color spaces, SPAD and LNC were correlated in order to eliminate the indices that showed lower correlations. Then multivariate linear regression (selected by previous studies of Baresel *et al.*, 2017) was performed under the model $Y=a+b_1x_1+b_2x_2+\ldots+b_nx_n$, in which LNC was the dependent variable and the CIEL*a*b* color coordinates, RGB and SPAD measurements were the independent ones, therefore, the regression coefficient was estimated with least squares; For comparison of models, the coefficient of determination, the model error, Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were implemented. Finally, the best color prediction equation was selected for each color and SPAD model was validated according to Valverde and Arias (2020a), the random cross-validation method was used. All the analyzes were developed through the R software version 3.6.2 (R Core Development Team 2020), with a significance of 0.05.

RESULTS AND DISCUSSION

LNC and CIEL*a*b* color space relationship

Figure 1 shows the different relationships between LNC and L*a*b* color space in the two tree species; similar behaviors were determined (All data met the assumptions of normality and homoscedasticity). The LNC values ranged from 0.8 to 2.4%. The luminosity (L*) ranged between 20 and 60 considering a medium to low luminosity (color trend in low light), a* values were negative and ranged from -20 to 0, it can be considered a greenish color trend. On the other hand, b* values obtained tended to yellow colorations, since they ranged from 0 to 60.

A relationship between LNC and luminosity (L*) was determined as the L* value increased, the percentage of LNC tended to decrease (Figure 1a and 1b), showing a coefficient of determination that ranged from 0.705 to 0.743 considered as significant. Regarding the color a* parameter (Figure 1c and 1d), a clear trend was not found, since a flat behavior was exhibited in *E. tereticornis* and moderately increasing in *E. saligna,* but with coefficients of determination lower than 0.13, which may be considered as a poor relationship. Finally, the increase of b* parameter generated decreases in the LNC values (Figure 1e and 1f), being a significant relationship with coefficients of determination higher than 0.722.

The results obtained were similar to those presented by Barrantes *et al.* (2018) with shrub and tree species for industrial purposes. The a* parameter is not very functional for the development of LNC estimates, because it is a variable of low sensitivity; the greenness of this variable showed low variability compared to the L* or b* parameter. This is because nitrogen losses in the plant tend to generate yellowing, which in the CIEL*a*b*

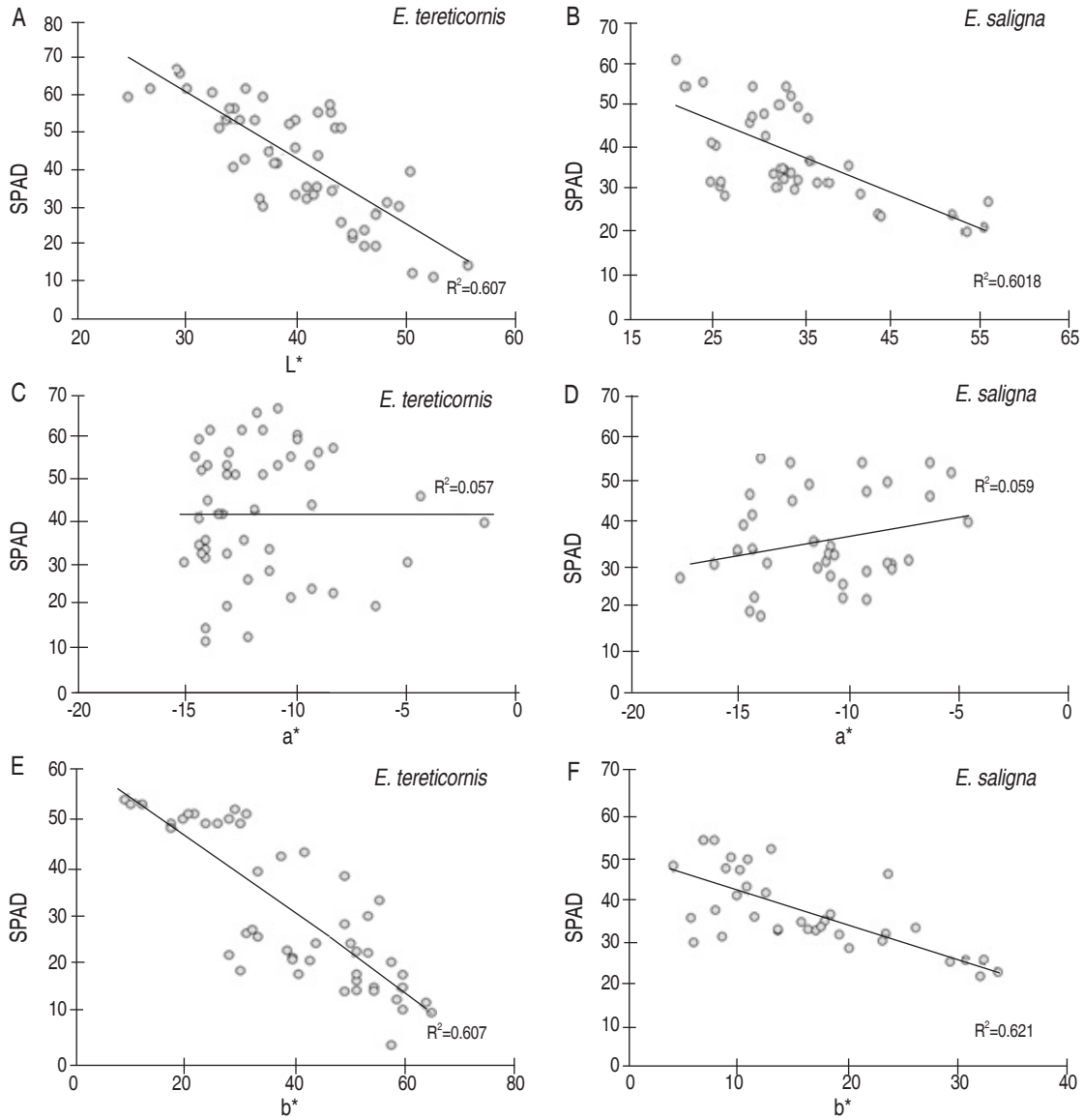

Figure 1. Relationship between color by L*a*b* format with leaf nitrogen content (LNC) of plants of *E. tereticornis* (A, C, E) and *E. saligna* (B, D, F).

color model, would be perceived by b*, which showed strong correlation with LNC. At high LNC concentrations, yellowing is scarce and increases as LNC decreases, an aspect that Hu *et al.* (2010) mentioned as a common behavior because low levels of nitrogen generate foliar discoloration due to a lower concentration of chlorophyll and the presence of amino acids and proteins, aspects that Conesa *et al.* (2019) highlight as indicators of the discoloration process. Therefore, the b* parameter is the best colorimetric indicator to denote the concentration of LNC (Nieto *et al*., 2017).

In luminosity, the relationship with LNC is due to two reasons as was mentioned by Wang *et al*. (2014) and Nieto *et al.* (2017): i) It is a color correction index, in very high or low luminosities, it tends to generate errors in the estimation of the LNC; therefore, it is common to have values close to 50 for a correct color determination and to relate it to LNC. Very high or low values of L* induce variations in the green tone of the plant, which generates under or overestimation of the a* and b* indices. ii) It is a parameter that shows a relationship with the concentrations of chlorophyll in

the leaf. High concentrations of chlorophyll, which is an indirect indicator of LNC, tend to have a dark leaf color and low luminosity, it is in leaves with chlorosis in which the luminosity is increased due to low concentrations of chlorophyll.

LNC and RGB color space relationship

Regarding the RGB color space (Figure 2) (All data met the assumptions of normality and homoscedasticity), it was determined that the R (Red) index varied between 40 and 140, while the G (Green) index ranged between 60 and 150 and the B (Blue) index was found within the range from 0 to 80. Relationships between the RGB and LNC color space were determined using the R index (Figure 2a and 2b), a decreasing trend of the LNC as the value of R increased. This behavior was the same in both studied species and with a significant relationship, obtaining a coefficient of determination ranged from 0.736 to 0.746. Regarding the G index (Figure 2c and 2d), a negative behavior was presented. As G value increased, the LNC decreased, presenting a coefficient of determination higher than 0.70 in both species. On the

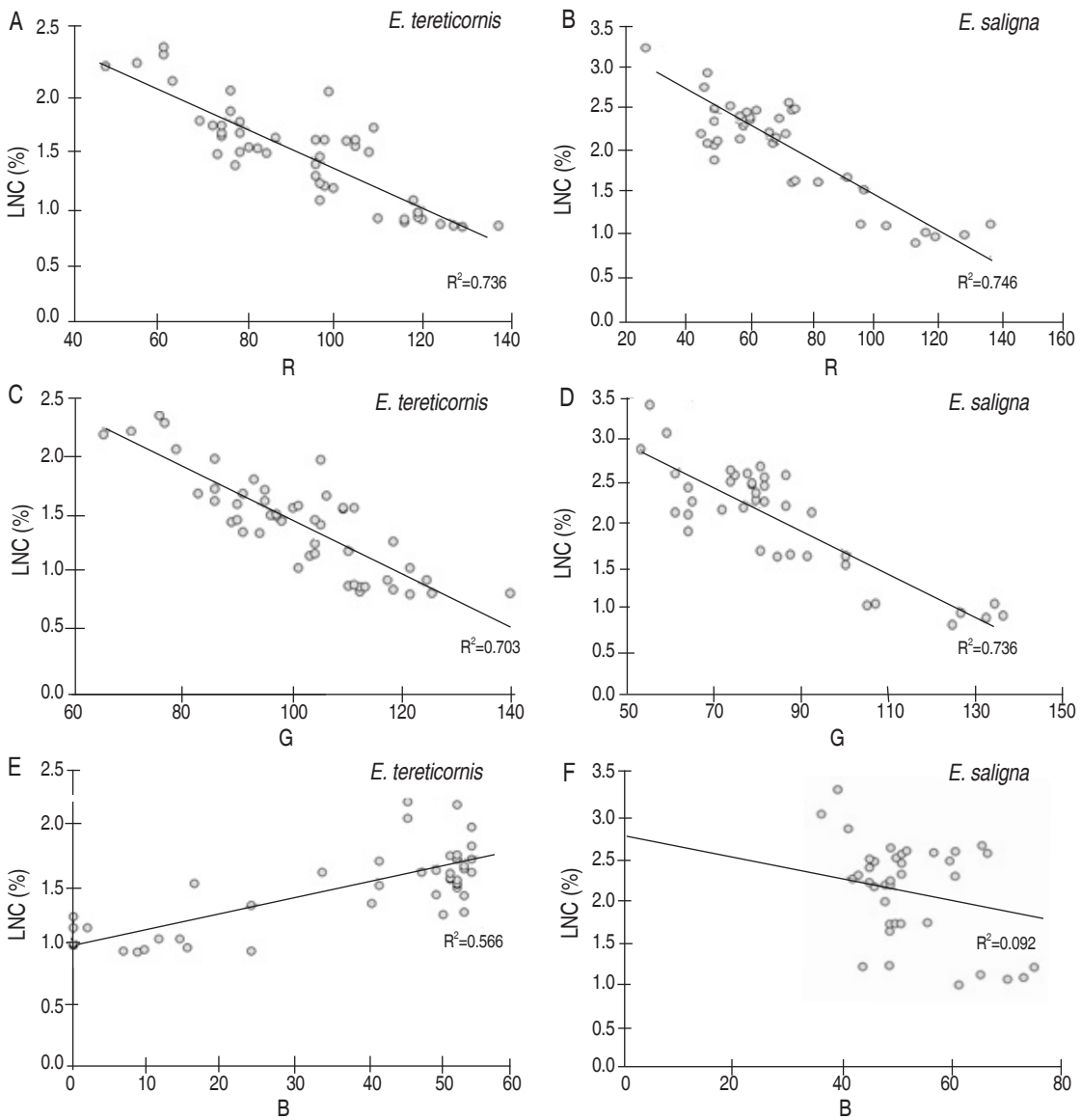

Figure 2. Relationship between color by RGB format and leaf nitrogen content (LNC) of E. tereticornis (A, C, E) and E. saligna (B, D, F).

other hand, the behavior varied among species regarding the B index (Figure 2e and 2f). For *E. tereticornis* (Figure 2e), a slightly positive trend was determined, which as B increased, the LNC increased, with a moderate coefficient of determination of 0.56. In contrast, with *E. saligna* there was no clear pattern of behavior, and it presented a poor coefficient of determination of 0.09.

The relationship between LNC, R and G indices were similar to the results obtained by Ali *et al.* (2013) with agronomic species; the leaves with the highest nitrogen content presented a dark greenish, which increases in the R and G indices, whereas the B index remains with a scarce variation due to the low sensitivity to changes in green. In colorimetric prediction models, Jannoura *et al.* (2015) and Baresel *et al.* (2017) mention that R and G layers are very susceptible to leaf color change, as slight variations of both indices can generate significant visual variations of the green color. B Index is not very sensitive and is generally constant in the different shades of green, limiting its use in equations.

LNC and SPAD relationship

Regarding the SPAD evaluation, the values ranged from 10 to 65, finding a direct proportional relationship in both species (Figure 3); as the SPAD value increased, the LNC also increased, with a high ratio the estimated coefficients of determination (0.720). These results are similar to those presented by Coste *et al.* (2010) with tree species, finding a positive linear relationship. The reason is that the SPAD equipment is developed to measure chlorophyll at red wavelengths (640 to 650 nm) and near-infrared (920-930 nm), points where is possible to find chlorophyll exposure, which is directly related to LNC. Pinkard *et al.* (2006) reported that nitrogen measurements with instrument are accurate due to the linear relationship with nitrogen; however, they found limitations because in the first stages of stress in the plant, they could not accurately identify changes in nitrogen concentration. It requires moderate periods of stress to identify the variation, since it is a method that requires the development of regression equations to link the indicator with nitrogen directly.

Correlation between LNC, color indices and SPAD

Correlations between LNC, the two-color models used and SPAD measurement showed the same behavior in both study species (Table 2). A strong correlation between LNC and SPAD was determined, being positive and with values higher than 0.850. On the other hand, with the CIEL*a*b* model, the correlations of the L* and b* were high and negative with a value higher than 0.840,

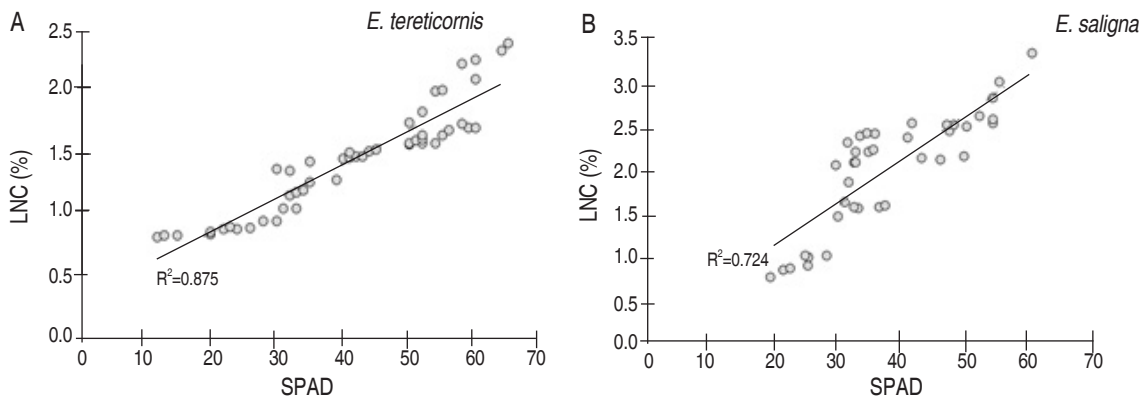

Figure 3. Relationship between SPAD values and leave nitrogen content (LNC) in plants of *E. tereticornis* (A) and *E. saligna* (B).

whereas with the a* index, no significant correlation was found with values lower than 0.350. Moreover, in the RGB model, R and G indices have a negative correlation with significant LNC with values higher than 0.830. In contrast, the B index varies with the species; for *E. tereticornis* a strong and negative relationship was found (value of -0.720), while for *E. saligna* no relationship was found, being an irrelevant indicator.

Using CIEL*a*b* space, the L* and b* indices showed a high correlation with LNC, similar to the result presented by Pinkard *et al.* (2006), Wang *et al.* (2014) and Nieto

et al. (2017) with shrub and tree species, where the relationship of both variables is due to the sensitivity of perception of yellowing, something that the a* index is not able to predict, and which generated its low correlation. Ali *et al.* (2013) highlighted the b* parameter as the optimal rate of LNC change in the short-term due to its high sensitivity in leaves and easy measurement. By contrast, with the RGB space, the R and G indices showed a high relationship due to the perceived bands of both indices. G is 520– 600 nm, while R is 630 nm, which allows seeing changes in the leaf greenness generated by changes in chlorophyll and nitrogen concentration. Finally, the SPAD is a device that has the functional capability to develop relationships with the LNC, as it was developed to measure chlorophyll in the visible and infrared field.

Table 2. Coefficients of correlation between the LNC values, SPAD measurements and color indices of the CIEL * a * b * and RGB models in leaves of *E. tereticornis* and *E. saligna*.

	LNC	L*	a*	b*	SPAD	R	G	B
LNC	1	-0.862 **	0.355 ns	-0.851 **	0.851 **	-0.863 **	-0.858 **	-0.304 ns
L*	-0.840 **	1	-0.379 ns	0.898 **	-0.663 **	0.975 **	0.998 **	0.544 ns
a*	0.035 ns	0.040 ns	1	-0.579 ns	0.361 ns	-0.220 ns	-0.426 ns	0.095 ns
b*	-0.876 **	0.885 **	0.017 ns	1	-0.669 **	0.860 **	0.906 **	0.237 ns
SPAD	0.936 **	-0.779 **	0.000 ns	-0.827 **	1	-0.653 **	-0.661 ns	-0.191 ns
R	-0.858 **	0.974 **	0.114 ns	0.917 **	-0.810 **	1	0.961 **	0.468 ns
G	-0.838 **	0.990 **	-0.052 ns	0.874 **	-0.764 **	0.952 **	1	0.545 ns
B	0.752 **	-0.629 **	0.010 ns	-0.911 **	0.742 **	-0.719 **	-0.615 **	1

The values above the diagonal correspond to the correlations obtained for *E. saligna*, the values below the diagonal correspond to *E. tereticornis*. **: significance at 0.01, ns: no statistical significance.

Equations to estimate LNC from color indices and SPAD

The prediction equations of the LNC from the color indices and SPAD measurements are presented in Table 3. For *E tereticornis* in CIEL*a* b* model, the combination of the L* and b* indices was determined, generating an equation with a higher coefficient of determination (0.786) and a lower error of 0.198 on developing individual models with each index, it means, CIEL*a*b* color model is the best option to estimate the LNC. In addition, a model with a high coefficient of determination of 0.875 and a deficient error of just 0.147 was generated from SPAD measurement, showing the effectiveness of this methodology to estimate LNC in the studied species. Also, RGB color model was another suitable combination to predict LNC. This combination presented a significantly higher coefficient of determination and a notably lower error than the equations with each index alone or their combinations.

For *E. saligna* species (Table 3), through CIEL*a*b* model, L* and b* indices in the same equation presented the highest coefficient of determination and the lowest error, AIC and BIC, being the optimal evaluation model. At the same time, an equation with a strong coefficient of determination of 0.716 and a moderate error of 0.337 was determined using SPAD measurements, (AIC and BIC), which showed the potential of SPAD in forest species to predict LNC. Finally, with RGB color model, the RG indices presented the best LNC prediction model, with the highest coefficient of determination (0.742) and the lowest model error (0.322), AIC and BIC, being the RGB color model the best option.

The selection of equations with the indices L* and b* were similar to those selected by Pagola *et al.* (2009); the accuracy of the prediction of the appearance of LNC is due to the precision of both color indices in predicting the color change of leaves by nitrogen. In the case of SPAD, Coste *et al.* (2010) showed similar equations due to the ease of the equipment to estimate LNC, making it a good choice for forest species. In the RGB case, the behavior was different from that reported by Baresel *et al.* (2017), and who determined that the equations with R and G were sufficient to estimate changes in LNC. In the case of the study, B index had a significant relationship with the LNC appearance due to the characteristics of the species.

Table 3. LNC estimation equations from CIEL*a* b* and RGB model color indices and SPAD measurements for *E. tereticornis* and *E. saligna* plants.

Species	No.	Equation	R^2	RMSE	AIC	BIC	P-value
E. tereticornis	1	LNC=3.591-0.052L*	0.705	0.224	96.877	80.655	0.002
	2	LNC=2.193-0.021b*	0.767	0.198	90.788	73.455	0.001
	3	LNC=2.724-0.018L*-0.016b*	0.786	0.192	88.901	70.899	0.001
	4	LNC=0.389+0.026SPAD	0.875	0.147	60.112	50.001	0.001
	5	LNC=2.896-0.016R	0.736	0.2	92.655	76.122	0.001
	6	LNC=3.729-0.023G	0.696	0.225	103.2	82.35	0.001
	7	LNC=0.937+0.014B	0.556	0.271	145.455	90.707	0.002
	8	LNC=3.507-0.010R-0.011G	0.806	0.179	78.877	61.566	0.001
	9	LNC=2.827-0.016G+0.007B	0.783	0.189	91.43	70.933	0.001
	10	LNC=2.349-0.012R+0.006B	0.809	0.178	78.9	62.344	0.002
	11	LNC=2.905-0.008R-0.009G+0.005B	0.846	0.159	75.443	56.99	0.001
E. saligna	1	LNC=4.009+0.059L*	0.735	0.326	68.766	75.456	0.002
	2	LNC=3.018-0.058b*	0.716	0.337	74.679	86.899	0.001
	3	LNC=3.652-0.034L*-0.027b*	0.76	0.31	55.899	49.988	0.001
	4	LNC=0.043+0.052SPAD	0.716	0.337	68.9	70.345	0.001
	5	LNC=3.603-0.022R	0.738	0.324	69.122	75.233	0.001
	6	LNC=4.047-0.024B	0.703	0.33	62.892	62.775	0.001
	7	LNC=3.826-0.001R-0.10B	0.742	0.322	70.344	73.79	0.001

Validation of the best LNC estimation equations

The validation of the studied models (Table 4) showed the same behavior for both species. The coefficient of determination and the R^2 of the error tended to decrease between 10 and 15% in all equations, finding the best result for *E. tereticornis*. LNC estimation method was performed with SPAD, as it showed the highest coefficient of determination and the lowest error, AIC and BIC, followed by RGB color model and the lowest accuracy, represented by CIEL*a*b* model. For *E. saligna*, the B index showed the best statistical values, being the best option for estimating LNC, followed by SPAD and finally the equation of the L* and b* indices.

Advantages and disadvantages of the proposed methodology

The nondestructive methodology implemented showed acceptable estimation values compared to destructive methods or high-cost instrumentation developed for N estimation. Ali *et al*. (2013) mentioned that the development of nondestructive measurements that estimate N values y are fundamental for intensive crop sampling with the advantage of reducing the cost of sampling, immediate data for making quick decisions, no impact on the crop and short time to generate information.

Nevertheless, the limitations of the photographic technique or SPAD must be considered, such as the dependence on climatic conditions (Baresel *et al*., 2017), the need for equipment calibration (Ali *et al.* (2017) and the dependence of the leaf color on the appearance of the plant, for instance, the competition for space, water stress, the availability of light and phytosanitary status, so the selection of sampling plants must be essential in the study and environmental conditions must be controlled (Chang and Robinson, 2003). If the cultivation conditions are controlled and the protocol is clearly managed, equations with significant precision can be obtained, as mentioned Sun *et al*. (2018) and Valverde and Arias (2020a) for agronomic and forest species.

The applicability of this system simplifies the nutritional assessment process. Equations can be created by species or group of species (which have characterization studies, and distinguished nutritional patterns. This is essential to be able to develop general equations. In case of not having

Table 4. Values of calibration and validation of the coefficient of determination and the error of the best equations for estimating the LNC from color indices and SPAD measurements in *E. tereticornis* and *E. saligna*.

Species	No.	Equation	Calibration				Validation			
			R^2	RMSE	AIC	BIC	R^2	RMSE	AIC	BIC
E. tereticornis	3	LNC=2.724-0.018L*-0.016b*	0.786	0.192	88.901	70.899	0.763	0.19	89.889	74.033
	4	LNC=0.389+0,026SPAD	0.875	0.147	60.112	50.001	0.858	0.129	61.233	52.988
	11	LNC=2.905-0.008R-009G+005B	0.846	0.159	75.443	56.99	0.834	0.102	76.973	78.741
E. saligna	3	LNC=3.652-0.034L*-0.027b*	0.76	0.31	55.899	49.988	0.762	0.155	56.32	52.19
	4	LNC=0.043+0.052SPAD	0.716	0.337	68.9	70.345	0.712	0.98	70.457	73.455
	6	LNC=3.826-0.001R-0.10B	0.742	0.322	62.892	62.775	0.723	0.65	65.733	67.873

this information parameterized, the generation of unique models is not recommended). It can avoid the process of sending samples to the laboratory and destructive analysis. With photographs collected in the field that follow a luminosity and image homogeneity protocol, nutritional analyses can be developed. Following the methodology proposed by Valverde *et al.* (2020b), the next step is the use of cameras and equipment of low-cost and greater accessibility that maintains representativeness, which would reduce the costs of the method and expand its application (it is possible to use smartphone or lower-cost cameras with a resolution greater than 8 megapixels).

CONCLUSIONS

It is possible to estimate LNC from indirect, low-cost, fast methods and without generating significant damage to the study plant. Furthermore, in CIEL*a*b* color space, the L* and b* indices showed a strong negative correlation with LNC, because they are two indicators susceptible to leaf chlorosis. In the case of RGB space, the R and B indices showed the most significant negative relationship with LNC, thus, a photographic image can be used in equations for the estimation of nitrogen with an acceptable precision for both study species. Regarding SPAD, its potential for LNC estimation is confirmed once again with positive linear relationships with nitrogen and a correlation higher than 0.75. Finally, the best model for *E. tereticornis* was LNC=0.389+0.026SPAD with SPAD equipment, while for *E. saligna* was LNC=3.826- 0.001R- 0.10B generated with digital photography.

The analysis of tropical tree species used in reforestation programs for commercial purposes should be considered for future research, they should be analyzed with different concentrations of nitrogen and water stress to generate different models to estimate nitrogen with nondestructive methods.

In addition, it is recommended to continue with these types of studies in which lower-cost cameras and analysis software are implemented in order to determine if the accuracy in the equations and models with good fit is still maintained.

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Response of soybean crop with different combinations of seed treatment and application of nitrogen, cobalt, and molybdenum topdressing

UNIVERSIDAD NACIONAL DE COLOMBIA

Respuesta del cultivo de soja con diferentes combinaciones de tratamiento de semillas y aplicación de nitrógeno, cobalto y molibdeno como cobertura

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ABSTRACT

Nitrogen is the element most demanded by the soybean crop, and the biological fixation of atmospheric nitrogen is the main means to supply it. In contrast, micronutrients and chemical treatments applied on seeds together with the inoculant can alter the phenomenon of biological fixation of atmospheric nitrogen. This work aimed to evaluate the effect of chemical products, micronutrients, and nitrogen fertilization on the nodulation, development, and yield of soybean. The experiment was developed in a field and a greenhouse in the municipality of Toledo, Brazil. A randomized block with four repetitions was used as an experimental design. This design had eight treatments, namely: T1 - Control (seeds treated with insecticide); T2 - Seeds treated with insecticides and inoculated with *Bradyrhizobium*; T3 - Untreated seeds inoculated with *Bradyrhizobium*; T4 - Seeds treated with insecticides and cobalt-molybdenum (CoMo), inoculated with *Bradyrhizobium*; T5 - Seeds with CoMo inoculated with *Bradyrhizobium*; T6 - Seeds treated with insecticides, inoculated with *Bradyrhizobium* and with foliar application of CoMo; T7 - Seeds treated with insecticides, inoculated with *Bradyrhizobium* and with the application of nitrogen in cover; T8 - Seeds treated with nitrogen by broadcast. No significant differences were observed between treatments on the nodules numbers, stem diameter, plant height, root length, the mass of 1000 grains, and yield. The application of nitrogen at the R2 stage (a plant with an open flower in one of the two uppermost nodes of the main stem with a fully developed leaf) and in association with the inoculant + CoMo without seed treatment provided a greater number of nodes, pods, and grains per plant.

Keywords:
Biological nitrogen fixation
Bradyrhizobium japonicum
Glycine max L.
Nodules
Seed treatment

RESUMEN

El nitrógeno es el elemento que presenta mayor demanda por parte del cultivo de soja, y la fijación biológica del nitrógeno atmosférico el principal medio para abastecerlo. En cambio, micronutrientes y tratamientos químicos aplicados a la semilla conjuntamente con el inoculante pueden alterar dicha fijación biológica de nitrógeno. El objetivo de este trabajo fue evaluar el efecto de productos químicos, micronutrientes y de la fertilizacion nitrogenada, en la nodulacion, desarrollo y rendimiento de la soya. El experimento fue desarrollado en campo y en invernadero en el municipio de Toledo, Brasil. El diseño experimental utilizado fue el de bloques al azar, con cuatro repeticiones. Los tratamientos fueron: T1 - Control (semillas tratadas con insecticida); T2 - Semillas tratadas con insecticidas e inoculadas con *Bradyrhizobium*; T3 - Semillas no tratadas inoculadas con *Bradyrhizobium*; T4 - Semillas tratadas con insecticidas y cobalto-molibdeno (CoMo), inoculadas con *Bradyrhizobium*; T5 - Semillas con CoMo inoculadas con *Bradyrhizobium*; T6 - Semillas tratadas con insecticidas, inoculadas con *Bradyrhizobium* y con aplicación de CoMo vía foliar; T7 - Semillas tratadas con insecticidas, inoculadas con *Bradyrhizobium* y con aplicación de nitrógeno en cubierta; T8 - Semillas tratadas con nitrógeno al voleo. No se observaron diferencias significativas entre los tratamientos sobre el número de nódulos, diametro de la tallo principal, altura de planta, longitud de raiz, masa de 1000 granos y rendimiento. La aplicacion de nitrógeno en la etapa R2 y la asociación del inoculante + CoMo sin tratamiento de semilla proporcionó mayor número de nudos, vainas y granos por planta.

Palabras clave:
Fijación biológica de nitrógeno
Bradyrhizobium japonicum
Glycine max L
Nódulos
Tratamiento de semillas

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One of the main agents of productivity increase in recent years has been the research on soil fertility and the scientific and technological innovations that have allowed the efficient use of agricultural soil corrective and fertilizers (Balafoutis *et al.,* 2017).

Nitrogen (N) is one of the most important elements that was introduced in large quantities as fertilizer, increasing agricultural production by up to 40%, since it is a constituent of amino acids, proteins, nucleic acids, among others (de Mello-Prado, 2021).

In plants, N is the component responsible for several reactions, besides being part of the structure of chlorophyll, enzymes, and proteins. Being an essential element, N has an impact on root formation, photosynthesis, production and translocation of photoassimilates, and the growth rate between leaves and roots, being primarily involved the leaf growth (Bang *et al.,* 2021).

According to Hungria *et al.* (2006), the rates of N obtained through biological fixation of atmospheric nitrogen (BNF) are between 60 and 250 kg ha^{-1}, which represents between 70 to 85% of all N accumulated in the soybean plant. N constitutes 80% of the gases of the atmosphere that is also in the rhizosphere, in the pores of the soil; however, plants cannot easily take it, due to the triple bond between the atoms of N. Meanwhile, some bacteria such as *Bradyrhizobium* through the dinitrogenase enzyme can break this connection, providing the reduction until ammonia (NH_3), even when N is supplied by chemical products.

These bacteria are chemically attracted by molecules exuded by germinating seeds and the root system of the soybean plants, which subsequently, penetrate these roots and stimulate the growth of cells in the host plant forming the nodules, where they remain (Santi *et al.*, 2013).

Ions of hydrogen (H^+) that are abundant in bacteria cells are incorporated into the ammonia forming ammonium (NH_4), which are distributed to host plants and incorporated for the formation of various substances (Parente *et al.*, 2015).

The use of these bacteria in the composition of commercial agricultural inoculants is an efficient way to increase agricultural production, which guarantees productivity to supply the growing demand for food and also the need to reduce dependence on chemical fertilizers, thus, promoting sustainable agriculture (Nosheen *et al.*, 2021). According to Hungria *et al.* (2006), the main Fabaceae commercially planted in Brazil, totally free of nitrogen fertilization, under normal growing conditions, can be supplied with the N requirements of the crop by BNF.

In Brazil, BNF is the main form to provide N; nevertheless, the peak fixation occurs in grain filling, and after this stage, there is a considerable reduction of BNF, given that, the preferential absorption process becomes the translocation of N from leaves and stems to pods (Moreno *et al.,* 2018).

Excess of nutrients can trigger negative environmental impacts that cause contamination and eutrophication of water bodies, and reduction of microflora and native microfauna. Also, the acidification of the soil, especially when it has the availability of elements in different forms (Sharpley, 2016). This negative interference can occur not only with the supply of N but also with other nutrients that participate in the BNF process such as molybdenum (Mo) and cobalt (Co). For both, there are variable responses of soybean to complementary fertilization, even with the importance of these nutrients for the process of symbiotic fixation of N, there are doubts in its application to obtain a higher yield of grains in the crop (Marcondes and Caires, 2005).

For BNF to be effective, bacteria must be highly dependent on these micronutrients. Mo acts on the enzyme nitrate reductase that is responsible for the reduction of nitrate to nitrite in the cellular cytoplasm and for participating in nitrogen metabolism as a cofactor of the enzymes nitrogenase and nitrite reductase (Marcondes and Caires, 2005). Co is part of the structure of vitamin B12 that is fundamental for the synthesis of leghemoglobin, which determines the activity of nodules, also has an influence on the nitrogen absorption through symbiotic (Marcondes and Caires, 2005). Concerning the forms of fertilization of the crop, there is uncertainty about the mineral and biological fertilization consortium. According to Embrapa (2008), the application of nitrogen fertilizer at any stage of plant development, in addition to the reduction in nodulation

and the effectiveness of biological nitrogen fixation, does not cause productivity increases.

In this context, this study aimed to evaluate the interference of chemicals products, micronutrients, and nitrogen fertilization on the efficiency of biological nitrogen fixation in soybean inoculated with *Bradyrhizobium.*

MATERIALS AND METHODS

This study was carried out in the experimental farm of the Pontifícia Universidade Católica do Paraná PUCPR, Toledo campus, located at the coordinates 24°43'14"S and 53°43'56"W, with an average altitude of 557 m, in the agricultural year 2016/17. The local climate is subtropical humid (Cfa) according to Koppen, with hot summers and infrequent frosts with trends in rainfall concentrations in the summer months, without a defined dry season, and the soil is classified as a Dystropherric Red Latosol (Embrapa, 2013).

The research was developed in two scenarios: directly in the field and in a pot arrangement under a protected environment. In the field, the soil was collected using the sampling methodology proposed by Raij *et al*. (2001) and showed the following results in the shallow layers from 0 to 20 cm: pH ($CaCl_2$) 4.75; 4.71 $cmol_c\ dm^{-3}$ of $H^+ + Al^{3+}$; 4.36 $cmol_c\ dm^{-3}$ of Ca^{2+}; 1.88 $cmol_c\ dm^{-3}$ of Mg^{2+}; 0.26 $cmol_c\ dm^{-3}$ of K^+; 6.39 mg dm^{-3} of P (Mehlich-1); and 57.98% of saturation per base.

The experimental area was approximately 555 m², previously planted with oats. Treatments were conducted in plots of 22.05 m² (3.15×7.0 m), arranged in randomized blocks with three replications and eight treatments (Table 1).

For the implantation of the experiment were used seeds without treatments and seeds previously treated with 6 mL kg^{-1} of Tiodicarbe 45 % + Imidacloprid 15%, the inoculation was performed shortly before sowing by mixing the inoculant with the seed in a plastic bag. A mixed liquid inoculant was used, which was composed of *B. japonicum* CPAC 15 (SEMIA 5079) and *B. diazoefficiens* CPAC 7 (SEMIA 5080) at the concentration of 6×10^9 colony-forming units mL^{-1} with a dose of 2 mL kg^{-1} of seed. The application of CoMo consisted of 3 mL of the commercial product composed of 15% of Mo and 1.5% of Co kg^{-1} of seed before inoculation using a plastic bag, and also, 240 mL ha^{-1} via foliar at R2 stage. N was applied manually by the end of the afternoon, the source used was ammonium sulfate composed of 21% N and 24% S, the dose was 100 kg ha^{-1}.

Table 1. Treatments applied to soybean crops via seed, foliar, and cover.

T1 - Control (seeds treated with insecticide)
T2 - Seeds treated with insecticides and inoculated with *Bradyrhizobium*
T3 - Untreated seeds inoculated with *Bradyrhizobium*
T4 - Seeds treated with insecticides and CoMo, inoculated with *Bradyrhizobium*
T5 - Seeds with CoMo inoculated with *Bradyrhizobium*
T6 - Seeds treated with insecticides, inoculated with *Bradyrhizobium* and with the application of CoMo via foliar
T7 - treated with insecticides, inoculated with *Bradyrhizobium* and with the application of nitrogen in cover
T8 - treated with nitrogen by broadcast

The sowing of the soybean was carried out manually on October 01, 2016, in which the cultivar Monsoy 5947 IPRO was used, with space between lines of 0.45 m using 36 seeds m^{-1}. Two days before sowing, the area was fertilized using a no-tillage seeder with the same space between lines using 300 kg ha^{-1} of the formula 00-20-18 of N-P-K. At the V3 stage (a plant with three nodes on the main stem with fully developed leaves beginning with the unifoliolate nodes), a reduction was carried out maintaining 10 to 12 plants m^{-1}. The average air temperature and rainfall that occurred during the crop cycle were 24.7 °C and 1125 mm, respectively.

The cultural treatments were according to the need of the crop. Two applications of herbicide, the first one at V3 with 2 L ha^{-1} of glyphosate 48% + 0.45 L ha^{-1} Cletodim

24% and the second one with 2 L ha^{-1} of glyphosate 48% at pre-flowering. Three applications of fungicides were also made, the first at R2, the second at R4, and the third at R5.4; all with 200 g ha^{-1} of Azoxystrobin 30% + Benzovindiflupir 15% as well as four applications of insecticide for bedbugs control.

In the first application, 500 g ha^{-1} of 75% acephate at the R2 stage was used, the second one, 1 L ha^{-1} of imidacloprid 10% + beta cyfluthrin 1.25% was applied at R4, the third application was performed at the R5.4 stage using 200 g ha^{-1} of 75% acephate and the last application at R6 using 200 g ha^{-1} of 75% acephate, for caterpillar it was not necessary to apply since the cultivar was resistant.

All applications were performed at the freshest times of the day, early in the morning, or late afternoon, with a yellow Teejet spray tip with an application angle of 110° and a flow rate of 0.46 to 0.91 L min^{-1}.

After maturation, the three central lines were collected manually with a length of 5 m in each plot, totaling 6.75 m² of useful area, discarding 1 m of each end of the plot.

The material was threshed, pre-cleaned, and moisture determined on a digital electronic device. After being weighed, the result of each plot was adjusted to 13% moisture and extrapolated to bags ha^{-1}. The evaluation of the production components: the number of pods and the number of grains $plant^{-1}$ was performed using 10 plants of each plot collected at harvest time. The mass of 1000 grains was determined according to the Seed Analysis Rule. Stem diameter was measured using a caliper, the height of the plant was measured by measuring tape. The number of pods, grains, and nodes per plant were counted manually, then the mean of each variable was calculated.

In the other scenario, 5 L of capacity perforated polyethylene pots were used, performing the same treatments that were carried out in the field; however, without fertilization and leaving only one plant per pot after emergence. The substrate used was soil from the field experiment site and sand in a ratio 4:1, respectively. Pots were kept in the university's greenhouse (PUCPR) and irrigated daily, in which they were distributed in a completely randomized experimental design with three replications. From the pot experiment, some variables were analyzed such as the number of nodules, which after washing the roots, the nodules were detached and counted one by one, and root length, which was measured by a measuring tape.

The data were tabulated and submitted to analysis of variance, according to the level of 5% of significance by Test F, and the qualitative averages grouped by the Tukey test at 5% probability. The analyzes were performed using the statistical software SISVAR 5.6 (Ferreira, 2011).

RESULTS AND DISCUSSION

According to Table 2, there were no significant effects among treatments (P>0.05), mainly highlighting that the variable number of nodules did not have a significant variation, proving that the experimental area may have had seeds with *Bradyrhizobium* strains previously. That is, the bacterium was not native to the soil after the first inoculation, given that the results of subsequent inoculations were lower. According to Gris *et al*. (2005), the untreated seeds and those treated with the commercial inoculant may not present a significant difference due to the presence of bacterial populations already existing in the soil, generating good nodulation and BNF.

Santos *et al*. (2013) observed that seeds treated with thiamethoxam, fludioxonil + metalaxyl-m, and thiabendazole molecules have a phytotoxic effect on bacteria and can inhibit the biological fixation or decrease inoculant viability. Also, Silva *et al*. (2011) affirm that the smaller number of nodules may be related to the active principles used in the treatment of seeds as fungicides, insecticides, and inoculants, compromising the diazotrophic bacteria results that differ from those found in this study.

According to Embrapa (2008), the presence of nitrogen fertilizers can reduce the efficiency of bacteria, and N, in mineral form affects the fixation and also the nodules in the plants. This is related to the decrease in available oxygen used in nodular respiration and also to the limitation of carbohydrates in the nodule. Another factor that may compromise nodulation is soil salinization, which was observed by Velagaleti *et al*. (1990) who reported that the salinity affects the infection process and initial development of the nodules, this may be due to the inhibition of calcium absorption by excess salts, which

Table 2. Averages of the variables number of nodules, stem diameter, and plant height with different combinations of seed treatment and application of N, Co, and Mo. Crop 2015/16. Toledo, PR.

Treatment	Number of nodules *	Stem diameter (mm)	Plant height (cm)
T1 - Control	260.0	6.76	99.6
T2 - *Bradyrhizobium*+Treatment	273.7	7.26	99.5
T3 - *Bradyrhizobium*+untreatment	283.2	7.56	105.3
T4 - *Bradyrhizobium*+Treatment+CoMo	250.0	7.63	105.3
T5 - *Bradyrhizobium*+CoMo+untreatment	223.0	7.96	112.0
T6 - *Bradyrhizobium*+Treatment+CoMo Foliar	277.7	7.03	105.7
T7 - *Bradyrhizobium*+Treatment+N in R2	219.5	8.33	110.7
T8 - Treatment+N in R2	268.7	8.10	110.6
mean	257	7.58	106.1
F value	0.813 ^{ns}	3.456^{ns}	1.893^{ns}
CV (%)	21.03	6.75	5.74

ns= non-significant; CV= coefficient of variation
* greenhouse experiment

reduces the growth of root and root hairs and decreases the potential for *Bradyrhizobium* infection.

According to Santos *et al*. (2013), the presence of the active ingredients in the rhizosphere causes the exudates of the roots to change, thus decreasing the emission of molecular signals, generating fewer nodules and lower BNF.

For the stem diameter, there was no statistical difference (P>0.05) possibly because only one cultivar was evaluated with the same population density, maintaining a standard of stem diameters.

For plant height, there was no significant difference (P>0.05) This occurs because the soil could have supplied the plant requirements of N through the satisfactory availability of this nutrient in the environment. However, if T5 (seeds with CoMo inoculated with *Bradyrhizobium*) is compared to T1 (control), the difference in plant height is 12.4 cm (Table 2). A similar result was found by Parente *et al*. (2015), who verified that the plant height in BRS Valiosa RR cultivar did not present a significant difference, but its height was above the average stipulated by the genotype providers.

T5, T7, and T8 had a higher number of nodes, pods, and grains (P<0.05) than the rest of the other treatments, according to Table 3. The application of N at the R2 stage of T7 and T8 could have provided a possible BNF deficiency, complementing the demand of the plant and resulting in a greater vegetative development as shown by the greater number of nodes.

Regarding the treatments of seeds, T5, T7, and T8 no differences were observed, that is, the treatments of seeds with different chemicals did not have influence, which could lead to the reduction of symbiotic microorganisms, and decrease the number of pods and grains. A different result was presented by Santos *et al*. (2013), who observed that, when the seeds are treated with chemical insecticides and fungicides, and then the inoculation of bacteria is used, the viability of the seeds is lower, and the inoculated bacteria cannot perform their function of BNF. The non-inoculation with *Bradyrhizobium* in T8 (only the chemical treatment of the seeds and application of nitrogen topdressing was carried out) allowed similar results to the other two treatments in which the inoculation was performed (T5 and T7). Therefore, after the first inoculations, the results are inferior, however, in many cases, they can be economically efficient.

Vieira *et al*. (2017) in their work on reinoculation of the soybean crop in a no-tillage area, observed that there was no difference with the control without inoculant. This result may be due to the efficiency of the natural *Bradyrhizobium* population of the soil, which can supply the nitrogen needs of the crop.

Table 3. Averages of the variables number of nodes, number of pods, and number of grain with different combinations of seed treatment and application of N, Co, and Mo. Crop 2016/17. Toledo, PR.

Treatment	Number of nodes	Number of pods	Number of grain
T1 - Control	31.93 b	51.66 b	127.96 b
T2 - *Bradyrhizobium*+Treatment	34.03 b	56.96 b	133.26 b
T3 - *Bradyrhizobium*+untreatment	35.30 b	59.20 b	143.53 b
T4 - *Bradyrhizobium*+Treatment+CoMo	35.93 b	55.70 b	138.73 b
T5 - *Bradyrhizobium*+CoMo+untreatment	40.42 a	64.66 a	159.36 a
T6 - *Bradyrhizobium*+Treatment+CoMo Foliar	35.77 b	55.53 b	135.53 b
T7- *Bradyrhizobium*+Treatment+N at R2	41.13 a	65.60 a	158.30 a
T8 - Treatment+N at R2	43.57 a	69.10 a	166.40 a
mean	37.26	59.80	145.38
F Value	5.342*	4.120*	2.795*
CV (%)	8.03	10.4	10.07

*: significant at the 5% probability level by the F test. Means followed by the same letter do not differ by Tukey test at 5% significance; CV= coefficient of variation

In the variables root length, the mass of a thousand grains, and productivity, the application of Co and Mo was not highlighted (Table 4). It can be stated that the soil is possibly supplied with these two micronutrients, without limiting the viability of the nodules.

Pessoa *et al*. (1999) found no significant difference in their work, but when the authors applied a dose of 80 g ha^{-1} of Mo, there was an increase in yield, although it did not differ from the other doses tested.

Although the number of nodes presented a significant difference, pods and grains $plant^{-1}$ did not differ in terms of productivity, which can be explained by the fact that there was no significance for the mass of 1000 grain. Another factor that may favor a similar result is the low

Table 4. Averages of the variables root length, the mass of 1000 grain, and productivity regarding the seed treatments and the application of N, Co, and Mo. Crop 2016/17. Toledo, PR.

Treatments	Root length* (cm)	Mass of 1000 grain (g)	Productivity (kg ha^{-1})
T1 - Control	21.87	149.23	3.097
T2 - *Bradyrhizobium*+Treatment	17.12	156.36	4.680
T3 - *Bradyrhizobium*+untreatment	19.37	152.66	4.619
T4 - *Bradyrhizobium*+Treatment+CoMo	16.25	156.10	4.358
T5 - *Bradyrhizobium*+CoMo+untreatment	21.75	159.43	4.618
T6 - *Bradyrhizobium*+Treatment+CoMo Foliar	32.50	152.40	4.912
T7 - *Bradyrhizobium*+Treatment+N at R2	25.87	157.06	4.440
T8 - Treatment+N at R2	24.62	153.66	4.139
mean	44.48	154.61	4.483
F value	1.117 ns	1.027 ns	0.459 ns
CV (%)	22.42	3.57	15.91

ns= not significant; CV= coefficient of variation
* greenhouse experiment

pH of the soil since according to Farias *et al.* (2016) a higher pH, produced by liming and soil cultivation, seems to trigger ecological modifications, which benefit the appearance of strains of *Rhizobium* spp.

Furthermore, Parente *et al*. (2015), also found no significant difference when applying N at R1 for the variables mass of 1000 grains and yield of BMX Potência cultivar. These results corroborate those obtained by Aratani *et al*. (2008), who did not obtain an increase in productivity with the application of N regardless of the application stage. Bahry *et al*. (2013) in their work with nitrogen fertilization in soybean crop coverage concluded that there was no higher yield of grains in the crop, a similar result to this work. Still, Pessoa *et al*. (1999) did not obtain a significant difference for soybean yield after Mo application and did not find a significant interaction between inoculant plus application of CoMo for grain mass and yield.

Golo *et al.* (2009) reported that when working with inoculant plus application of CoMo, there was no significant interaction for plant height, insertion height of the first pod, number of pods per plant, number of seeds per plant, number of seeds per pod, the mass of 1000 grains and productivity. Nevertheless, according to the authors, the doses of CoMo significantly influenced the mass of 1000 seeds and productivity. For the root length, the results were similar for all treatments, showing no significant difference; however, the treatment that stood out in root length was T6 (*Bradyrhizobium* + Treatment + CoMo Foliar application) with 32.50 cm.

CONCLUSION

Combinations of inoculant, CoMo, seed treatment with insecticide, and nitrogen application did not influence nodulation, stem diameter, plant height, root length, a mass of 1000 grain, and productivity. Application of nitrogen at R2 and seed treatment can provide a greater number of nodes, pods, and grains per plant.

It is worth mentioning that the use of chemical seed treatment together with inoculation with *Bradyrhizobium* and foliar application of CoMo can result in greater root development of the crop, which can allow productivity increases when not subjected to stress.

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Comparison of statistical indices for the evaluation of crop models performance

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Comparación de índices estadísticos para la evaluación de modelos de cultivos

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ABSTRACT

This study presents a comparison of the usual statistical methods used for crop model assessment. A case study was conducted using a data set from observations of the total dry weight in diploid potato crop, and six simulated data sets derived from the observations aimed to predict the measured data. Statistical indices such as the coefficient of determination, the root mean squared error, the relative root mean squared error, mean error, index of agreement, modified index of agreement, revised index of agreement, modeling efficiency, and revised modeling efficiency were compared. The results showed that the coefficient of determination is not a useful statistical index for model evaluation. The root mean squared error together with the relative root mean squared error offer an excellent notion of how deviated the simulations are in the same unit of the variable and percentage terms, and they leave no doubt when evaluating the quality of the simulations of a model.

Keywords:
Crop simulation model
Deviation statistics
Efficiency coefficient
Index of agreement
Model evaluation
RMSE

RESUMEN

Este artículo presenta una comparación de los métodos estadísticos habituales que se utilizan para la evaluación de modelos de cultivos. Se realizó un estudio de caso utilizando un conjunto de datos observados del peso seco total en un cultivo de papa diploide y seis conjuntos de datos simulados destinados a predecir las observaciones. Los parámetros estadísticos evaluados fueron el coeficiente de determinación, la raíz cuadrada del cuadrado medio del error, la raíz cuadrada del cuadrado medio del error relativo, el error medio, el índice de concordancia, el índice de concordancia modificado, el índice de concordancia revisado, el índice de eficiencia y el índice de eficiencia revisado. Los resultados mostraron que el coeficiente de determinación no es un índice estadístico útil para la evaluación de modelos de cultivo. La raíz cuadrada del cuadrado medio del error junto a la raíz cuadrada del cuadrado medio del error relativo, ofrecen una excelente idea de cuánto están desviadas las simulaciones en la misma unidad de medida de la variable y en términos porcentuales. La raíz cuadrada del cuadrado medio del error y la raíz cuadrada del cuadrado medio del error relativo no dejan dudas al evaluar la calidad de las simulaciones de un modelo respecto a las observaciones

Palabras clave:
Modelo de simulación de cultivos
Estadísticas de desviación
Coeficiente de eficiencia
Índice de concordancia
Evaluación del modelo
RMSE

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The traditional research based on field experiments has a high investment in infrastructure, equipment, labor, and time. Alternatives to conventional studies are the development and application of crop models in agriculture, which show a simplified representation of the processes that occur in a real system, including variables that interact and evolve, showing dynamic and real behavior over time (Thornley, 2011). Crop models allow experimentation, complementing traditional research based on field experiments, and allowing an economical and practical evaluation of the effect of different environmental conditions and several agricultural management alternatives, reducing risk, time, and costs (Ewert, 2008).

Several simulation models have been developed for crops such as cassava (Moreno-Cadena *et al*., 2020), potato (Fleisher *et al*., 2017; Saqib and Anjum, 2021), wheat (Asseng, 2013; Iqbal *et al*., 2014), rice (Li *et al*., 2015), and corn (Abedinpour *et al*., 2012; Bassu *et al*., 2014; Kumudini *et al*., 2014). Moreover, models are continuously evaluated under different environmental conditions, cultivars, and treatments. These crop models are useful tools for simulations of real crop growth and development processes (Yang *et al*., 2014). The used models are assumptions that have best survived the unremitting criticism and skepticism that are an integral part of the scientific process of construction and development (Thornley, 2011).

In general, the datasets used to develop a crop model are different from the real inputs in which the model is expected to be used. For a crop simulation model to represent a real process, it must be evaluated considering the differences between crop systems, soils, climate, and management practices; otherwise, the conclusions may be speculative and incorrect (Yang *et al*., 2014).

The growth dynamics represented by crop models are based on a set of hypotheses, which could result in simulation biases or errors (Yang *et al*., 2014). Thus, the model performance evaluation is crucial by comparing model estimates to actual values, and this process includes a criteria definition that relies on mathematical measurements of how well the estimates produced by the model simulate the observed values (Ramos *et al*., 2018). This statistical analysis is considered as the critical method to compare the model outputs with the measured data (Montoya *et al*., 2016; Reckhow *et al*., 1990; Willmott *et al*., 1985; Yang *et al*., 2000).

The most common methods for assessing the reliability of simulation models are based on the analysis of differences between measured and simulated values, and on regression analysis, also between measured and simulated values (Lin *et al*., 2014; Willmott, 1982; Yang *et al*., 2000). However, many authors who research crop modeling use such methods without detailing methodological basis and using terminology and symbols that create confusion. For example, in the analysis of the difference, statistics such as relative error (*RE*), index of agreement (*d*), and modeling efficiency (*EF*) may be useful when comparing the simulation capability of one model with another, but not when comparing what is observed with what is simulated in the same model (Ramos *et al*., 2018; Yang *et al*., 2014). Relative error (*RE*), which relates the error between measured and simulated values, concerning the measured average, represents the relative size of the average difference (Willmott, 1982), indicating whether the magnitude of the root-mean-square error (*RMSE*) is low, medium or high. However, it has the disadvantage that it can be affected by the magnitude of the values, by outliers, and the number of observations. It may be the case that two groups of data with high and low values, present a similar RMSE. However, having different averages, *RE* values will also be different (Cao *et al*., 2012).

Because of its simplicity, regression analysis is often misused to evaluate simulation models. In some cases, the *RMSE* that measures the average difference between measured and simulated values tends to be used indiscriminately, without considering that it is different from the *RMSE* obtained in regression analysis (Willmott, 1982). The coefficient of determination (R^2) is a measure of the linear regression adjustment, which, when used in isolation, makes no sense since the goal is to evaluate the crop simulation model, not the regression model obtained.

The magnitude of R^2 does not necessarily reflect whether the simulated data represent well the observed data since it is not consistently related to the accuracy

of the prediction (Willmott, 1982). This is because an R^2 can be obtained close to 1.0 but below or above the 1:1 line, tending to simulate high values or underestimates the observed values, respectively.

Many statistical indices are frequently used in model evaluation, and this paper aimed to compare and improve the understanding and interpretation of these conventional statistical indices in a case study.

MATERIALS AND METHODS

The performance of nine statistical indices was computed to evaluate the simulations of actual observations and simulations of total dry weight (kg ha^{-1}) obtained in a diploid potato field experiment conducted in Medellín, Colombia. This data set were taken from Saldaña-Villota and Cotes-Torres (2020). Besides, from the actual observed data, six data sets were generated with arbitrary deviations appropriately imposed to illustrate the behavior of the statistical indices under evaluation. (Table 1). In case 1, the first half of the simulations is overestimated, and the second half is underestimated in the same amount (200 kg ha^{-1}). In case 2, the first half of the simulations is overestimated 1.5 times, and the second half of the simulations is underestimated 0.5 times. In case 3, all simulations are overestimated in 100 kg ha^{-1}. In case 4, all simulations are overestimated 2.5 times. In case 5, most of the simulations are overestimated in different proportions, and an outlier 3.4 times larger than its corresponding observation is presented. Finally, in case 6, all simulations are overestimated in different proportions, and they do not have any relationship with the observations.

The indices are expected to inform the researcher of the accuracy of any model in simulating the observations.

Table 1. Actual observations of diploid potato total dry weight (kg ha^{-1}) and simulated data sets.

Days after planting (DAP)	Actual observed data set[a]	Simulated Data Sets Cases					
		1	2	3	4	5	6
23	57.43	257.44	86.15	157.43	143.59	52.29	549.89
30	153.20	353.20	229.79	253.19	382.99	467.99	3212.52
37	315.10	515.10	472.65	415.10	787.75	429.79	1649.67
43	547.59	747.59	821.39	647.59	1368.99	1736.02	2950.30
51	804.70	1004.70	1207.05	904.70	2011.75	1832.45	6468.99
58	1166.00	1366.00	1749.00	1266.00	2915.00	3151.93	7949.38
65	1338.00	1138.00	669.00	1438.00	3345.00	4608.72	3849.24
72	1837.00	1637.00	918.50	1937.00	4592.50	3432.99	8595.41
79	2740.00	2540.00	1370.00	2840.00	6850.00	6263.89	25702.79
85	4103.00	3903.00	2051.50	4203.00	10257.50	3968.15	5946.69
91	5657.00	5457.00	2828.50	5757.00	14142.50	18991.13	17439.52
100	6738.00	6538.00	3369.00	6838.00	16845.00	6088.44	17071.64
Mean	2121.42	2121.42	1314.38	2221.42	1314.38	4251.98	8448.84

[a] Total dry weight in diploid potato crop. Source: Saldaña-Villota and Cotes-Torres (2020).
Case 1: The first half of the simulations is overestimated, and the second half is underestimated in the same amount (200 kg ha^{-1}).
Case 2: The first half of the simulations is overestimated 1.5 times, and the second half of the simulations is underestimated 0.5 times.
Case 3: All simulations are overestimated in 100 kg ha^{-1}.
Case 4: All simulations are overestimated 2.5 times.
Case 5: Most of the simulations are overestimated in different proportions, and an outlier 3.4 times larger than its corresponding observation is presented.
Case 6: All simulations are overestimated in different proportions, and they do not have any relationship with the observations.

The statistical indices are expected to allow decisions to be made regarding the acceptance or rejection of the models. In this study, with the modifications applied to generate the six cases, the statistical indices must accept cases 1 and 3 and reject the other cases without ambiguity.

Many statistical indices are commonly used in model evaluation, and they have been classified depending on their mathematical formulation. In this study, nine indexes were evaluated and classified into two categories. The first one corresponds to the 'test statistics', and the second one corresponds to measures of accuracy and precision called 'deviation statistics' (Ali and Abustan, 2014; Willmott *et al*., 1985; Yang *et al*., 2014).

Test statistics

Linear regression and coefficient of determination (R^2) are used to explain how well the simulations (y) represent the observations (x) (Kobayashi and Salam, 2000; Moriasi *et al*., 2007; Willmott, 1982). The linear model follows Equation 1.

$$y = \alpha + \beta x + \varepsilon \qquad (1)$$

where α is the regression intercept, β is the slope, and ε represents the random error.

The R^2 assesses the goodness of fit of the linear model by measuring the proportion of variation in y, which is accounted for by the linear model. R^2=1.0 indicates a perfect fit of Equation 1, and R^2=0 means there is no linear relationship.

However, many researchers have reported the limitation of R^2 in the appropriate evaluation of the models, remarking that R^2 estimates the linear relationship between two variables, and it is no sensitive to additive and proportional differences between model estimates and measured data (Kobayashi and Salam, 2000; McCuen and Snyder, 1975; Willmott, 1981). The authors also indicate that the relationship may be non-linear, which would be an additional problem.

Deviation statistics

Some deviation statistics correspond to measures developed to test the deviation directly (*deviation = y-x*) and surpass the limitation of correlation-based statistics (Yang *et al*., 2014). The Mean Error (*E*) (Equation 2) indicates whether the model simulations (*y*) overestimate or underestimate the observations (*x*). When *E*>0 means that the model is overestimating, while *E*<0 means that model underestimates the measured data. *E* has a drawback: the positive and negative errors can negate each other, and large positive and negative deviations can still obtain *E*-0 (Addiscott and Whitmore, 1987; Yang *et al*., 2000).

$$E = n^{-1}\sum(y_i - x_i) \qquad (2)$$

where *i*-1,2,...,*n* .

Due to *E* disadvantage, some measures based on the sum of squares were developed (Yang *et al*., 2014). The root mean square error (*RMSE*) (Equation 3) has the same unit of deviation *y-x*, and it is frequently used in both model calibration and validation process (Hoogenboom *et al*., 2019; Hunt and Parsons, 2011)

$$RMSE = \left[n^{-1}\sum_{i=1}^{n}(y_i - x_i)^2 \right]^{0.5} \qquad (3)$$

The relative root mean square error (*rRMSE*) (Equation 4) is a relative measure used for comparisons of different variables or models. indicating whether the magnitude of the root-mean-square error (*RMSE*) is low, medium, or high (Priesack *et al*., 2006).

$$rRMSE = \frac{RMSE}{\bar{x}} x100 \qquad (4)$$

Nash-Sutcliffe modeling efficiency coefficient (*EF*) (Equation 5) (Nash and Sutcliffe, 1970). This index is a dimensionless measure (– ∞ to 1.0). A perfect fit between simulations and observations produces an *EF*–1.0. Any value between 0 and 1.0 is obtained for any realistic simulation. *EF*<0 is obtained if the simulated values are worse than merely using the observed mean $(\bar{x})$ to replace the simulated y_i.

$$EF = 1 - \frac{\sum(y_i - x_i)^2}{\sum(x_i - \bar{x})^2} \qquad (5)$$

Another index that is commonly used in crop model evaluation is the index of agreement (*d*) (Equation 6) a dimensionless measure (0 to 1.0) proposed by Willmott (1982). This index has been recommended by researchers in modeling to carry out comparisons between simulated values and measured data (Krause *et al*., 2005; Moriasi *et al*., 2007).

$$d = 1 - \frac{\sum (y_i - x_i)^2}{\sum (|y_i - \bar{x}| + |x_i - \bar{x}|)^2} \quad (6)$$

EF and *d* are more sensitive to larger deviations than smaller deviations. The main disadvantage of both statistics is the fact that the differences between model estimates and observations are calculated as squares values; thus, these sums of squares-based statistics are very sensitive to outliers or larger deviations due to the squaring of the deviation term (Krause *et al*., 2005; Legates and McCabe Jr, 1999; Willmott *et al*., 2012).

To overcome the difficulty of the statistics based on the sum of squares that are inflated by the squaring deviation term, statistics based on the sum of absolute values were proposed (Krause *et al*., 2005; Willmott *et al*., 2012). The modified efficiency coefficient (EF_1) (Equation 7) replaces the sum of squares term with the sum of absolute values of $y–x$. EF_1 is less sensitive to outliers, and it takes also values between $-\infty$ and 1.0 (Legates and McCabe Jr, 1999).

$$EF_1 = 1 - \frac{\sum |y_i - x_i|}{\sum |x_i - \bar{x}|} \quad (7)$$

Willmott *et al*. (1985) proposed the modified index of agreement (d_1) (Equation. 8), to avoid the critical effect of outliers in the sum of squares used on *d*. The author remarks that d_1 yields 1.0 more slowly than *d*. *d* and d_1 show relative high values even if a substantial deviation is evident, and to overcome this issue, Willmott *et al*. (2012) proposed a refined index of agreement (d_1') (Equation 9), which is ranged –1.0 to 1.0. When , d_1'=0.5, the sum of the magnitude of the errors is half of the sum of the perfect-simulated-deviation and observed-deviation magnitude.

$$d_1 = 1 - \frac{\sum |y_i - x_i|}{\sum (|y_i - \bar{x}|) + |x_i - \bar{x}|} \quad (8)$$

$$d_1' = 1 - \frac{\sum |y_i - x_i|}{2\sum |x_i - \bar{x}|} \quad (9)$$

The calculation of the statistics indices to evaluate the six simulated data sets, and figures were made with R statistical software (R Core Team, 2020).

RESULTS AND DISCUSSION

This study shows a comparison of nine statistical indexes used during model evaluation. The actual data of the total dry weight measured in a diploid potato field experiment and the six simulated data set are shown in Figure 1 to facilitate the visualization of the data and their analyzes.

Coefficient of determination (R^2)

In the simulated data cases 1, 2, 3, and 4 (Figure 1A-D), different scenarios were presented in which the actual observations are overestimated or underestimated. The simulations preserved the trend of the measurements, which is the reason why the R^2 was high. Although simulations considerably overestimated the measurements in case 4, the fact that the simulated data follow the trend of the observations even if they are overestimated or underestimated, the R^2 will be close to 1.0. Consequently, this index is not adequate to evaluate the quality of the simulations in growth variables in crop models. The coefficient of determination was lower in cases 5, and 6 (Figures 1E and F), indicating that the simulated data did not follow the observed data trend.

Mean error (*E*)

E indicates whether the model overestimates or underestimates the measurements. This index presented difficulty to indicate what happened in case 1, in which half of the simulations were overestimated, and half were underestimated in the same proportion. In this case,*E*–0, and this value gives no indication of over or underestimation. In case 2, *E* indicates that the simulated data underestimate the total dry weight by 807,040 kg ha^{-1}. In the remaining cases, *E*>0, indicating that the simulations overestimate the measurements. According to *E*, case 6 was the one that registered the maximum overestimation, exceeding 6000 kg ha^{-1}.

Root mean squared error (*RMSE*) and the relative-RMSE (*rRMSE*)

The *RMSE* indicates how deviated the simulated mean is from the observed mean. This index does not indicate whether there are overestimates or underestimates. Nevertheless, if the *RMSE* is close to zero or less than the amount assigned by the researcher according to the expertise in the crop studied, the model performs better in predicting the measured data. If the researcher is not an

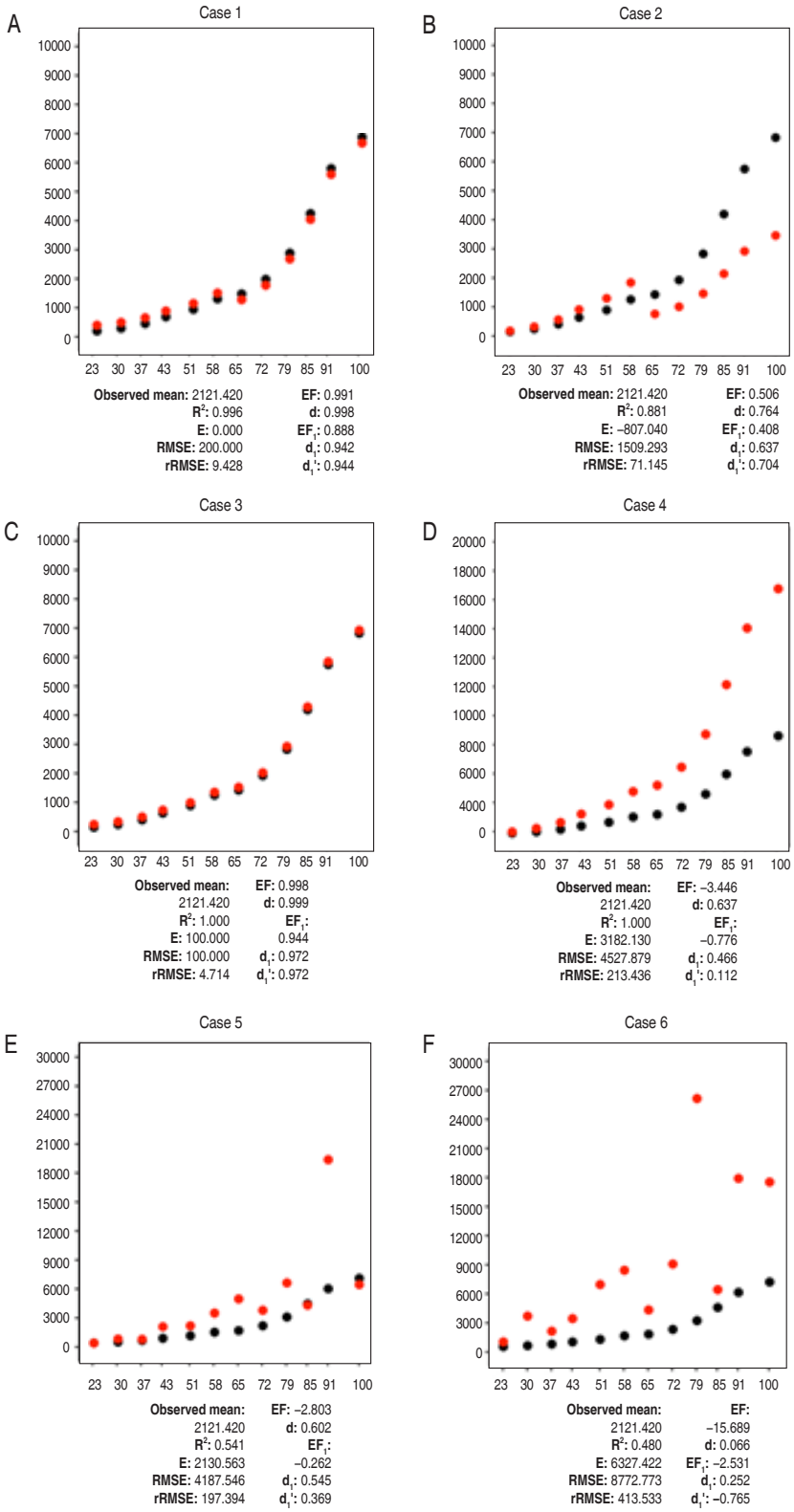

Figure 1. Comparison between real observations and six simulated data set of total dry weight in diploid potato crop (kg ha^{-1}) over time (days after planting). Black circles correspond to the real observations, and red ones correspond to the simulated counterpart.

expert about the range of values that a growth variable can reach, the *RMSE* should be evaluated together with *rRMSE*, which indicates the deviation of the simulations from the general mean of the observations in percentage terms. In this sense, according to the characteristics of these two indices, unquestionably cases 1 and 3 had the best performances when simulating the observations, where the deviation from the mean was 200 and 100 kg ha^{-1}, corresponding to 9.428 and 4.714%, respectively.

Regarding case 2, where the simulations underestimated the total dry weight from 65 DAP, the *RMSE* was affected, recording a value of 1509.293 kg ha^{-1}, meaning a deviation higher than 70% (*rRMSE*). In case 4, although as mentioned, the simulations overestimated the observations even though they followed their trend. This overestimation significantly influenced the *RMSE*, which registered a value of 4527.879 kg ha^{-1}, equivalent to a deviation of more than 200% concerning the general mean of the observations (2121.42 kg ha^{-1}). Case 5 exemplifies the effect that outliers have on statistical indices. At 91 DAP, a very high datum was recorded in the simulations compared to the other simulations and, of course, to the observations. Together with the other predicted data, this outlier generated *RMSE*–4187.516 kg ha^{-1}, and *rRMSE*–197.394%. If the researcher, after exploring different explanations for this extreme data, decides not to consider the outlier, the *RMSE* would be equal to 2320 kg ha^{-1} and the *rRMSE*–10939%, indicating that in the same way, the model does not predict the observations in an acceptable way and these are overestimated at 2130 kg ha^{-1} (keeping the outlier) and at 547.96 kg ha^{-1} (eliminating the outlier). Finally, the *RMSE* and *rRMSE* obtained in case 6 are definitive to consider that the simulations are unacceptable. Although the graphical representation (Figure 1F) is a clear indication of the low quality of the predictions, an *RMSE*–8772.773 kg ha^{-1} and an *rRMSE* higher than 400% are enough to rule out the model. Besides, this data set had outliers, but in general, the simulated data had no relationship with the observations.

Nash-Sutcliffe coefficient (*EF*) and the modified Nash-Sutcliffe coefficient (EF_1)

The analysis of the following indices that are dimensionless, such as the Nash-Sutcliffe coefficient (*EF*) and the modified Nash-Sutcliffe coefficient (EF_1), confirm that simulations in cases 1 and 3 are close to perfection with values very close to 1.0 (*EF*–0.991 and 0.998; EF_1–0.888 and 0.972, respectivelty).

According to Nash and Sutcliffe (1970), *EF* and EF_1 values between 0 and 1 are expected in any modeling scenario. However, in case 2, for instance, *EF* and EF_1 reached values of 0.506 and 0.408, which are values higher than zero, but by themselves, they are not clear with the reality of the simulation quality. Nonetheless, values less than zero in these two indices are indicators of wrong predictions; thus, cases 2, 5, and 6 achieved values <0, confirming what *E*, *RMSE*, and *rRMSE* had indicated. Also, the more negative values suggest that the simulated data were worse. The clearest example is case 6, which reached –15.689 in *EF*, but EF_1 was –2.531. *EF* reached higher values (both positive and negative) because when considering sums in terms of the sum of squares in its formulation, it is more affected by outliers. EF_1 is calculated considering the sum in terms of absolute values, that means less sensitivity to extreme data.

Index of agreement (*d*), modified index of agreement (d_1), and revised index of agreement (d_1')

Finally, from the group of indices *d*, d_1, and d_1', the best simulations reach values close to 1.0 (Cases 1 and 3). In the same way as *EF* and EF_1, the statistics of group *d* must be estimated in association with other indices to make better inferences about the accuracy of the simulation. In case 2, *d*=0.764, and if this value is analyzed by itself, it would suggest that the model is adequate, but d_1 is stricter than *d*, and its value is clearer suggesting that the simulations are not adequate (d_1=0.637). *d* and d_1 in cases 5 and 6 were less than 0.75, suggesting that these models are not suitable for simulating the measured data set. Case 6 was the only one that reached a negative d_1' value (–0.765), again indicating that the simulations, in this case, are not adequate when predicting the measurements.

General performance of the statistical indices in evaluating the quality of the simulations of a model

Summarizing the previous results (Table 2), the *RMSE*, *rRMSE*, *EF*, EF_1, and d_1 are the best indices for evaluating the quality of simulations because,

they accepted the simulations in cases 1 and 3, and rejected the other cases, which was expected in this study when comparing the behavior of the statistical indices. However, given the simplicity in the interpretation of *RMSE* and *rRMSE*, they are preferred over dimensionless statistics.

Table 2. Acceptance or rejection of the simulations defined by different statistical indices for each data set.

Statistical parameter	Simulated Data Sets					
	1	2	3	4	5	6
R^2	✓	✓	✓	✓	✗	✗
E	-	-	-	-	-	-
RMSE	✓	✗	✓	✗	✗	✗
rRMSE	✓	✗	✓	✗	✗	✗
EF	✓	✗	✓	✗	✗	✗
EF_1	✓	✗	✓	✗	✗	✗
d	✓	✓	✓	✗	✗	✗
d_1	✓	✗	✓	✗	✗	✗
d_1'	✓	✓	✓	✗	✗	✗

✓ Simulations accepted
✗ Simulations rejected

Statistical analysis is a crucial procedure during model calibration and evaluation, and there are many statistical methods useful to support crop model researchers. It is unquestionably that R^2 is not a suitable parameter for model evaluation because it is not sensitive to additive (regression intercept) and proportional differences (regression slope) (Willmott *et al*., 2012; Yang *et al*., 2013). The linear regression should be employed to evaluate the simulated outputs with the observed inputs when the time series data follow the assumptions of independence, normality, and homoscedasticity in the error term (Yang *et al*., 2014). The error term does not follow these assumptions in the deviation statistics because they are not hypothesis tests (Willmott *et al*., 1985).

The mean error (*E*) is a good statistical parameter to quickly determine if the model under or overestimates the observations. Unfortunately, it does not offer clarity on the quality of the simulations. Nevertheless, *RMSE* and *rRMSE* are very suitable for model evaluation because they provide the researcher with a useful decision-making guide. It is important to highlight the advantages that the *RMSE* and the *rRMSE* offer, which together offer a better idea of how deviated the simulations are in the same unit of the variable and percentage terms.

If only the group index of agreement is considered during the evaluation of a model, it is possible to make bad decisions or assume that the model predicts the measured data with quality when in reality, the predictions are not adequate. *d* can quickly reach 1.0 without considering significant discrepancies between simulations and observations because the sum of squares-based deviations easily inflates *d*. A researcher could consider case 2 a suitable model to simulate the observations according to *d* and d_1' values, even when d_1' seems to be stricter than *d* in mathematical terms. d_1 and *EF* showed well behaviors, and they have a sharp meaning and interpretation when values tend to zero. Yang *et al*. (2014) suggested for plant growth variables simulations *EF*>0 and *d*, d_1, and d_1' as minimum values for dry weight of leaves, stems, yield, tubers, total in the case of the potato crop.

Both modeling efficiency coefficients (*EF* and EF_1) and indices of agreement (*d*, d_1, and d_1') are widely used in modeling evaluation. Although *d* and *EF* are sensitive to the sum of squares and, in consequence, they achieve higher values even with not accurate simulations. The researcher should use these dimensionless indices carefully. Alternatively, use *RMSE* and *rRMSE* as good guides to evaluate the quality of the models.

CONCLUSION

The *RMSE* and the *rRMSE* offer a better idea of how deviated the simulations are in the same unit of the variable and percentage terms; for this reason, these

indices are the most appropriate to reflect the quality of the simulations of a model. This pair of indices was the only one that unquestionably established that cases 1 and 3 are almost perfect with deviations less than 200 kg ha^{-1}, which is less than 10% concerning the mean of the observations. *RMSE* and *rRMSE* leave no doubt that cases 2, 4, 5, and 6 correspond to models that reflect very poorly or do not reflect the observations.

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The water footprint of coffee production in Colombia

Huella hídrica de la producción de café en Colombia

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Keywords:
Climate change
Coffee
Water consumption
Water contamination
Water risks
Water scarcity

ABSTRACT

The problem of water availability and its important role in the agricultural sector, specifically in the cultivation of coffee, which has historical, cultural, and economic importance for Colombia, requires a study of the water footprint in this country. This paper presents the results of a study of the water footprint of coffee production (cultivation and wet processing) in Colombia by the traditional and ecological wet-processing methods. To this purpose, the Water Footprint Network methodology was followed according to the Water Footprint Assessment Manual (2011). The green water footprint of coffee production in Colombia was 8,746 $m^3\ t^{-1}$ and does not have a blue water footprint as it does not require irrigation, while the gray water footprint was 7,000 $m^3\ t^{-1}$. When the traditional wet-processing method is used, the blue water footprint is 4 $m^3\ t^{-1}$ and the gray water footprint is 3,200 $m^3\ t^{-1}$, while if the ecological Becolsub® technology is used, the blue water footprint is 0.60 $m^3\ t^{-1}$ and the gray water footprint is 1,739 $m^3 t^{-1}$. For the Ecomill® technology, the blue one is 0.55 $m^3\ t^{-1}$ and had no gray water footprint because it does not generate any water discharge and the little leachate that it produces is reincorporated into the process. This implies that the Becolsub® ecological processing method reduces the water footprint by 45.7% and 99.9% with the ecological Ecomill® process (no wastewater discharge) compared to traditional wet processing technology. Compared to other countries, Vietnam has the lowest green footprint in coffee cultivation, followed by Colombia, Ethiopia, Brazil, Peru, and Indonesia. The water footprint of coffee depends on the climate and yields, consequently, the water footprint of the coffee crop varies significantly between locations and the evaluation period.

Palabras clave:
Cambio climático
Café
Consumo de agua
Contaminación de agua
Riesgos hídricos
Escasez de agua

RESUMEN

El problema de la disponibilidad de agua y su importante papel en el sector agrícola, específicamente en la presión que existe actualmente por el recurso hídrico, pero además en países como Colombia donde el cultivo del café tiene una importancia histórica, cultural y económica, lo que hace necesario un estudio de la huella hídrica de este cultivo en el país. Aquí se presentan los resultados de la huella hídrica de la producción de café (cultivo y beneficio) en Colombia, por el método de beneficio tradicional y ecológico. Para su cálculo se siguió la metodología propuesta por Water Footprint Network. La huella hídrica verde promedio del cultivo de café en Colombia es de 8.746 $m^3\ t^{-1}$, no tiene huella hídrica azul porque no requiere riego y la huella hídrica gris es del orden de 7.000 $m^3\ t^{-1}$. El beneficio tradicional de café no tiene huella hídrica verde, la huella hídrica azul es de 4.00 $m^3\ t^{-1}$ y tiene una huella hídrica gris de 3.200 $m^3\ t^{-1}$. El beneficio ecológico Becolsub® tiene una huella hídrica de azul de 0,60 $m^3\ t^{-1}$ y una huella hídrica gris de 1.739 $m^3\ t^{-1}$; mientras la tecnología Ecomill® sin vertimientos de aguas residuales tiene una huella hídrica azul de 0,55 $m^3\ t^{-1}$ y no tiene huella hídrica gris porque no presenta vertimientos. Esto implica que el método de procesamiento ecológico Becolsub® disminuye la huella hídrica en un 45,7% y en un 99,9% con el proceso ecológico Ecomill® (sin descarga de aguas residuales) en comparación con la tecnología tradicional de procesamiento húmedo. A nivel mundial, Vietnam cuenta con la menor huella hídrica, seguido por Colombia, Etiopia, Brasil, Perú e Indonesia. La huella hídrica del café, depende del clima y el rendimiento del cultivo, por esta razón, la huella hídrica del cultivo de café varia significativamente con el lugar y el periodo de evaluación.

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Global water use has increased by a factor of six over the past 100 years and continues to grow at a rate of about 1% per year as a result of increasing population, economic development, and shifting consumption patterns. Combined with a more erratic and uncertain supply, climate change worsens the current water-stressed situation of regions and generates water stress in regions where water resources are still abundant today. Physical water scarcity (WS) is often a seasonal phenomenon, rather than a chronic one, and climate change is likely to cause shifts in seasonal water availability throughout the year in several places (UNESCO, 2020). Around 1.6 billion people, or nearly a quarter of the world's population, face economic water shortage, which means they lack the necessary infrastructure to access water (UN-Water, 2014; UNESCO, 2020). The number of people tackling low, moderate, significant, and severe WS during a given number of months per year at the global level is about 71% of the global population (4.3 billion people), who live under conditions of moderate to severe water scarcity (WS>1.0) at least 1 month of the year, and about 66 % (4.0 billion people) live under severe water scarcity (WS>2.0) at least 1 month of the year. The number of people facing severe WS for at least 4 to 6 months per year is 1.8 to 2.9 billion. Half a billion people live severe WS all year round. Of those half-billion people, 180 million live in India, 73 million in Pakistan, 27 million in Egypt, 20 million in Mexico, 20 million in Saudi Arabia, and 18 million in Yemen (Mekonnen and Hoekstra, 2016).

From the different sectors of the worldwide economy, agriculture is the most sensitive to WS (FAO, 2013). This sector is occasionally considered as a “wasteful” water user after the domestic and industrial sectors accounting for 70% of global freshwater use and more than 90% of consumptive use. However, it is also the sector with the most possibilities for adjustment options (FAO, 2013). The extraction of water for different agricultural processes has a direct relationship with greater consumptive use as after the agricultural use, it is not available for other uses because it is contaminated or evaporated (Perry, 2007).

Cultivation and production of coffee are stages that have a significant environmental impact, which is especially attributed to the consumption and contamination of water (water footprint). Moreover, coffee is one of the crops with the greatest water footprint compared to others, such as wheat, corn, soybeans, sugar cane, and cotton, in terms of the volume of water consumed and polluted per quantity produced but also it has an especially high green water footprint (WFgreen**)** due to its water consumption and a high gray water footprint as a result of the wet-processing method required by this crop (Arévalo and Sabogal, 2012; Arévalo and Campuzano, 2013; IDEAM, 2015; Martins *et al*., 2018).

The water footprint is a measure of the appropriation of freshwater by humans in volumes of water consumed or polluted. This indicator is used to evaluate the amount of direct and indirect water used and contaminated to generate a product or service (Hoekstra *et al*., 2011).

On the other hand, coffee is historically and culturally Colombia’s primary product and the main national crop in terms of growing area, with approximately 24% of the total area of the country under coffee cultivation in 20 of the 33 Colombian departments. It represents approximately 2% of the national Gross Domestic Product (GDP) and 22 % of agricultural GDP (Federación Nacional de Cafeteros de Colombia, 2015). This is one of the main export products and contributes 8% to the total exported value of the country, generating more than 500,000 direct jobs that correspond to 36% of total agricultural employment (Ciro *et al*., 2011). Moreover, Colombia is the third-largest coffee exporter country in the world, according to the International Coffee Organization (2021).

In this context, this paper aimed to determine the water footprint of coffee production in Colombia and conduct a sustainability analysis of this water footprint, including the entire production process, from cultivation through the production of dry parchment coffee, which is the final export product. This information can help to identify, at the national level, the impacts associated with the water footprint of coffee production, its sustainability, and ways to address the associated impacts.

MATERIALS AND METHODS

Study site

For this study, four Colombian departments with a long

coffee tradition (Antioquia, Cauca, Caldas, and Quindío) were selected. These departments were chosen based on the fact that they are representative of the entire country in terms of coffee production and with a wide range of environmental conditions of the coffee zone in Colombia (Table 1).

Table 1. Basic information related to the departments, meteorological stations used in the calculations of coffee cultivation and their environmental conditions.

Department	Municipality	Weather Station	Location			Mean annual precipitation (mm year^{-1})	ETa (mm year^{-1})	Field capacity of soils (%)	Permanent wilting point of soils (%)
			Latitude (N)	Longitude (W)	Altitude (masl)				
Caldas	Chinchiná	Naranjal	04° 58'	75° 42'	1,381	1,921	1,018	48	29.4
Antioquia	Venecia	El Rosario	05° 58'	75° 42'	1,635	1,724	969	36.8	26
Cauca	El Tambo	Manuel Mejía	02° 24'	76° 44'	1,735	1,270	770	58.8	46.8
Quindío	Buenavista	Paraguaicito	04° 24'	75° 44'	1,203	1,455	1,008	19.7	10

Source: Cenicafé (2011-2020) and Ramírez *et al.* (2010).

Calculation and analysis of the water footprint

The methodology of the Manual for Water Footprint Assessment of the Water Footprint Network (WFN) (Hoekstra *et al.*, 2011) was used to calculate the water footprint of coffee production in Colombia. The total water footprint (WF) is the sum of the green, blue, and gray components, as shown in Equation 1:

$$WF\text{– total} = WF(\text{green} + \text{blue} + \text{gray}) \quad (1)$$

The WFgreen corresponds to the volume of rainwater that does not become runoff, is stored in the soils satisfying the demands of the vegetation. This shallow groundwater allows the existence of natural vegetation, and it returns to the atmosphere through the processes of evapotranspiration. The WFgreen for an agricultural crop corresponds to the actual evapotranspiration of the respective crop in a defined study area and evaluation period. Therefore, to calculate the WFgreen component in this study was necessary to determine the actual evapotranspiration of the coffee crops in Colombia.

To estimate the actual evapotranspiration (ETa) of the coffee crops in the different producing areas of the country, the methodology developed by Cenicafé was applied, which is based on the study of moisture balance in the soils of Colombian coffee growers, especially those under shaded systems and those with an open exposure (Jaramillo, 2006). The reference evapotranspiration (ET_0) was estimated from the expression of García and López (1970) that was modified by Jaramillo (1982) (Equation 2), where RH=relative humidity (%), T=temperature (°C), both are given in daily means and $n=(7.45T)x(234.7+T)^{-1}$.

$$ET_0=[1.22x10^{n}\times(1-0.01RH)]+0.2T-1.8 \quad (2)$$

The potential evapotranspiration (ET_p) of the crop is mandatory to calculate ET_0, which was estimated by Equation 3 and 4 (Ramírez *et al.*, 2010), where E_V=evaporation (mm), PP=precipitation (mm) and K_c=crop coefficient. As this K_c has not been measured for Colombia, therefore, the value reported by (Ramírez *et al.*, 2010) were used in this case, given the planting density and age of the plants.

$$E_v=1.071\times ET_0 \quad (3)$$

$$\begin{cases} ET_a = ET_0 \times K_c & \text{if } PP \le E_v \\ ET_p = 0 & \text{if } PP > E_v \end{cases} \quad (4)$$

The crop coefficient K_c was estimated based on the age and planting density, as proposed by Ramírez *et al.* (2010). ρ is the adjustment to the evapotranspiration by soil moisture (Equation 5).

$$\rho = \frac{\theta_{i-1}}{\theta_s} \quad (5)$$

Where θ_i is the soil moisture of the previous day, and θ_s the saturation moisture. When the estimation of $\rho<0.35$, the crop evapotranspiration is lower than the atmospheric demand due to a lack of water in the soil, and its value

is that given by the estimate; on the contrary case, the estimation uses ρ=1.

The actual crop evapotranspiration (ETa) was estimated using the water balance model of Thornthwaite-Mather and modified by Jaramillo (2006) (Equation 4). This equation was applied in conjunction with the expressions of Equation 6 to Equation 11, where P_{eff}=effective precipitation (mm), E_{run}=runoff (mm), $\theta_{v.cc}$=volumetric soil moisture at field capacity or water retained at -33 kPa (%), $\theta_{v.pmp}$=volumetric soil moisture at the permanent wilting point or water retained at 1.500 kPa (%), C.W.=water storage capacity, RDe=effective root depth, S.M=stored moisture, and C.N.=cumulative negative function. Under the condition that when the water retained in the soil in the effective range of the roots equals the retention capacity, the crop transpires at maximum capacity (ET_a=ET_p), and the above equations, which are given in terms of effective precipitation (P_{eff}), correspond to the condition of open crop exposure. This condition was assumed because it is the most unfavorable scenario concerning crop evapotranspiration (compared to a shaded condition). The details of the expressions described above can be found in Jaramillo (2006); Ramírez *et al.* (2010).

$$ET_a=(P_{eff}-E_{sc})+S.M_{i-1}-S.M_i \quad (6)$$

$$S.M.=C.W\times e^{\left(C.N/C.W\right)} \quad (7)$$

$$C.N.=(P_{eff}-ET_p) \quad (8)$$

$$C.W.=RDe(\theta_{v.cc}-\theta_{v.pmp}) \quad (9)$$

$$\begin{cases} E_{run}=\dfrac{5.16}{1+16.52e^{(-0.072\times PP)}} & \text{if } PP > 6.0mm \\ E_{sc}=0 & \text{if } PP \leq 6.0mm \end{cases} \quad (10)$$

$$\begin{cases} P_{eff}=\dfrac{69.13}{1+12.45e^{(-0.040xPP)}} & \text{if } PP > 6.0mm \\ P_{eff}=0 & \text{if } PP \leq 6.0mm \\ P_{eff}=PP & \text{if } PP > 44mm \end{cases} \quad (11)$$

The effective root depth was considered to be 0.50 m, given that this part of the soil profile represents 96% of the absorbing roots and more than 89.9% of the total roots, although the roots of a coffee plant can be as deep as 1.0 to 1.5 m (Pulgarín, 2007).

The temperature and rainfall information were extracted from the Coffee Meteorological Calendar from 2010 to 2019 from four stations located in the Colombian coffee zone (Cenicafé, 2011-2020). Based on this information, the actual daily evapotranspiration of the crop was estimated using the previously referenced expressions developed for the Colombian coffee zone.

The field capacity and permanent wilting point values used in this study are presented in Table 1 (Ramírez *et al.*, 2010). A crop coefficient value of K_c=1.1 was taken from the above calculation period, corresponding to a mature and dense coffee plantation, (Ramírez *et al.*, 2010).

In the case of coffee, ETgreen (the actual green total evapotranspiration) was assumed as the value of ET_a because for the period in which the water balances were carried out (at daily scale), ETgreen corresponds to the relationship between the effective precipitation, the crop evapotranspiration, and the soil moisture content, which has great importance to how the crop transpires, and ET_a considers these three mechanisms.

To evaluate the sustainability of coffee production in Colombia, in terms of its WFgreen, the green WS was calculated as the ratio of WFgreen to green water availability, here considered as annual rainfall.

The minimum coffee productivity reported for the study period was 0.76 t ha^{-1} in the department of Cauca in 2013. The maximum was 1.56 t ha^{-1} in the department of Antioquia in 2019, while the total average productivity was 1.10 t ha^{-1}.

The blue water footprint is the volume of water extracted from a surface water or groundwater source and consumed to produce goods and services to cover an unsatisfied water demand due to a deficit in the availability of rainwater.

The gray water footprint of agricultural production, which is an indicator of the volume of water pollution, was calculated by quantifying the volume of water necessary

to assimilate the nutrients that reach the ground or surface waters due to the leaching of nutrients from crops is the main pollutant from non-point sources of surface water and groundwater bodies. To calculate the gray water footprint in coffee cultivation, the contamination of the water resource due to the application of fertilizers was taken as a reference. Pesticides and other agrochemicals were not considered because, in Colombia, there is no information about them. The calculation of the gray water footprint for coffee cultivation was performed only by nitrogen (N) contamination because phosphorous, which is applied as a fertilizer that is not absorbed by the plant, generally accumulates in the soil, and only a very small fraction is transported to subsurface water and groundwater sources (Ercin *et al*., 2011).

For the calculation of the gray water footprint of the coffee crop from nitrogen fertilization, the following information was considered:

- Nitrogen application range in coffee crops from 28 to 154 kg ha^{-1} $year^{-1}$, although the recommended amount is 300 kg ha^{-1} $year^{-1}$ (Cenicafé *et al*., 2015). In this study, the maximum value applied by farmers (154 kg ha^{-1} $year^{-1}$) was used.
- Nitrogen leaching rate: 10% of the total nitrogen applied (Ercin *et al*., 2011).
- The maximum allowable concentration of nitrogen, C_{max} (kg m^{-3})= 2 mg L^{-1} (Área Metropolitana del Valle de Aburrá, 2011).
- The natural concentration of nitrogen, C_{nat}= 0 mg L^{-1}. When natural concentrations are not known, but it is estimated that they are low, it can be assumed that C_{nat} = 0 for simplicity (Hoekstra *et al*., 2011).
- Productivity of coffee cultivation in Colombia: 1.10 t ha^{-1} (Agronet, 2021).

For the calculation of the gray water footprint (WFgray) in m^3 ha^{-1}, the amount of applied fertilizer or load (L) (in kg ha^{-1} of nitrogen) is divided into the difference between the maximum allowable concentration and the natural concentration (C_{nat} of N, kg m^{-3}) according to the following expression:

$$WFgray = \frac{L}{C_{max} - C_{nat}} \quad (12)$$

The value obtained from Equation 12 is divided by the crop yield (t ha^{-1}). In this way, the amount of water required (m^3 t^{-1}) to dilute the pollutant load of nitrogen is obtained.

Wet coffee processing is a sequence of operations performed to transform the coffee cherry (from the crop) into dry parchment coffee (the export product). Thus, to obtain 1 kg of parchment coffee, approximately 5 kg of coffee are required. The present study analyzed three existing technologies in Colombia for wet coffee processing: conventional, ecological (Becolsub® and Ecomill®), and natural (dry process) methods. The conventional coffee mill uses approximately 40 L of water per kg of coffee produced (between transport and washing), the Belcosub® mill consumes 5 L kg^{-1} in the transportation of the grain and between 0.50 and 1.00 L kg^{-1} in the wash (in this study 1.00 L kg^{-1} were used), the Ecomill® mill consumes 5 L kg^{-1} in transport and between 0.30 and 0.50 L kg^{-1} (0.50 L kg^{-1} were used for the calculation) and the natural mill is done completely dry and does not use water (Cenicafé, 2015). The present work included the calculation of the WF blue and gray for coffee processing by the following Equation 13 and 14 respectively:

$$WFblue = BlueWaterEvaporation + BlueWaterIncorporation + LostReturnflow \quad (13)$$

For the calculation of the blue water footprint of the coffee mill, around 10% of its demand was considered, according to Ariza and Arevalo (2018), for a study of the water footprint in coffee farms in Colombia.

$$WF\,gray = \frac{C_{eff} - C_{act}}{C_{max} - C_{nat}} \times Effl \quad (14)$$

Where:
C_{eff} is the effluent concentration.
C_{act} is the current concentration of the water source.
C_{max} is the maximum acceptable concentration in the water source.
C_{nat} is the natural concentration of contaminants in water, without intervention by humans.
Effl is the effluent of the process.

The natural concentrations of each parameter in the surface water sources of the coffee zone are low, specifically in those sites where this study was carried out.

Therefore, the C_{max}, or maximum acceptable concentration, was determined as the value defined by the regional environmental authority (Área Metropolitana del Valle de Aburrá, 2011) in the water quality targets for the Aburrá River; these targets propose a BOD_5 (Biological Oxygen Demand) value between 5 to 100 mg L^{-1} according to the section of the river where the discharge takes place (Área Metropolitana del Valle de Aburrá, 2011). For this study, the C_{max} value was taken as a BOD_5=5 mg L^{-1}, and C_{nat} was taken as a BOD_5=0 mg L^{-1}, considering this latter when the natural concentrations of a given water quality variable are not known with precision but are estimated to be low, it can be assumed that C_{nat}=0 for simplicity. However, this will result in an underestimated WFgray when C_{nat} is not truly equal to zero (Hoekstra *et al*., 2011). For C_{effl}, the permissible limits of the applicable discharge standard (Ministerio de Medio Ambiente, 2015) were taken for the respective activity, for the traditional processing BOD_5=400 mg L^{-1} and ecological one BOD_5=1,449 mg L^{-1} (based on the calculation of the limit permissible for COD and a biodegradability ratio of the process discharges of 2.07 (Cenicafé, 2015).

From the results of the water requirements of the coffee crop in Colombia for the study sites during the period between 2010 and 2019, the water footprint of the crop was calculated for the respective departments, by using data on coffee crop productivity or yield reported from each department.

RESULTS AND DISCUSSION

Tables 1 and 2 show the results of the actual evapotranspiration calculation and the water requirements for the cultivation or production of coffee in the four

Table 2. Water requirements for coffee cultivation in the selected departments.

Year	ETgreen (m^3 ha^{-1})						
	Antioquia	Caldas	Cauca	Quindío	Mean	Minimum	Maximum
2010	9,781	10,154	6,848	10,161	9236	6,848	10,161
2011	9,113	9,827	7,882	10,057	9220	7,882	10,057
2012	9,409	9,751	7,736	10,230	9282	7,736	10,230
2013	9,684	10,199	7,586	9,637	9277	7,586	10,199
2014	9,559	10,033	7,673	10,405	9418	7,673	10,405
2015	9,941	9,682	7,696	10,046	9291	7,696	10,046
2016	10,478	10,944	8,149	10,554	10031	8,149	10,944
2017	9,653	10,618	8,002	9,955	9557	8,002	10,618
2018	9,567	10,613	7,551	9,939	9418	7,551	10,613
2019	9,756	10,166	7,921	9,769	9403	7,921	10,166
Mean	9,694	10,179	7,704	10,075	9413		
Minimum	9,113	9,482	6,848	9,637	9277		
Maximum	10,478	10,944	8,149	10,554	10031		

Colombian departments with a wide-ranging coffee tradition between 2010-2019. According to this information, there are marked differences between the locations, with actual total evapotranspiration values between 770 and 1,018 mm $year^{-1}$ (Table 1) and crop water requirement values between 6,848 and 10,944 m^3 ha^{-1} (Table 2).

Considering the results of the water requirements of the coffee crop in Colombia for the departments of Antioquia, Caldas, Cauca, and Quindío for the period between 2010 and 2019, as shown in Table 2, and the productivity or yield of the coffee crop for each department and the same period, as shown in Table 3, the results of WFgreen of coffee cultivation in Colombia for the four departments between 2010 and 2019 are shown in Table 4. According to the results shown in Table 4, the WFgreen of the coffee crop for the sites and the study period varied between 6,254 m^3 t^{-1} (in Antioquia - 2019) and 11,978 m^3 t^{-1}

Table 3. Production and productivity of coffee cultivation in the selected departments.

Department	Year	Area harvested (ha)	Production (t)	Yield (t ha^{-1})	National production contribution (%)	Harvested area national contribution (%)
Antioquia	2010	111,602.71	121,253.38	1.09	15.56	14.99
	2011	106,419.57	115,267.98	1.08	18.00	14.94
	2012	112,221.14	91,621.30	0.82	14.72	15.85
	2013	109,755.50	102,403.24	0.93	15.70	14.22
	2014	110,115.86	111,452.98	1.01	15.30	13.84
	2015	109,649.61	120,365.78	1.10	14.15	13.69
	2016	105,666.61	119,970.64	1.14	14.05	13.59
	2017	99,311.53	140,398.62	1.41	16.49	13.18
	2018	95,899.72	125,075.63	1.30	15.38	13.32
	2019	116,439.78	181,814.64	1.56	18.57	14.98
Caldas	2010	72,240.58	95,957.90	1.33	12.31	9.71
	2011	66,331.61	78,805.87	1.19	12.31	9.31
	2012	52,206.88	49,627.46	0.95	7.98	7.38
	2013	60,264.29	58,634.21	0.97	8.99	7.81
	2014	59,757.18	62,869.38	1.05	8.63	7.51
	2015	58,376.40	67,231.37	1.15	7.90	7.29
	2016	56,022.06	66,661.14	1.19	7.81	7.20
	2017	51,854.59	68,668.20	1.32	8.06	6.88
	2018	49,281.53	61,062.81	1.24	7.51	6.84
	2019	53,194.00	73,192.25	1.38	7.47	6.84
Cauca	2010	55,162.00	45,113.00	0.82	5.79	7.41
	2011	54,246.42	41,645.39	0.77	6.50	7.61
	2012	56,825.00	50,588.14	0.89	8.13	8.03
	2013	74,105.64	56,303.93	0.76	8.63	9.60
	2014	77,068.46	63,365.77	0.82	8.70	9.69
	2015	77,405.83	83,626.46	1.08	9.83	9.66
	2016	78,421.96	87,642.49	1.12	10.26	10.08
	2017	80,289.56	97,922.49	1.22	11.50	10.66
	2018	79,610.48	86,005.63	1.08	10.57	11.06
	2019	82,333.80	92,015.83	1.12	9.40	10.59
Quindío	2010	18,159.00	21,065.00	1.16	2.70	2.44
	2011	20,139.30	20,814.11	1.03	3.25	2.83
	2012	21,109.83	18,030.13	0.85	2.90	2.98
	2013	21,203.03	20,599.27	0.97	3.16	2.75
	2014	21,462.81	22,518.43	1.05	3.09	2.70
	2015	21,491.21	24,694.55	1.15	2.90	2.68
	2016	20,041.68	23,791.30	1.19	2.79	2.58
	2017	17,699.67	18,792.05	1.06	2.21	2.35
	2018	15,502.95	19,996.48	1.29	2.46	2.15
	2019	14,742.96	17,951.31	1.22	1.83	1.90
Mean		64,090.82	70,620.41	1.10	9.09	8.48
Minimum		14,742.96	17,951.31	0.76	1.83	1.90
Maximum		116,439.78	181,814.64	1.56	18.57	15.85

Source: Agronet (2021)

(in Quindío – 2012). The department with the lowest mean water footprint was Cauca, with 8,168 m^3 t^{-1}, and the department with the highest mean WFgreen was Quindío, with 9,304 m^3 t^{-1}. There is a marked correlation between WFgreen and actual evapotranspiration, considering that the places with the lowest and highest green water

Table 4. Green water footprint (WFgreen) of the coffee crop for the study departments.

Year	WFgreen (m^3 t^{-1})						
	Antioquia	Caldas	Cauca	Quindío	Mean	Minimum	Maximum
2010	9,002	7,644	8,373	8,759	8,445	7,644	9,002
2011	8,413	8,272	10,266	9,731	9,171	8,272	10,266
2012	11,525	10,258	8,689	11,978	10,612	8,689	11,978
2013	10,380	10,482	9,984	9,919	10,191	9,919	10,482
2014	9,444	9,537	9,333	9,917	9,558	9,333	9,917
2015	9,056	8,233	7,123	8,743	8,289	7,123	9,056
2016	9,229	9,197	7,292	8,891	8,652	7,292	9,229
2017	6,846	8,044	6,559	9,391	7,710	6,559	9,391
2018	7,359	8,559	6,992	7,705	7,654	6,992	8,559
2019	6,254	7,367	7,072	8,008	7,175	6,254	8,008
Mean	8,751	8,759	8,168	9,304	8,746		
Minimum	6,254	7,367	6,559	7,705	7,175		
Maximum	11,525	10,482	10,266	11,978	10,612		

footprint are also those with the lowest and highest average evapotranspiration for the study period (Table 4). Cauca showed the lowest values of evapotranspiration (Table 2) but also observed the lowest annual values of WFgreen, while Quindío showed the highest values for these two variables during the study period and departments. The mean WFgreen of the coffee crop in Colombia for the study period indicated that the lowest WFgreen was 7,175 m^3 t^{-1} in 2019, the highest was 10,612 m^3 t^{-1} in 2012 and the mean green was 9,274 m^3 t^{-1}. These results coincide with the occurrence of La Niña phenomenon in 2011 and 2012, when WFgreen was considerably higher for all departments studied (Table 4), except for Cauca where this phenomenon was very week, while during El Niño event (2018 and 2019), the WFgreen was the lowest observed during the studied period for most departments, region where was observed a less reduction on precipitation during these years (NOAA, 2021) through the quarterly average of the Oceanic Niño Index (ONI, 2021). When analyzing the results of the WFgreen of the coffee cultivation in Colombia with the reports of the climatic phenomena La Niña as a highlighted event, there is a tendency to reduce the water footprint and when a marked incidence of El Niño is presented, a tendency to increase the water footprint is noted. This could be explained because of the increase in rainfall and decrease in temperature, and therefore, in the evapotranspiration of the crop, while during El Niño rainfall decreases and increases in temperature and evapotranspiration; conditions that affect the use and contamination of water by coffee production.

Results from the green WS calculations for the studied departments in Colombia indicated that although WS varies within regions and among years, values were always over 4, with an average of 6.4±1.6. This implies that for the rainfall conditions in the coffee production region in Colombia, the WFgreen is relatively low, and coffee production under these rainfall conditions is environmentally sustainable.

The behavior of the WFgreen of the coffee crop in the four departments over time is very similar, except for Cauca, where WFgreen is the lowest. This seems to be related to the noticeably low evapotranspiration and the not markedly low crop productivity compared to the average.

Due to the high rainfall in the coffee-growing areas of Colombia, which is generally sufficient for the development of this crop, additional irrigation is not required or applied to coffee crops, thus, the supply of water to coffee crops in Colombia is an exclusive function of rainfall. Nevertheless, as it can be seen in Figure 1 (A, B, C,

and D), in some periods, and almost in the four weather seasons, the crop water needs were not met, and the difference between precipitation and evapotranspiration has been increasing, which implies that in the future irrigation must be considered.

According to Herrón (2013), a coffee plant requires for its normal growth an annual rainfall between 1,500 to 3,000 mm, depending on its geographical location (latitude and altitude) and the type of soil (texture and structure). Moreover, the water requirement from the coffee plant is approximately 125 mm month^{-1}; likewise, daily evaporation fluctuates (Andean zone) between 90 and 120 mm day^{-1}. When analyzing a potential coffee zone, without irrigation possibilities, it can be concluded that if the annual water balance, expressed in terms of the difference between the total annual rainfall and the annual evaporation is 150 mm year^{-1} or more, the region is considered suitable for coffee cultivation; otherwise, the region is not. Therefore, irrigation options in certain months of the year should

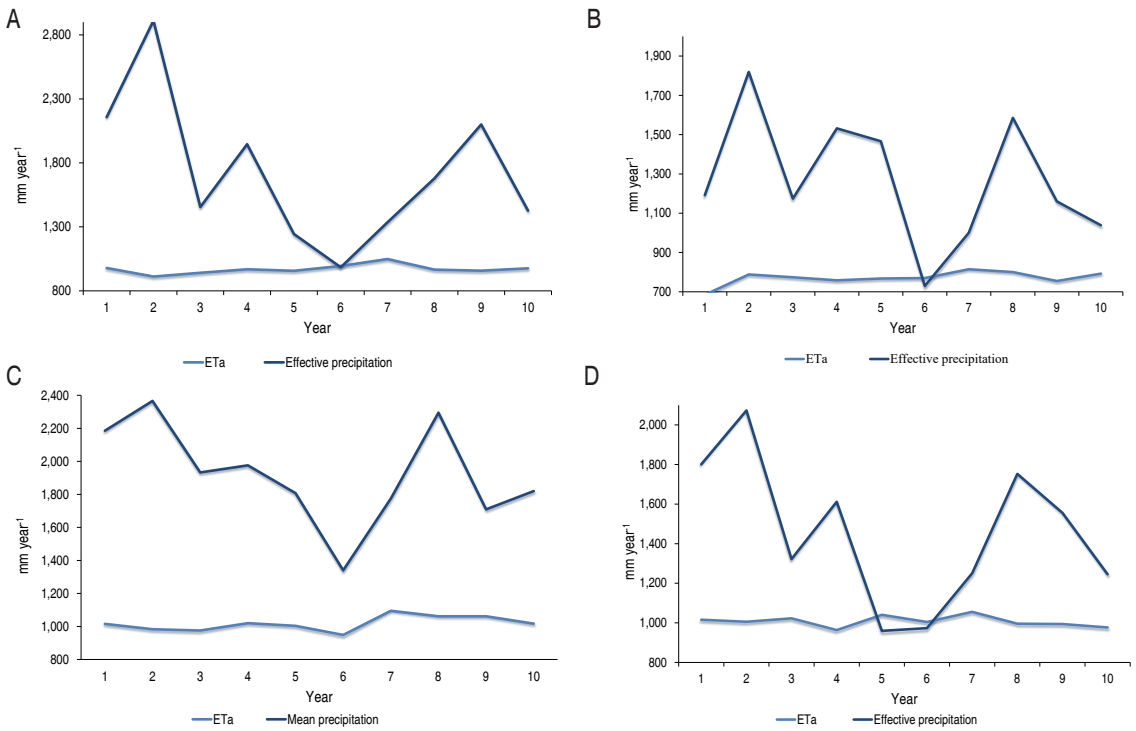

Figure 1. Mean precipitation vs. actual evapotranspiration of the crop in: A. Antioquia, B. Cauca, C. Caldas, D. Quindío.

be studied in non-traditional coffee growing areas, where the amount of rainfall does not satisfy the water demand. Therefore, WFblue for coffee cultivation in Colombia can be considered to be zero or negligible concerning the other two components of the water footprint (green and gray), according to the applied methodology (Hoekstra *et al.*, 2011).

The WFgray per unit of cultivated area (Equation 13) was around 7,700 m^3 ha^{-1}, assuming mean productivity of coffee in Colombia of 1.10 t ha^{-1} for the area and study period, which leads to a gray water footprint of coffee cultivation in Colombia of approximately 7,000 m^3 t^{-1}. Table 5 presents the results of the water footprint for the wet processing of coffee in Colombia.

The WFgreen of the crop was 8,746 m^3 t^{-1}. The crop has no WFblue, as it does not require irrigation, and the WFgray was 7,000 m^3 t^{-1}. If the traditional wet-processing method is used, the WFblue is 4 m^3 t^{-1} and the WFgray is 3,200 m^3 t^{-1}, while if the ecological Becolsub® technology is used, the WFblue is 0.60 m^3 t^{-1} and the WFgray is 1,739 m^3 t^{-1}. For the Ecomill® technology, the WFblue is 0.55 m^3 t^{-1} and does not have WFgray because it does not generate any polluted water, according to the local environment water quality standards, and the little leachate, which is produced and reincorporated into the process. This implies that the Becolsub® ecological processing method reduces the water footprint by 45.7% and 99.9% with the ecological Ecomill® process (no wastewater discharge)

compared to traditional wet processing technology. The natural processing of coffee is a totally dry process, it does not use water to transport and wash the coffee and it does not generate spillage; therefore, it does not have a water footprint. Nonetheless, it is no longer a widely used practice.

Table 5. Water footprint for coffee with traditional and ecological wet-processing methods.

Product/Process	WF green (m^3 t^{-1})	WF blue (m^3 t^{-1})	WF gray (m^3 t^{-1})	WF Total (m^3 t^{-1})
Coffee cherry	1,749	0.0	1,400	3,149
Coffee parchment	8,746	0.0	7,000	15,746
Traditional wet process	0.0	4.0	3,200	3,204
Belcosub® ecological wet process	0.0	0.6	1,739	1,740
Ecomill® ecological wet process	0.0	0.6	0.0	0.6
Natural benefit (dry process)	0.0	0	0.0	0.0

A comparison of the estimates obtained in this study with those from other coffee producer countries (Figure 2), shows that the order of magnitude is similar in all studies. According to the studies reviewed for the green and blue water footprint of coffee cultivation in Colombia, were estimated range between 6,328 and 13,033 m^3 t^{-1}, and the results of this study are at the lower range (8,746 m^3 t^{-1}).

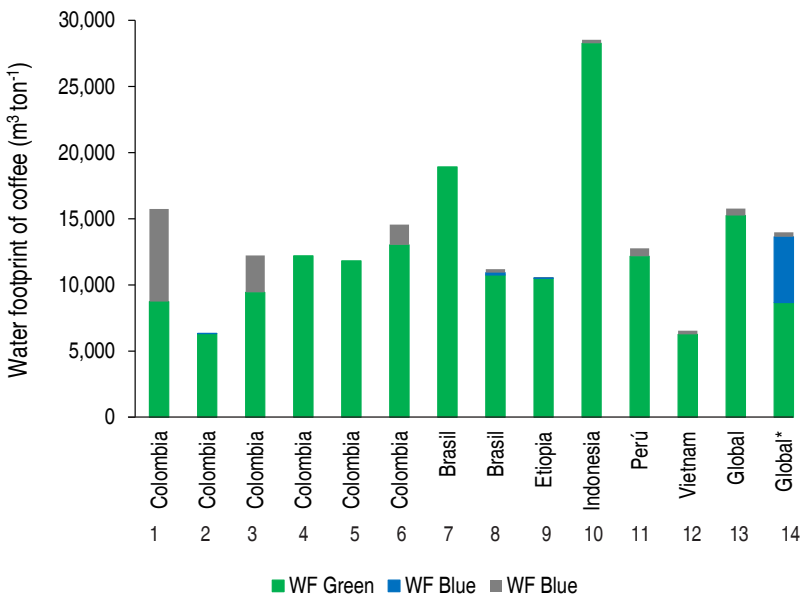

Figure 2. Comparison of values for water footprint of coffee production found in this study with those from other studies in Colombia and other coffee-producing countries around the world.
[1]This study; [2]Ariza and Arevalo, 2018; [3]Arévalo and Sabogal, 2012; [4]Arévalo and Campuzano, 2013; [5]IDEAM. 2015; [6]Mekonnen and Hoekstra, 2010; [7]Martins et al., 2018; [8]Mekonnen and Hoekstra, 2010; [9]Mekonnen and Hoekstra, 2010; [10]Mekonnen and Hoekstra, 2010; [11]Mekonnen and Hoekstra, 2010; [12]Mekonnen and Hoekstra, 2010; [13]Mekonnen and Hoekstra, 2010; [14]Mekonnen and Hoekstra, 2010
Global* = Mean global irrigation. Source: Mekonnen and Hoekstra (2010).

Only 3 of the 6 studies of the water footprint of coffee in Colombia analyzed in this study published results of the gray water footprint, with data of 1,533 m^3 t^{-1} (Mekonnen and Hoekstra, 2010), 2,778 m^3 t^{-1} (Arévalo and Sabogal, 2012) and the present study with the highest value reported (7,000 m^3 t^{-1}).

When comparing the water footprint obtained in the present study with those obtained by Mekonnen and Hoekstra (2010) for the main coffee-producing countries (Figure 2), Colombia has the second-lowest water footprint for coffee cultivation in the five main producing countries. There is a strong inversely proportional relationship between the coffee crop productivity at each study site and the water footprint, where the lowest water footprint was reported in Vietnam, which reported the highest crop productivity (1.85 t ha^{-1}) for 1996-2005. The largest water footprint was in Indonesia (28,520 m^3 t^{-1}), with the lowest crop productivity (0.51 t ha^{-1}) for the years 1996-2005. When comparing the results of the water footprint of the coffee crop obtained in this study with the global average, differences were found since the global average with irrigation is 0.20% lower and without irrigation, it is 11% higher than in this research. This difference between the global mean coffee cultivation with and without irrigation indicates a greater efficiency in water use and contamination in the technology with irrigation; however, the real impact on the resource will depend on the situation of each location in terms of the availability of green and blue water.

According to the above results, the coffee productivity in Vietnam (1.85 t ha^{-1}) is 3.6 times higher than in Indonesia (0.51 t ha^{-1}) and almost 2 times higher than in Colombia (1.10 t ha^{-1}), and its direct effect on the water footprint in the cultivation of this product is evidenced, given that the mean precipitation of the main coffee producers is similar, except for Ethiopia, which is considerably lower than that of the other countries included in the analysis.

Although there are differences in the results of these studies, they all agree with the dominant role of the WFgreen in the global production of coffee. The differences in the results of the reviewed studies may be due to a variety of causes, including the type of model, the spatial resolution, the period considered and the data related to cultivated and irrigated surfaces, growth periods, crop parameters, soil, climate and the season for which the water footprint was calculated. Some studies use a calculation for a specific base year, and other studies (e.g. this study) use a mean calculation for a given time range, making a comparison of the results complex.

The WFgreen of the coffee crop, at the national scale, corresponds approximately to 0.55% of the total available green water in the country (IDEAM, 2015), indicating that the WFgreen of the coffee in Colombia is sustainable.

Additionally, the traditional wet-processing of coffee (the process after cultivation), reported a WFgray of 3,200 m^3 $t^{-1}$and the blue water footprint is about 4.0 m^3 t^{-1} and it does not have a WFgreen because rainwater is not used in this process. The Ecological wet-processing with the Belcosub® technology has a WFgray of 1,739 m^3 t^{-1} and a WFblue of 0.60 m^3 t^{-1} and with the Ecomill® technology, the WFgray is 0.55 m^3 t^{-1} and does not have WFblue because it has no sewage discharge. Out of the six largest coffee producer countries in the world, Colombia ranks second in terms of coffee's WFgreen.

To sum up, the water footprint of coffee depends on the climate and yields per hectare at the specific site of production. The latter is due to the climatic conditions of each site but also the soil conditions and management practices; therefore, the water footprint of the coffee crop can vary markedly depending on the location and the evaluation period.

CONCLUSIONS

The water footprint of coffee cultivation in Colombia is about 8,746 m^3 t^{-1} for the WFgreen, 7,000 m^3 t^{-1} for the WFgray (due to leaching of fertilizers to water sources), and does not have a blue water footprint, given that the coffee-growing sites of Colombia do not require irrigation because the water requirements are supplied with annual rainfall

The conventional coffee mill has a WFblue of 4 m^3 t^{-1}, a WFgray of 3,200 m^3 $t^{-1,}$ and does not have WFgreen because generally the mill does not incorporate atmospheric water in the process or is insignificant. The ecological Belcosub® coffee mill has a WFblue of 0.60 m^3 t^{-1} and a WFgray 1,739 m^3 t^{-1}, while the ecological mill Ecomill® has a WFblue of 0.55 m^3 t^{-1} and does not have WFgray because it has no discharges (few leachates are generally used as fertilizer for cultivation). The natural benefit of coffee is a totally dry process, it does not use water to transport and wash the coffee and it does not generate spillage; therefore, it does not have

a water footprint, however, it is no longer a widely used practice.

Finally, the WFgreen of the coffee crop in Colombia, at the national scale, corresponds approximately to 0.55% of the total available green water in the country, indicating that the WFgreen of the coffee in this country is sustainable.

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Use of effective microorganisms and FitoMas-E® to increase the growth and quality of pepper (*Capsicum annuum* L.) seedlings

UNIVERSIDAD NACIONAL DE COLOMBIA

Uso de microorganismos eficientes y FitoMas-E® para aumentar el crecimiento y la calidad de plántulas de pimiento (*Capsicum annuum* L.)

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ABSTRACT

The agricultural bio-products based on effective microorganisms (EM) are a suitable alternative to reduce the use of chemical fertilizers and have a positive effect on the growth and development of plants. The aim of the present work was to evaluate the effect of EM and FitoMas-E® on the production of pepper seedlings (*Capsicum annuum* L.). The experiment was carried out in an organoponic garden in the municipality of Matanzas, Cuba. It was constituted for four treatments: control (without application of bio-products), EM (4 mL m^{-2}), FitoMas-E® (0.1 mL m^{-2}) and EM (4 mL m^{-2}) + FitoMas-E® (0.1 mL m^{-2}). A randomized block design was performed with three replications per treatment. One-way analysis of variance was performed to determine differences among treatments and Duncan´s Multiple Range Test for media comparison. Seedling height, number of leaves per seedling, stem diameter, root length, slenderness index, fresh and dry weight of leaf and root and shoot/root ratio based on dry weight data were determined. The application of EM and FitoMas-E® had a positive effect on the growth and quality of the pepper seedlings under nursery condition. The treatment 4 showed the best results regarding the morphological parameters: plant height (17.19 cm), number of leaves (6.01), stem diameter (3.98 mm), root length (8.82 cm) as well as fresh and dry weight of leaves and roots. The combined application of EM and FitoMas-E showed to be effective in promoting the growth of roots and aerial organs but maintaining a shoot/root ratio ranged from 1.28 to 2.5, which are suitable values in order to obtain quality pepper seedlings.

Keywords:
Bio-products
Capsicum annuum
Nursery
Organoponic

RESUMEN

Los bioproductos agrícolas basados en microorganismos eficientes (EM) constituyen una alternativa viable para disminuir el uso de fertilizantes químicos, y tienen un efecto positivo sobre el crecimiento y desarrollo de las plantas. Este trabajo se realizó con el objetivo de evaluar el efecto de microorganismos eficientes (EM) y FitoMas-E® en la producción de plántulas de pimiento (*Capsicum annuum* L.), para lo cual se realizó un experimento en un jardín organopónico en el municipio de Matanzas, Cuba. Se estudiaron cuatro tratamientos: control (sin aplicación de los bio-productos), EM (4 mL m^{-2}), FitoMas-E® (0.1 mL m^{-2}) y EM (4 mL m^{-2}) + FitoMas-E® (0.1 mL m^{-2}). Se estableció un diseño de bloques completamente al azar con tres repeticiones por tratamiento. Se realizó un análisis de varianza para determinar diferencias entre los tratamientos y la prueba de Rangos Múltiples de Duncan para la comparación entre las medias. Se evaluaron los indicadores altura de las plántulas (cm), número de hojas por plántula, diámetro del tallo (mm), longitud de la raíz (cm), el índice de esbeltez, peso fresco y seco foliar y de la raíz (g) y la relación parte aérea/raíz con relación al peso seco. La aplicación de EM y FitoMas-E® tuvo un efecto positivo sobre el crecimiento y la calidad de las plántulas de pimiento en semillero. El tratamiento 4 mostró los mejores resultados con relación a los indicadores morfológicos: altura de la planta (17.19 cm), número de hojas (6.01), diámetro del tallo (3.98 mm), longitud de la raíz (8.82 cm), así como en el peso fresco y seco de las hojas y de las raíces. La aplicación combinada de EM y FitoMas-E fue efectiva para estimular el crecimiento de la raíz y los órganos aéreos, manteniendo una relación parte aérea/raíz entre 1.28 y 2.5, lo cual es importante en función de obtener plántulas de pimiento con calidad.

Palabras clave:
Bioproductos
Capsicum annuum
Semillero
Organopónico

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Current crops production operates under an intensive agriculture model, characterized by the indiscriminate use of chemical fertilizers and pesticides, which increase the costs of production (Meena *et al*., 2017). The overuse of those agrochemicals has a negative impact on the environment, reducing soil fertility and microbiota diversity (Modi *et al*., 2017). Interest in the development of new plant-based products that promote microorganisms has increased, which allows reducing the application of agrochemicals while maintaining high crops yield (Akintokun *et al*., 2019).

Pepper (*Capsicum annuum* L.) is one of the most important fruit worldwide, due to its exquisite flavor and nutritional values (Guilherme *et al*., 2020). In Cuba, this fruit may be used fresh or processed, and it is extensively cultivated in all regions with a total area of 7,029 ha in 2019 and a production of 79,726 t (ONEI, 2020).

Organoponic garden technology, as a production model in urban, suburban and personal agriculture, has been developed with very good results in the production of vegetable and fruits (Terry *et al*., 2014). In this context, to obtain quality seedlings, it is necessary to apply a proper amount of organic matter that ensures soil fertility and the availability of nutrients, which satisfy the nutritional demand of the crops.

The crop nursery stage is a fundamental step in growing several crops under tropical conditions. The use of bio-products that stimulate plants growth is usually underestimated, however, it can be an key element for an optimal growth. Bio-products based on EM have been used on pepper and other crops, due to several advantages, such as low costs, simple technology, minimal impact on the environment and positive effect on growth and productivity (Pradhan *et al*., 2019). Moreover, many EM-based products have shown to be a plant root promoter, which increase seedling survival during stressful conditions of transplanting (Jochum *et al*., 2019).

FitoMas-E® is a natural bio-nutrient product based on high-energy active substances (aminoacids, nitrogenous bases and polysaccharides), as well as minerals containing nitrogen, phosphorus and potassium. It has been successfully supplied to crops to stimulate growth and development (Díaz *et al*., 2021). Recently, the combined application of FitoMas-E and EM enhanced the agronomic response in common bean, by improving morphological, physiological and reproductive parameters (Calero-Hurtado *et al*., 2019). Hence, this work aimed to evaluate the effect of Effective Microorganisms and FitoMas-E® on the production pepper seedlings.

MATERIALS AND METHODS

Plant material and nursery preparation

The experiment was carried out under organoponic conditions in the municipality of Matanzas, Cuba, from January to February 2018. Seeds of *Capsicum annuum* L., Español Liliana variety were supplied by the Liliana Dimitrova Horticultural Research Institute. Three beds were used, each divided into four plot nurseries, which were prepared by making furrows transversely to the plot, with 15 cm between rows. The substrate consisted of organic matter (filter cake, 50%) and red ferralitic soil (50%). The agro-technic management during the experiment was performed following the recommendations of Rodríguez *et al*. (2011), on intensive vegetable production in gardening and semi-covered technology (Technical Handbook for Organoponic). A daily watering was carried out by a micro-jet irrigation system in order to achieve a homogenous moisture of the substrate. After 7 days germination, the seedbed was subjected to a thinning process to obtain a proper number of seedlings m^{-1}. Weeds were manually removed during the experiment.

Bio-products

The EM were supplied by the Bio-pesticide Production Laboratory LABIOFAM, province of Matanzas, Cuba, which consisted of a mixture of bacteria (*Bacillus* spp. and *Pseudomonas* spp.) at $13x10^8$ CFU mL^{-1}, fungi from the genera *Trichoderma*, *Aspergillus*, *Rhizopus*, *Mucor* and *Penicillum* ($18x10^5$ CFU mL^{-1}) and the yeast (*Saccharomyces* sp.) at $21x10^6$ CFU mL^{-1}. FitoMas-E® was supplied by the Station of Sugarcane Research "Antonio Mesa", province of Matanzas. A foliar application of both bio-products was performed in the morning (7:00-9:00 am), 5 and 15 days after seeds germination by using a fumigation backpack (MATABI).

Treatments and evaluated parameters

The studied treatments consisted of: control (without application of bio-products) (T1), EM

at 4 mL m^{-2} (T2), FitoMas-E® at 0.1 mL m^{-2} (T3), EM at 4 mL m^{-2} + FitoMas-E® at 0.1 mL m^{-2} (T4). The concentrations of the bio-products were those recommended by Montano (2008) for FitoMas-E® and Álvarez *et al*. (2012) for EM-based bioproduct.

Plant height (cm) was determined measuring the length from the base of the root up to the shoot tip by a measuring tape; number of leaves per seedling by direct counting, stem diameter (mm) by using a caliper (Vernier) at 1 cm above the base of the stem, root length (cm) measured from the base of the stem up to the root tip using a measuring tape. Slenderness Index (SI) was calculated as the ratio between plant height (H) and diameter of the stem (D) (SI=H/D, according to Birchler *et al*., 1998). Fresh weight of leaves and roots (g) was measured by a digital balance (Sartorius, ALC-110.4). Dry weight of leaves and roots (g) was also evaluated. Samples were dehydrated using an oven at 70 °C for 72 h and dry matter was successively weighed by a Sartorius balance until a constant weight was achieved. Plant shoot/root ratio was calculated according to Romero *et al*. (2012) and expressed as the ratio between shoot dry weight and root dry weight. For this purpose, 25 seedlings of 35-days-old were randomly taken from each experimental plot to evaluate.

Experimental design and statistical analysis

The experiment was conducted in a randomized block design with three replications. The collected information was processed by Statgraphic plus 5.1 software. Normality data were first analyzed by Kolmogorov Smirnov´s and Bartlett´s Tests and subjected to one-way analysis of variance (ANOVA) and means separated by Duncan´s Multiple Range Test (P<0.05).

RESULTS AND DISCUSSION

Effect of EM and FitoMas-E® on morphological parameters

Table 1 shows the effect of both bio-products and their combination on distinct growth parameters. Plant height is an important indicator of seedling quality to achieve a successful transplant. The tallest seedlings were recorded for those treated with FitoMas-E® (T3) and the combination of EM and FitoMas-E® (T4), with no statistical difference between them; whereas T2 showed no positive response compared to the control. Similarly, T3 and T4 reported the highest number of leaves per plant and highest stem diameter among all treatments. Root length increased with the application of EM and FitoMas-E alone or with the combination of these bio-products. The highest value was observed with the combined application (T4), followed by T3 and T2.

Table 1. Effect of effective microorganisms and FitoMas-E® on morphological parameters of pepper seedlings.

Parameters	Treatments				SE
	T1	T2	T3	T4	
Plant height	15.20 b	15.93 b	16.64 a	17.19 a	0.13
Number of leaves/plant	4.72 c	5.03 bc	5.47 b	6.01 a	0.11
Stem diameter	3.36 c	3.51 bc	3.65 b	3.98 a	0.05
Root length	7.18 d	7.80 c	8.23 b	8.82 a	0.04

T1: control (without the application of bio-products), T2: EM (4 mL m^{-2}), T3: FitoMas-E® (0.1 mL m^{-2}), T4: EM (4 mL m^{-2}) + FitoMas-E® (0.1 mL m^{-2}). SE: standard error. Different letters indicate statistical differences among treatments of each row (Duncan test, P<0.05).

These results are consistent with other authors who reported an increase in plant height after application of EM on *Phaseolus vulgaris* L. (Calero *et al*., 2017) and *Daucus carota* L. (Núñez *et al*., 2017). The increase in plant height and stem diameter was also observed in tomatoes (*Solanum lycopersicum* L.) after the application of FitoMas-E® (0.7 L ha^{-1}), after 7 and 30 days of transplantation (Ricardo and Aguilar, 2015).

Santana *et al*. (2016) studied the effect of FitoMas-E® and *Trichoderma harzianum* Rifai on the germination and seedling growth of tomatoes and reported a better response on stem length after 25 days of seed germination, compared with non-treated plants. However, the combination of both bio-products did not increase height; on the contrary, an increase was observed in the stem diameter, as well as fresh and dry weight.

The increase in stem diameter after the application of EM and/or FitoMas-E® was previously reported by Lescaille *et al.* (2015), combining two strains of EcoMic® with EM. Similarly, the application of FitoMas-E® and *Trichoderma harzianum* enhanced the germination stage, seedling growth and stem diameter of tomatoes (Santana *et al.*, 2016).

Díaz *et al.* (2019) reported a stimulating effect of EM on morphological, physiological and biochemical parameters of *Sorghum bicolor* L. (Moench). After seed immersion into a solution of EM-based product IHPLUS® 6%, root and stem length, seedling vigor and the activity of α-amylase were significantly promoted.

The application of FitoMas-E® (0.1 mL m^{-2}) on *Cucumis melo* L. seedlings raised plant height, stem diameter, number of leaves per plant and root length (Pérez, 2018). The ability of FitoMas-E® to stimulate the growth of roots and shoots was also demonstrated under *in vitro* conditions by Gallego (2016), who suggested the use of FitoMas-E® as an alternative for *in vitro* rooting and shoot induction of *Saccharum* spp. cultivar.

The positive effect of FitoMas-E® on pepper seedlings growth may be associated with the presence of various active components, such as amino acids and carbohydrates chelating compounds, which may act as a delivery system of those substances that can be used by the cells as energetics or in the synthesis of new polypeptides or metabolites (Batista *et al.*, 2015). The rooting properties of FitoMas-E® could be explained due of the presence of tryptophan in its formulation, which is used by plants and soil microorganisms as a precursor for the biosynthesis of indole-3-acetic acid (IAA), which stimulate root growth by enhancing cell division and elongation (Jeyanthi and Kanimozhi, 2018).

EM could have contributed to the plant promoting effect observed in the present research. *Bacillus* and *Pseudomonas* species were reported to release plant regulatory metabolites such as gibberellins and IAA, which play an important role in plant biological processes such as cell differentiation, expansion and division as well as regulation of genes (Hernández-Montiel *et al.*, 2017; Yousef *et al.*, 2018). Moreover, both genera are able to release low molecular weight organic acids and binding to phosphate through their hydroxyl and carboxyl groups, which provoke the conversion of the insoluble phosphates to soluble forms (Florez-Márquez *et al.*, 2017; Nithyapriya *et al.*, 2021). This element is an essential part of the chemical structures of important macromolecules such as nucleic acids and antioxidant compounds, and it also plays a key role in several ATP-depending metabolic pathways.

Pseudomonas species are well known to produce siderophorous compounds, which commonly chelate iron in the rhizosphere forming a complex and increasing the availability of iron to plants. This element is vital for plant metabolism since it acts as a cofactor in enzymatic reactions and redox reactions involved in the processes of aerobic respiration and photosynthesis; thus, enhancing plant growth and productivity (Sharma *et al.*, 2020). *Pseudomonas* genus has also been documented to control several phytopathogens, due to their ability to produce a wide range of inhibitory substances such as hydrogen cyanide (Lakshmi *et al.*, 2015) and siderophores (Singh, 2018). Similarly, *Bacillus* spp. was also found to produce those substances (Chinakwe *et al.*, 2019). These mechanisms allow controlling the population of pathogen microorganisms in the soil, that negatively impact plant growth and development.

The positive effect of EM-based bioproduct on the growth of pepper seedlings, may also be associated with the presence of various fungi and yeasts that promote plant growth, which have been described to have several mechanisms to enhance plant growth. The production of various types of phytohormones has been recorded in *Trichoderma* spp. (Contreras-Cornejo *et al.*, 2014), *Rhizopus* spp. (Evstatieva *et al.*, 2020) and *Penicillium* sp. (Khan *et al.*, 2009). In addition, *Trichoderma* and *Penicillium* have been found to be a good biofertilizers (Wakelin *et al.*, 2007; Zin and Badaluddin, 2020). Similarly, *Aspergillus* was reported to convert the insoluble tri-calcium phosphate into soluble forms by processes of acidification, chelation and exchange reactions (Padmavathi, 2015). On the other hand, *Saccharomyces* has shown plant growth promotion activity through similar mechanisms described above, such as IAA production and phosphate solubilization (Shih-Feng *et al.*, 2017).

Fresh and dry weight

Figure 1 shows the effect of EM and FitoMas-E® application on foliar fresh weight of pepper seedlings. The combination

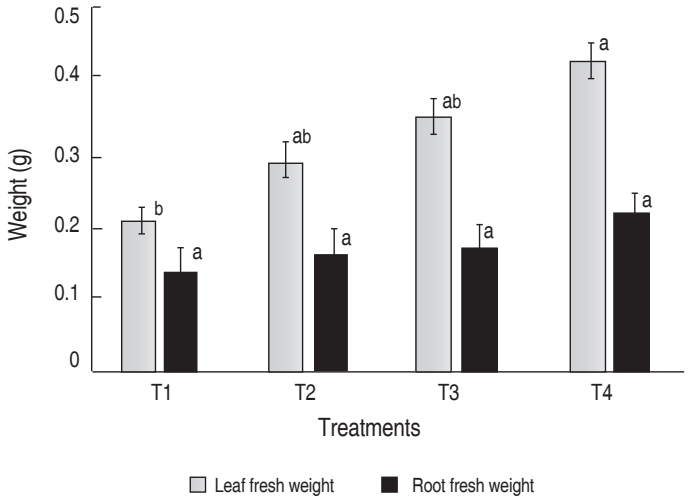

Figure 1. Effect of EM and FitoMas-E® on the foliar and root fresh weight of seedlings of *Capsicum annuum* L. T1: Control (without application of bio-products), T2: EM (4 mL m^{-2}), T3: FitoMas-E® (0.1 mL m^{-2}), T4: EM (4 mL m^{-2}) + FitoMas-E® (0.1 mL m^{-2}). Different letters indicate statistical differences among treatments for each organ (Duncan test, $P<0.05$).

of EM and FitoMas-E® (T4) increased the foliar fresh weight up to 0.43 g, which did not differ from the other treatments but was higher than the control. This result may be attributed to the promoted effect of combined application of EM and FitoMas-E® on the root growth, which allow the plant to absorb a higher amount of water and nutrients, that can be translocated to the upper parts of the plant and thus, increasing plant metabolism and fresh weight. The root fresh weight showed no difference among treatments and the control.

Foliar and root dry weights significantly increased after the application of EM and FitoMas-E® (Figure 2). In the case of foliar dry weight, there was no difference between T3 and T4. However, the application of EM (4 mL m^{-2}) by itself did not increase the foliar dry weight, in comparison with the control. Root dry weight showed a positive response with the combined application; however, no differences were found between control, T2 and T3.

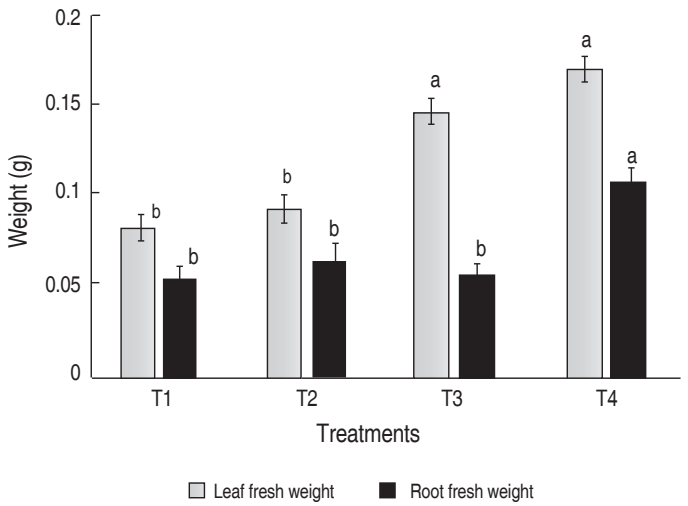

Figure 2. Effect of EM and FitoMas-E® on leaf and root dry weight of seedlings of *Capsicum annuum* L. T1: control (without application of bio-products), T2: EM (4 mL m^{-2}), T3: FitoMas-E® (0.1 mL m^{-2}), T4: EM (4 mL m^{-2}) + FitoMas-E® (0.1 mL m^{-2}). Different letters indicate statistical differences among treatments for each organ (Duncan test, $P<0.05$).

According to Santana *et al*. (2016), root and total fresh weight of tomatoes were improved after the application of FitoMas-E® and *T. harzianum*. Similarly, Calero *et al*. (2017) reported an increase in dry weight in *Phaseolus vulgaris* L. with the application of FitoMas-E® and the mixture of this bio-product with other bio-preparations based on effective microorganisms. The enrichment of the substrate with amino acids supplied by FitoMas-E®, may play an important role in the protein metabolism, which is essential for the accumulation of plant biomass generating a higher dry weight.

Slenderness index (SI) and shoot/root ratio

Slenderness index has been used to evaluate the seedling quality in various plant species such as *Moringa oleifera* Lam. (Castillo *et al*., 2013), *Pithecellobium dulce* (Roxb.) Benth and *Platymiscium diadelphum* S. F. Blake (Parra and Maciel, 2018). Although this parameter has not been employed for this fruit species, it was considered useful in order to assess pepper seedlings growing under organoponic conditions.

Table 2 shows the slenderness index (SI) and the shoot/root ratio (S/R) for pepper seedlings after the application of FitoMas-E® and EM. SI showed similar results for all treatments and control. The application of FitoMas-E® at 0.1 mL m^{-2} (T3) increased the shoot/root ratio, whereas this relation was similar among the rest of the treatments and control. In the present work, SI ranged from 4.32 up to 4.56 without differences among treatments with the application of the bio-products and control. The fact that FitoMas-E® and/or EM did not affect SI, may indicate a proportional increased of plant height and stem diameter, and the suitability of SI to be used to select pepper seedlings with quality.

Table 2. Effect of EM and FitoMas-E® on the Slenderness Index (SI) and shoot/root ratio (S/R) in seedlings of pepper.

Variable	Treatments				SE
	T1	T2	T3	T4	
Slenderness index (SI)	4.53 a	4.55 a	4.56 a	4.32 a	0.05
S/R ratio	1.28 b	1.41 b	2.11 a	1.66 b	0.007

T1: control (without application of bio-products), T2: EM (4 mL m^{-2}), T3: FitoMas-E® (0.1 mL m^{-2}), T4: EM (4 mL m^{-2}) + FitoMas-E® (0.1 mL m^{-2}). SE: standard error. Different letters indicate statistical differences among treatments for each parameter (Duncan test, $P<0.05$).

Terán (2014) demonstrated when working with tomatoes seedlings, that plants of high quality are those with a proportional ratio between plant height and stem width, which indicate a better support to cope with the stressful conditions created during seedling transplantation. Studies with forest species reported the highest seedling qualities for those with a low SI (short and width seedling). On the contrary, a high SI is associated with tall and thin seedlings and less possibilities of survival (Rodríguez, 2008).

The shoot/root ratio is expressed on the base of the dry weight for both organs and represents a balance between the use of water by the foliage and its absorption capacity by the roots. Cano and Cenita (2004) recommended that the ratio should not exceed the value of 2.5. According to this index, the higher quality of the plant corresponds to that of a minor ratio shoot/root. This allows to the seedlings a better probability of survival under field conditions, based on a regulation between transpiration and water absorption processes (Soriano, 2011). Literature showed few works concerning the optimal S/R ranges for different vegetable species. However, there are reports on forest species which described the optimal range between 1.5 to 2.5 for a better plant performance (Rodríguez, 2008; Mateo *et al*., 2011).

The S/R ratio in the present work ranged from 1.28 up to 2.11, which indicates a good balance between shoot transpiration and water absorption by root. It permits the aerial organs to be supplied with water and nutrients to carry out the photosynthesis and others metabolic reactions in leaves (Rodríguez, 2008). Similar results were reported in *Pinus patula* Schiede ex Schltdl. & Cham (Romero *et al*., 2012) and *Pinus montezumae* Lamb. (Hernández *et al*., 2014) on nursery conditions.

CONCLUSIONS

The application of effective microorganisms and FitoMas-E® to pepper seedlings, revealed the potential of those bio-

products to improve morphological and physiological parameters, that lead the production of seedlings with quality at nursery stage and their subsequent establishment after transplantation. The combination of EM (4 mL m^{-2}) and FitoMas-E® (0.1 mL m^{-2}) showed the best results regarding plant height, number of leaves per plant, stem diameter, length of root and fresh and dry weight of leaves and roots. The results reinforce the novel approach of using those bio-products as biofertilizers, in order to replace agrochemicals and develop an effective and sustainable agriculture.

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Phenolic compounds and *in vitro* antioxidant activity of six accessions of mashua (*Tropaeolum tuberosum* R. & P.) from Puno Region, Peru

Compuestos fenólicos y actividad antioxidante *in vitro* de seis accesiones de mashua (*Tropaeolum tuberosum* R. & P.) de la Región Puno, Perú

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Keywords:
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Flavonoids
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Plant tubers
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ABSTRACT

Mashua (*Tropaeolum tuberosum* R. & P.) is an Andean crop of high nutritional value and medicinal properties, which presents a great diversity in morphology and color. The aim of the study was to evaluate the content of phenolic compounds and *in vitro* antioxidant activity of the most economically important mashua accessions in the Puno Region, Peru. Six accessions of mashua (three purple-colored and three yellow-colored) were evaluated. The content of total polyphenols, total flavonoids and identification of phenolic compounds was determined by the Folin-Ciocalteu assay, aluminum chloride colorimetric method and HPLC-DAD, respectively. *In vitro* antioxidant activity was evaluated using the FRAP and DPPH assays. In general, the purple-colored mashua had a significantly higher content of total polyphenols, total flavonoids, and *in vitro* antioxidant activity compared to the yellow-colored mashua; being the Tt-23 accession purple-colored (peel/pulp, purple/purple), which presented a significantly higher content of phenolic compounds and *in vitro* antioxidant activity compared to the other accessions evaluated ($P<0.05$). Furthermore, a significant correlation was observed between FRAP and DPPH activities with the total content of polyphenols and flavonoids ($P<0.01$), as well as between FRAP activity and the caffeic acid and rutin levels ($P<0.05$). These results suggest that purple-colored mashua, particularly the Tt-23 accession (peel/pulp, purple/purple), has better nutraceutical and antioxidant properties due to its higher content of phenolic compounds.

Palabras clave:
Antioxidantes
Flavonoides
HPLC
Tubéculos vegetales
Polifenoles

RESUMEN

Mashua (*Tropaeolum tuberosum* R. & P.) es un cultivo andino de alto valor nutricional y propiedades medicinales, que presenta una gran diversidad en morfología y color. El objetivo del estudio fue evaluar el contenido de compuestos fenólicos y la actividad antioxidante *in vitro* de las accesiones de mashua de mayor importancia económica en la Región Puno, Perú. Se evaluaron seis accesiones de mashua (tres de color púrpura y tres de color amarillo). El contenido de polifenoles totales, flavonoides totales e identificación de compuestos fenólicos se determinó mediante el ensayo de Folin-Ciocalteu, método colorimétrico de cloruro de aluminio y HPLC-DAD, respectivamente. La actividad antioxidante *in vitro* se evaluó mediante los ensayos FRAP y DPPH. En general, la mashuas de color púrpura presentaron un contenido significativamente mayor de polifenoles totales, flavonoides totales, y actividad antioxidante *in vitro* en comparación con las mashua de color amarillo; siendo la accesión Tt-23 de color púrpura (piel/pulpa, púrpura/púrpura), la que presentó un contenido significativamente mayor de compuestos fenólicos y actividad antioxidante *in vitro* en comparación con las otras accesiones evaluadas ($P<0,05$). Asimismo, se observó una correlación significativa entre las actividades de FRAP y DPPH con el contenido de polifenoles y flavonoides totales ($P<0,01$), así como entre la actividad de FRAP y los niveles de ácido cafeico y rutina ($P<0,05$). Estos resultados sugieren que las mashua de color púrpura, particularmente la accesión Tt-23 (piel/pulpa, púrpura/púrpura), presenta mejores propiedades nutracéuticas y antioxidantes debido a su mayor contenido de compuestos fenólicos.

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Due to the climate change, it is necessary to search for crops resistant to harsh climates, pests and poor soils that can replace popular crops (Zhang *et al*., 2018). The search for new plants with antioxidant compounds has increased considerably during the last 5 years (Pisoschi *et al*., 2016), mainly due to the ability of antioxidants to neutralize free radicals that help prevent cardiovascular and cerebrovascular diseases as well as cancer (Gul *et al*., 2016). Antioxidants can also prevent atherosclerosis, arthritis, diabetes, and other diseases (Zhang *et al*., 2015). In the food industry, antioxidants are used to reduce the rate of oxidation of the products and thus, extend their shelf life (Xu *et al*., 2017). Peru has a high biodiversity of food and medicinal plants with nutraceutical and antioxidant potential, among which are Andean tuberous as mashua (*Tropaeolum tuberosum* R. & P.) (Campos *et al*., 2018; Pacheco *et al.,* 2020). This tuber is a perennial herbaceous plant native to the Andean region with a high nutraceutical potential, which grows between 2,800 and 4,000 masl. Its spread and distribution includes Colombia, Ecuador, Peru, Argentina and Bolivia (Roca *et al*., 2007; Valle-Parra *et al*., 2018; Choquechambi *et al*., 2019; Apaza *et al*., 2020). In the Andean region, Peru and Bolivia represent the largest planting areas, which is generally grown in association with other tubers such as oca (*Oxalis tuberosa*), ulluco (*Ullucus tuberosus*) and potato (*Solanum tuberosum*) (Manrique *et al.,* 2014). Mashua has a high diversity in morphology and color, which ranges from beige to dark purple. In Peru, more than 100 accessions have been recognized due to their variability in morphology and color, which would be correlated with their levels of phenolic compounds (Campos *et al*., 2018). Economically, it is the less important Andean tubers; however, it contains phenolic compounds with high antioxidant activity (Campos *et al*., 2006). Previous studies show that mashua contains glucosinolates (Martin and Higuera, 2016; Villacrés *et al.,* 2016), phenolics and high antioxidant activity (Chirinos *et al*., 2015). Within the Andean tubers such as potatoes (*Solanum* sp.), oca (*O. tuberosa*) and olluco (*U. tuberosum*), mashua has the highest antioxidant activity (Campos *et al*., 2006). Furthermore, mashua has high resistance to pests and plant diseases, helps prevent soil erosion, adapts to cold temperatures and poor soils, has medicinal properties and can be used as a bioinsecticide. It is used in ethnomedicine to relieve kidney, liver, and prostate disorders, obtaining favorable results due to its bioactive compounds (Grau *et al*., 2003).

In Peru, Puno Region is the mean producer of mashua followed by the Cusco and Ayacucho Regions. It has a planting area of 4,828 ha that produces only 7,368 t year^{-1}, compared to the potato production that is 742,924 t year^{-1} in the same region (Ministerio de Agricultura y Riego, 2018). This low production is due to its minimal demand since it has a bitter taste because of the presence of its glucosinolates (Martin and Higuera, 2016). Despite the fact that its planting area is less than those other Andean tubers, its cultivation is still important, since it is part of the food security of thousands of peasant families in the Andes through self-consumption or generation of income from the sale of this product (Apaza *et al*., 2020). Several studies recommend using it as a nutraceutical product or in the food preservation industry (Campos *et al*., 2006; Chirinos *et al*., 2007; Chirinos *et al*., 2008; Chirinos *et al*., 2015). In the Puno Region, one of the provinces with the highest production of mashua is Yunguyo. Therefore, the aim of this study was to characterize the physicochemical and antioxidant properties of six accessions of mashua (*T. tuberosum* R. & P.) of greatest economic importance in the province of Yunguyo (Puno Region, Peru), which vary according to the shape and color.

MATERIALS AND METHODS

Plant material

Six accessions of mashua (*T. tuberosum* R. & P.) were collected in the Yunguyo district, Yunguyo Province, Puno Region, Peru (16°14′39″S, 69°05′34″W). Yunguyo is one of the 13 provinces of the Puno Region, it has an altitude of 3,826 m, an average temperature of 8 °C, a maximum temperature of 17.3 °C and a minimum temperature of -1.3 °C. Three purple-colored mashua: Tt-03 (peel/pulp, purple/purple), Tt-23 (peel/pulp, purple/purple) and Tt-25 (peel/pulp, purple/purple); and three yellow-colored mashua Tt-02 (peel/pulp, yellow/yellow), Tt-11 (peel/pulp, yellow/yellow) and Tt-19 (peel/pulp, yellow/yellow), were provided by the National Institute of Agricultural Innovation ILLPA-Puno of Peru and numbered using the prefix Tt (*T. tuberosum*) (Figure 1). Approximately five units of each accession were

Figure 1. Accessions of mashua (*T. tuberosum* R. & P.) from Yunguyo Province, Puno Region, Peru.

disinfected by submersion in 5% sodium hypochlorite for 15 min, then cut to 2.5 mm in thickness and lyophilized for 7.5 h with a minimum temperature of -40 °C and 13.33 Pa by a freeze dryer device (Stellar, Millrock Technology, NY, USA). Subsequently, grinding process was performed in a rotor mill (mesh 0.08 mm) and the lyophilized powder was stored at -20 °C until the extraction and purification of phenolic compounds took place.

Sample preparation

The extraction of phenolic compounds was performed according to Chirinos *et al.* (2007), with minor modifications. Briefly, 5 g of the lyophilized powder from each accession was weighed using an analytical balance (Sartorius Extend Scale, ED224S) and homogenized for 20 min with methanol (CH_3OH) (Sigma-Aldrich, USA), acetone (C_3H_6O) (Sigma-Aldrich, USA), and destilated water (45:45:10), then acidified with 5 drops of 1% chloride acid (HCl) (Sigma-Aldrich, USA). The purification of phenolic compounds was carried out by solid phase separation in columns RP-18 (Lichrolut, Germany) of 60 mL. The column was conditioned with 60 mL of acidified methanol and 50 mL of acidified water, pH 2. Then, 60 mL of the sample was added, the column was washed with 40 mL of acidified water, and the elution was performed with 40 mL of acidified methanol, all the solvent was removed under vacuum on a rotary evaporator (Selecta, Spain) at 38 °C for 30 min. The purified solid residue was diluted in methanol (10 mg mL^{-1}) and kept at -20 °C in a freezer (Thermo Scientific, USA) until use.

Total polyphenols

The content of total polyphenols was determined according to Herrera-Calderon *et al.* (2016). Briefly, 0.1 mL of sample was mixed with 1 mL of 10% Folin-Ciocalteu reagent for 5 min at 25 °C, then 1 mL of 5% sodium carbonate (Na_2CO_3) (Sigma-Aldrich, USA) was added and the mixture was placed in a water bath at 45 °C for 30 min. Absorbance was read by means of a spectrophotometer (Pharo 300, Spectroquant, USA) at 725 nm. The results were expressed in mg gallic acid equivalent 100 g^{-1} fresh weight (mg GAE 100 g^{-1} FW).

Total flavonoids

Total flavonoid content was performed according to Wolfe *et al.* (2008). To 0.250 mL of sample, 1250 mL of 5% sodium nitrite ($NaNO_2$) (Sigma-Aldrich, USA) was added and it was left to react for 5 min, then 0.150 mL of aluminum chloride ($AlCl_3$) was added, and the mixture could stand for 5 min. Finally, 0.5 mL of 1 M sodium hydroxide (NaOH) was added to the mixture and it was left in contact for 15 min. The reading was performed at 510 nm by a spectrophotometer (Pharo 300, Spectroquant, USA). The results were expressed in mg catechin equivalent 100 g^{-1} fresh weight (mg CE 100 g^{-1} FW).

Antioxidant capacity by ferric reducing antioxidant power (FRAP)

Antioxidant activity evaluated by the FRAP assay was performed according to the methodology proposed by

Szollosi and Varga (2002). Briefly, to 20 µL of sample, 1 mL of distilled water and 1 mL of FRAP reagent (Sigma-Aldrich, USA) were added, the mixture was placed in a water bath at 37 °C and allowed to react for 15 min. The reading was made using a spectrophotometer (Pharo 300, Spectroquant, USA) at 593 nm. A standard curve was prepared using different concentrations of Fe^{2+} ranged from 15 to 75 mM. The results were expressed in mM Fe^{2+} 100 g^{-1} fresh weight (mM Fe^{2+} 100 g^{-1} FW).

Antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

Antioxidant activity evaluated by the DPPH radical scavenging assay was determined through the method developed by Brand-Williams *et al*. (1995), with minor modifications. It was performed using different concentrations of sample, which were placed in test tubes containing 1 mL of 0.1 M Acetate buffer pH 6.0, 1.5 mL methanol and 0.5 mL of 0.1 mM DPPH, then the mixture was stirred at 2500 rpm for 1 min and incubated at 37 °C for 30 min. Absorbance was read by a spectrophotometer (Pharo 300, Spectroquant, USA) at 517 nm. The results were expressed as µM Trolox equivalent antioxidant capacity 100 g^{-1} fresh weight (µM TEAC 100 g^{-1} FW).

Identification of phenolics compounds by HPLC-DAD

HPLC analyses of caffeic acid, rutin, chlorogenic acid, quercetin, apigenin and kaempferol were performed according to Chirinos *et al*. (2008), with minor modifications. All phenolic compound standards were obtained from Sigma-Aldrich, USA. Briefly, a VWR HITACHI Chromaster 600 HPLC with a diode array detector (HPLC-DAD 300), autosampler and a reversed phase C18 column (5 µm particle size, i.d. 4.6x250 mm) was used. The mobile phase consisted of 0.1% acetic acid solution (A) and 100% acetonitrile (B). The gradient profile was from 10 to 90% B from 0 to the desired gradient time (28, 39 and 55 min) at a flow rate of 0.5 mL min^{-1}. Detection was performed at 272 and 414 nm using a photodiode array detector. Calibration curves were made in triplicate using five different concentrations (10, 20, 30, 40, 60 µg mL^{-1}) for all phenolic compounds evaluated (Seal 2016). Data were processed using OpenLAB CDS software (Agilent Technologies, USA).

Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean±standard deviation (SD), and analyzed using SPSS software for Windows version 26.0 (SPSS, Inc., Chicago, IL, USA). The means were compared by one-way ANOVA followed by Tukey's multiple comparison test, at a significance level of $P<0.05$. Statistical correlation among different variables was performed using the Pearson coefficient (r) and results were statistically significant when $P<0.05$.

RESULTS AND DISCUSSION

Total polyphenols

Table 1 shows the total content of poyphenols and flavonoids, DPPH radical scavenging and FRAP activity of all samples evaluated. The content of total polyphenols ranged from 75.08 to 221.07 mg GAE 100 g^{-1} FW. All three purple-colored mashua showed significantly higher levels of total polyphenols compared to the three yellow-colored mashua ($P<0.05$). Within the group of purple-colored mashua, the Tt-23 accession presented significantly higher levels of total polyphenols compared to the Tt-03 and Tt-25 accessions (220.83±0.42, 172.62±0.76 and 173.55±0.10, respectively).

These results agreed with a previous study (Chirinos *et al*., 2006) in three purple-colored mashua, which showed a total polyphenol content that ranged from 174.9 to 374.4 mg GAE 100 g^{-1} FW. In another study (Campos *et al*., 2006), the content of total polyphenols was evaluated in four Andean tubers species. In the native potato (*Solanum* sp.), oca (*O. tuberosa* Molina) and ulluco (*U. tuberosus* Caldas), the content of total polyphenols was ranged from 64 to 232 mg chlorogenic acid equivalent 100 g-1 FW, 71 to 131 mg chlorogenic acid equivalent 100 g-1 FW and 41 to 77 mg chlorogenic acid equivalent 100 g^{-1} FW, respectively; while in mashua, in the case of the 11 genotypes evaluated, the total polyphenol levels ranged from 92 to 337 mg chlorogenic acid equivalent 100 g^{-1} FW. Studies carried out on different foods show that content greater than 100 mg GAE 100 g^{-1} is recognized as a high content of polyphenols (Ovaskainen *et al*., 2008). These results showed that the Tt-23 accession purple-colored had a high content of total polyphenols, even higher than other Andean tubers previously evaluated (Campos *et al*., 2006).

Table 1. Total content of poyphenols and flavonoids, DPPH radical scavenging and FRAP activity of six accessions of mashua (*T. tuberosum* R. & P.) from the Puno Region, Peru.

Accessions	Total polyphenols (mg GAE 100 g^{-1} FW)	Total flavonoids (mg CE 100 g^{-1} FW)	FRAP activity (mM Fe^{2+} 100 g^{-1} FW)	DPPH activity (µM TEAC 100 g^{-1} FW)
Tt-02	90.06±1.83 c	3.10±0.48 f	985.63±4.62 d	43.00±1.44 c
Tt-03	172.62±0.76 b	77.30±0.20 b	1242.52±16.67 b	60.84±1.53 b
Tt-11	90.83±1.20 c	4.23±0.51 e	390.68±14.30 f	28.35±0.65 e
Tt-19	77.48±2.35 d	8.00±0.35 d	460.78±4.20 e	35.26±1.08 d
Tt- 23	220.83±0.42 a	79.66±0.19 a	2299.03±25.46 a	68.25±1.80 a
Tt- 25	173.55±0.10 b	10.03±0.26 c	1055.53±4.66 c	60.28±1.10 b

Values (mean±SD) in the same column with different letters (a–f) are significantly different (One-way ANOVA with Tukey's post hoc test, $P<0.05$). GAE: Gallic acid equivalent; CE: Catechin equivalent; TEAC: Trolox equivalent antioxidant capacity; FW: fresh weight.

Total flavonoids

In the mashua accessions evaluated, the total flavonoid levels were ranged from 2.54 to 79.77 mg CE 100 g^{-1} FW. As shown in Table 1, the purple-colored mashua (Tt-23 and Tt-03) presented significantly higher content of total flavonoids (79.66±0.19 and 77.30±0.20 mg CE 100 g^{-1}, respectively), approximately 8 times higher compared to the other mashua accessions evaluated ($P<0.05$). There are no reports on total flavonoid levels in mashua, however; some flavonoids such as flavan 3-ols, anthocyanins, flavones, flavonols and flavanones have been detected by high-performance liquid chromatography (Chirinos *et al*., 2008; Pacheco *et al*., 2019).

***In vitro* antioxidant activity by FRAP and DPPH radical scavenging assays**

In the different mashua accessions evaluated, the FRAP activity ranged 376.89 to 2327.18 mM Fe^{2+} 100 g^{-1} FW, while the DPPH radical scavenging activity varied from 376.89 to 68.25±1.80 µM TEAC 100 g^{-1} FW. As shown in Table 1, all the purple-colored mashua presented significantly higher FRAP and DPPH radical scavenging activity compared to all yellow-colored mashua ($P<0.05$), being again the Tt-23 accession purple-colored, the one that presented the highest FRAP and DPPH radical scavenging activity compared to the other accessions ($P<0.05$).

Mashua accesions present a high diversity in their morphology and color, ranging from beige to purple. Previous studies have shown that purple-colored mashua have 8 to 10 times more antioxidant activity than yellow-colored mashua. This increase in the *in vitro* antioxidant activity is due partially to its high anthocyanin content (Campos *et al*., 2006; Chirinos *et al*., 2006; Chirinos *et al*., 2008). Chirinos *et al.* (2006) reported that mashua anthocyanins contributed to the total antioxidant activity in only one of the three purple-colored mashua, which allows to hypothesize that other phenolic compounds could participate in its antioxidant activity. Some studies have evaluated the *in vitro* antioxidant activity in different genotypes of mashua using the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and oxygen radical absorbance capacity (ORAC) assays (Campos *et al*., 2006; Chirinos *et al*., 2006; Chirinos *et al*., 2008). A study in 11 pigmented genotypes of mashua (*T. tuberosum* R. & P.) reported that the *in vitro* antioxidant activity evaluated by the ABTS method ranged between 3.82 and 39.15 µmol Trolox equivalent g^{-1} FW, finding that the purple-colored DP-02-24, ARB-5241 and ARV-5366 genotypes showed the highest antioxidant activity (Campos *et al*., 2006). Another study in three purple-colored mashua showed an *in vitro* antioxidant capacity evaluated by the ABTS method, which ranged from 16.2 to 45.7 µmol Trolox equivalent g^{-1} FW (Chirinos *et al*., 2006). Furthermore, when the antioxidant activity was evaluated by the ORAC method in two purple-colored mashua, the values ranged from 221 to 359 µmol Trolox equivalents g^{-1} dry matter. These results agree with the present study, which among the different accessions of mashua evaluated, all purple-colored mashua (peel/pulp, purple/purple) showed an increased for the for the *in vitro* antioxidant activity.

Identification of phenolic compounds by HPLC-DAD

Table 2 shows the phenolic compounds analysis of the six accessions. The HPLC-DAD analysis showed detectable values for caffeic acid, rutin, chlorogenic acid and quercetin. As shown in Table 2, the highest levels of phenolic compounds were observed for caffeic acid, which ranged from 4.77 to 267.25 mg 100 g^{-1}, lower levels were detected for the other phenolic compounds.

Table 2. Phenolic compounds measured by HPLC-DAD in six accessions of mashua (*T. tuberosum* R. & P.) from the Puno Region, Peru

Accessions	Cafeic acid	Rutin	Chlorogenic acid	Quercetin
	(mg 100 g^{-1})			
Tt-02	251.01±15.74 a	5.58±0.40 c	7.76±0.05 e	2.53±0.04 c
Tt-03	39.54±3.38 c	7.30±0.44 b	25.43±0.36 a	7.66±0.12 a
Tt-11	8.44±0.37 d	7.89±0.16 b	18.55±0.08 b	3.89±0.05 b
Tt-19	5.30±0.48 d	6.75±1.09 b,c	8.73±0.22 d	0.11±0.01 f
Tt- 23	169.19±1.12 b	9.81±0.02 a	5.96±0.15 f	1.69±0.02 e
Tt- 25	8.97±0.19 d	5.82±0.14 c	12.37±0.13 c	2.11±0.04 d

Values (mean±SD) in the same column with different letters (a–e) are significantly different (One-way ANOVA with Tukey's post hoc test, $P<0.05$).

A previous study, in 3 genotypes of colored mashua from Peru, the presence of gallic acid, gallocatechin, epigallocatechin, procyanidin B2 and derivatives of epigallocatechin, rutin and/or derivatives of myricetin and different derivatives of hydroxycinnamic and hydroxybenzoic acid were identified in these accesions (Chirinos *et al.*, 2008). Furthermore, Pacheco *et al.* (2019) in a mashua from Ecuador reported the presence of flavonols and flavan 3-ols of 68.8 and 31.2%, respectively. Among the flavanols, (+)-gallocatechin, (-)-epigallocatech and (-)-epicatechin were identified, being the latter the most abundant (9.22 µg g^{-1} dry matter). Isorhamnetin 3-rutinoside, quercetin 3-rutinoside, and quercertin and myricetin derivatives were found. Furthermore, in purple-colored mashua, the presence of 11 anthocyanins such as delphinidin 3-glucoside-5-acetylrhamnoside, delphinidin 3-sophoroside-5-acetylrhamnoside, delphinidin 3-glucoside-5-rhamnoside, delphinidin 3-sophoroside-5-rhamnoside, delphinidin 3-glucoside, cyanidin 3-sophoroside, cyanidin 3-sophoroside-5-rhamnoside, cyaniding 3-glucoside, cyanidin 3-rutinoside, pelargonidin 3-sophoroside and pelargonidin 3-sophoroside-5-rhamnoside were identified; of which the first 2 pigments were found in a higher concentration. This anthocyanin-rich fraction was significantly correlated with the *in vitro* antioxidant activity evaluated by an ABTS assay and the content of phenolic compounds (r=0.6379 and r=0.9873, respectively) (Chirinos *et al.*, 2006).

Other phenolic compounds have not been evaluated in mashua. The present study found an association between *in vitro* antioxidant activity and the content of phenolic compounds other than anthocyanins. Table 3 exposes th ecorrelation between *in vitro* antioxidant activity and

Table 3. Correlation between *in vitro* antioxidant activity and levels of total polyphenols, total flavonoids and phenolic compounds identified by HPLC-DAD of six accessions of mashua (*T. tuberosum* R. & P.) from the Puno Region, Peru.

	Correlation (r)					
	Total polyphenols	Total flavonoids	Caffeic acid	Rutin	Chlorogenic acid	Quercetin
FRAP activity	0.883**	0.797**	0.508*	0.570*	-0.280	0.046
DPPH radical scavenging activity	0.945**	0.760**	0.227	0.296	-0.034	0.211

* $P<0.05$, ** $P<0.01$

levels of total polyphenols, total flavonoids and phenolic compounds identified. A significant correlation was observed between the FRAP activity and the total content of polyphenols and flavonoids (P<0.01), as well as with caffeic acid and rutin levels (P<0.05), while the DPPH activity was correlated with the total content of polyphenols and flavonoids (P<0.01) (Table 3). Furthermore, a significant correlation was observed between FRAP and DPPH radical scavenging activity (r=0.873, P<0.01, data not shown). These findings show that phenolic compounds other than anthocyanins could also contribute to the *in vitro* antioxidant activity presented by different accessions of mashua.

CONCLUSIONS

The present study shows that among the six mashua accessions evaluated in the Puno Region, Peru; the purple-colored mashua present a high content of total polyphenols and flavonoids, as well as a high *in vitro* antioxidant activity; the latter significantly correlated with the levels of total polyphenols and flavonoids, as well as phenolic compounds such as caffeic acid and rutin. These results highlight that the high antioxidant activity of this Andean tuber could be explained by bioactive compounds other than anthocyanins, which could be used as a nutraceutical in food and beverages to minimize the risk of diseases caused by oxidative stress, liver and kidney diseases, as well as urinary and prostate disorders.

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Use of phenolic compounds from cocoa pod-husks (*Theobroma cacao* L.) as inhibitors of *Salmonella* spp. in fresh cheese produced in Manabí, Ecuador

UNIVERSIDAD NACIONAL DE COLOMBIA

Uso de compuestos fenólicos del pericarpio de cacao (*Theobroma cacao* L.) como agentes inhibidores de *Salmonella* spp. en queso fresco producido en Manabí, Ecuador

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ABSTRACT

Cocoa pod-husk is a by-product of cocoa processing, underutilized despite its phenolic compounds that can be an alternative to preserve the microbiological quality of food. The aim of this work was to evaluate the *in vitro* inhibitory activity of phenolic compounds from the cocoa pod-husk against *Salmonella* spp, which is commonly found in fresh cheese produced in Manabí, Ecuador; as well as the effect on the sensory characteristics of cheese after immersion in a solution of phenolic compounds. *In vitro* microbiological analyzes of the inhibitory activity of phenolic compounds, showed that the concentrations 1 and 1.5% had the highest zone of inhibition against *Salmonella* spp., with mean diameters of 10.67 and 11.8 mm, respectively. On the other hand, the growth curve of *Salmonella* spp. indicated that 2 h were required for complete inhibition of bacteria by phenolic compounds at concentrations of 1 and 1.5%. For the sensory analyzes of cheese treated with phenolic compounds, 56.3% of the panelists accredited the firmness and odor with "I like it", while 37.5% of the panelists qualified the color of the cheese with "I neither like nor dislike". Firmness and odor had higher values of acceptance than color. For 25 and 12.5% of the panelists, firmness and odor were rated as "I like it a lot", respectively, and 56.3% of the panelists conferred the label of "I like it" to both attributes. Cheese color was the lowest rated attribute, given that 12.5% of the panelists chose "I like it a lot" and 25% for "I like it".

Keywords:
Cheese
Cocoa pod-husk
Fine aroma cocoa
Growth curve measurement
Phenolic compounds
Sensory evaluation

RESUMEN

El pericarpio del cacao es un subproducto generado en la transformación agroindustrial, comúnmente subutilizado, a pesar de los compuestos fenólicos presentes en él que pueden ser una alternativa para preservar la calidad microbiológica de los alimentos. El objetivo del presente trabajo fue evaluar el poder inhibitorio *in vitro* de compuestos fenólicos provenientes del pericarpio de cacao frente a *Salmonela spp.*, comúnmente presente en queso fresco producido en Manabí, Ecuador; así como el efecto en las características sensoriales del queso después de su inmersión en una solución de compuestos fenólicos. Los análisis microbiológicos *in vitro* del poder inhibitorio de los compuestos fenólicos mostraron que concentraciones de 1 and 1,5% tuvieron las mayores zonas de inhibición de *Salmonela* spp., con medias de 10,67 mm y 11,8 mm de diámetro, respectivamente. Por otro lado, la curva de crecimiento de *Salmonela* spp. indicó que se requirió de 2 h para una inhibición completa de la bacteria frente a concentraciones de compuestos fenólicos de 1 y 1,5%. Los análisis sensoriales del queso tratado con compuestos fenólicos presentaron que la firmeza y el olor tuvieron mejores calificaciones que el color. La firmeza y el olor se calificaron con "me gusta mucho" por 25% y 12,5% de los panelistas, respectivamente y con "me gusta" por un 56,3% de los panelistas para ambos atributos. El color del queso fue el atributo de menor calificación, con 12,5% de los panelistas que escogieron el nivel de agrado "me gusta mucho" y 25% para el nivel "me gusta".

Palabras clave:
Queso
Pericarpio de cacao
Cacao fino y de aroma
Curva de crecimiento
Compuestos fenólicos
Análisis sensorial

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Cocoa tree is cultivated in tropical countries with hot and humid environments. Three countries, Cote D'Ivoire, Ghana and Indonesia together cultivate 61% and produce 67% of global traded cocoa (Campos-Vega *et al*., 2018), considering the top ten countries account for approximately 93% of total world cocoa production (Table 1). The average yield in countries from Latin America and the Caribbean is 0.39 t ha^{-1} of cocoa and in the world is 0.44 t ha^{-1} of cocoa (Sánchez *et al*., 2019). The main varieties of cocoa produced and sold in Ecuador are cacao fino y de aroma (fine aroma cocoa) (Clones Nacionales, Pichilingue Tropical Experimental Station, Ecuador) and CCN-51 cocoa (Castro Naranjal Collection, Guerrero, 2015). Ecuador is an important producer of fine aroma cocoa worldwide and is currently recognized for providing more than 60% of its production. Fine aroma cocoa is recognized worldwide for its aroma, color and quality. The use of the fruit seed to produce chocolate, cosmetics, among others, triggers the generation of by-products. The agroindustry of cocoa generates between 17 and 20 kg of by-products for each 100 kg of cocoa fruit (López, 2014), which currently are used in soil fertilization (Munongo *et al*., 2017). Nonetheless, several phenolic compounds were identified by LC-MS/MS in cocoa pod-husk by Karim *et al.* (2014) such as phenolic acids, flavonoids, luteolin, apigenin and linarin. Despite cocoa pod-husk is an important source of phenolic compounds (Hii *et al*., 2009), with great potential for application in the field of nutrition, health and medicine, due to its antioxidant and antimicrobial characteristics (Niemenak *et al*., 2006), in many cases the by-products of cocoa are accumulated without any treatment, leading to the generation and transmission of diseases that may affect crops (FEDECACAO, 2013).

Table 1. Countries with the highest production of cocoa (Campos-Vega *et al*., 2018).

	Production (t)	% total	Cultivation (ha)	% total
Cote D'Ivoire	1,472,313	32.96	2,851,084	27.96
Ghana	858,720	19.23	1,683,765	16.51
Indonesia	656,817	14.71	1,701,351	16.69
Cameroon	291,512	6.53	723,853	7.10
Nigeria	236,521	5.30	838,046	8.22
Brazil	213,843	4.79	720,053	7.06
Ecuador	177,551	3.98	454,257	4.45
Peru	107,922	2.42	125,580	1.23
Dominican	81,246	1.82	172,940	1.70
Colombia	56,163	1.26	165,844	1.63

The fresh cheese is an Ecuadorian artisanal cheese with 37% moisture (Espinosa, 2012) and good acceptance among consumers, especially in the province of Manabí (Ecuadorian coast). The presence of pathogenic microorganisms such as *Salmonella* in this kind of cheese (Zambrano, 2014) together with commercialization of the cheese at inappropriate temperatures can affect the health of the consumer (Lee *et al*., 2015) and contribute to a high number of people suffering from Salmonellosis in the province of Manabí, Ecuador (Ministerio de Salud Pública, 2014, 2015, 2016, 2017, 2018).

Salmonella and other pathogenic microorganisms have been found in cheese of countries such as Colombia (Instituto Nacional de Salud, 2011), Turkey (Kahraman *et al*., 2010), France (Domínguez *et al*., 2009), Germany (Fretz *et al*., 2010) and Egypt (El-Baz *et al*., 2017). To deal pathogen microorganisms, natural antimicrobials can be an important alternative to preserve the microbiological quality of food and guarantee consumer safety (Abdalla *et al*., 2007).

Concerns about the use of synthetic chemical antimicrobials have renewed the interests of consumers using natural and

safe alternatives (Nazzaro *et al*., 2009). The antimicrobial activity of phenolic compounds is well documented by several authors (Erdemoglu *et al*., 2007; Xia *et al*., 2011). The growth of strains of *Salmonella* and *Escherichia coli* is inhibited by phenolic compounds extracted from vegetables, fruits, herbs and spices (Cetin-Karaca and Newman, 2015), cocoa bean (Todorovic *et al*., 2017) and bean shells (Nsor-Atindana et al., 2012). However, no studies of the use of phenolic compounds of cocoa pod-husk against *Salmonella* growth have been performed.

In the present work, the inhibition of *Salmonella* spp. in fresh cheese was studied, after immersing the cheese in a solution of phenolic compounds previously extracted from the pericarp of fine aroma cocoa. Additionally, sensory analyzes were used to examine whether cheese treated with phenolic compounds showed organoleptic differences compared to an untreated cheese.

MATERIALS AND METHODS

Samples of fine aroma cocoa pericarp (*Theobroma cacao* L.) were collected at the Alegría farm, located in Junín, Manabí province, Ecuador, with 0.9277 south latitude and 80.2058 western longitude. The samples were washed with water and dried in an oven at 50 °C, ground and stored for future analyzes. Fresh cheese was purchased at the Nuevo Tarqui shopping center in the city of Manta, Ecuador.

Alcoholic extraction of phenolic compounds

The extraction of phenolic compounds was done according to Santacruz *et al.* (2020). An amount of 15 g of the previously ground cocoa pericarp was mixed with 150 mL of ethanol (95% v/v) and kept under stirring at 130 rpm for 24 h at 25 °C. Afterwards, the sample was centrifuged (SIGMA 2-16P, Germany) for 10 min at 4000 rpm and the supernatant was filtered by vacuum, saving the filtered liquid fraction.

Quantification of phenolic compounds

The quantification of phenolic compounds was done according to the Folin-Ciocalteu method proposed by Mahmood *et al.* (2011). A stock solution was prepared by mixing 10 mL of the previous filtered fraction with 5 mL of ethanol (95% v/v) and distilled water to complete a total volume of 100 mL. From the stock solution, 0.1 mL was mixed with 0.5 mL of the Folin-Ciocalteau reagent, allowing it to stand for 5 min. Afterwards, 1 mL of a sodium carbonate solution (5%) was added and made up to 25 mL with distilled water. The resulting solution was left in dark for 1 h and its absorbance at 760 nm was measured (Spectrophotometer Jenway 6320D, China). The quantification of phenolic compounds was performed using a calibration curve with gallic acid standards, and the results were expressed in mg of gallic acid equivalent (GAE) g^{-1} cocoa pericarp.

Inhibition of *Salmonella* spp. growing by phenolic compounds

The antibacterial activity of phenolic compounds against *Salmonella* spp. was performed according to Santacruz and Castro (2018) and the Clinical and Laboratory Standards Institute (CLSI, 2009). *Salmonella* spp. was inoculated into Petri dishes using Salmonella-Shigella agar (HiMedia Laboratories, India) as culture medium and incubated at 37 °C for 24 h. Filter paper disks (Fisher Scientific Q2) of 5 mm diameter were immersed in a solution of phenolic compounds at a specific concentration (see experimental design). The disks were dried and placed in the center of the Petri dishes containing *Salmonella* spp. and incubated at 37 °C for 24 h. Afterwards, the zone of inhibition of the growth by phenolic compounds was measured in triplicate.

Bacterial growth curve measurement

Bacterial growth was performed according to Puupponen *et al.* (2001). 10 mL of fresh growth medium (Salmonella-Shigella agar) were inoculated with 5% of culture (frozen *Salmonella* spp. was transferred to liquid media and incubated for 24 h). Phenolic compounds, from alcoholic extraction, were added to the culture media to give final concentrations of 0.5, 1 or 5%. The culture was manually shaken, and afterwards, it was incubated at 37 °C for 24 h. Bacterial growth was followed by taking seven samples from the culture during the incubation period of 24 h (0, 0.5, 1, 2, 4, 6, and 24 h). The samples were plated in Petri dishes containing Salmonella-Shigella agar and incubated at 37 °C for 24 h before bacterial count was recorded.

Bacterial growth in cheese

Fresh cheese was immersed in a 1% phenolic compounds solution for 15 min. Afterwards, cheese samples were stored for 8 days, overnight under refrigeration conditions and during the daytime at room temperature (approximately 25 °C).

Microbiological analysis of *Salmonella* in cheese was performed on samples after 0, 2, 4, 6 and 8 days of storage, according to ISO 6579: 2002 method (ISO, 2002). For the test, 10 g of cheese were weighed and placed in a sterile resealable plastic bag, before adding 90 mL of sterile peptone water. The sample was homogenized manually, and 1 mL of the liquid was transferred to a glass test tube before adding 9 mL of peptone water (10^{-1} dilution). The process was repeated to get a dilution of 10^{-4}, and immediately 1 mL of the solution was inoculated into Petri dishes and incubated at 37 °C for 24 h prior CFU counting.

Sensory analyzes

Cheese samples were previously analyzed to verify the absence of *Salmonella*. Afterwards, the cheese was immersed for 15 min in a 1% phenolic compounds solution before sensory analyzes. First, a duo-trio test ISO 10399: 2004 (ISO, 2004) with 16 semi-trained panelists was done to determine organoleptic differences between cheese samples treated with phenolic compounds and a control sample (cheese with no immersion). Subsequently, attributes of firmness, color, odor and flavor were evaluated using a five-point hedonic scale, with scores ranging from "I like it a lot" (5) to "I dislike it a lot" (1).

Texture analysis

Puncture tests were performed according to Castro *et al.* (2017), using cheese cubes of edge 2 cm, with slight modifications. A Shimadzu texturometer (Model EZ-LX, Japan) together with a stainless-steel probe of 3 mm diameter and 8 cm length were utilized. The probe speed was 10 mm s^{-1} and the maximum penetration force was recorded. Results were the average of three measurements.

Experimental design and statistical analyzes

A completely randomized design with a unifactorial arrangement was used. The concentration of phenolic compounds obtained by alcoholic extraction at 3 levels (0.5, 1 and 1.5%) was established as the independent variable (Medrano, 2019), whereas the zone of inhibition and the bacterial growth measurement were the dependent variables. The concentration of phenolic compounds with the best *Salmonella* inhibition was applied to cheese samples, before sensory analysis.

ANOVA and the significance of the difference between means was determined by Tukey test ($P<0.05$) using InfoStat statistics software (Infostat version 2014, Argentina). All measurements were performed in triplicate.

RESULTS AND DISCUSSION

The results of the inhibition of the growth of *Salmonella* spp. by phenolic compounds showed that there was a difference between *Salmonella* inhibition zones at the three concentrations of phenolic compounds ($P<0.05$) with values between 7.78 and 11.78 mm (Table 2). A positive correlation was also found between the inhibitory effect and the concentration of phenolic compounds by Hernández *et al.* (2012). The results of the present study agree with those obtained by Nsor-Atindana *et al.* (2012) for an ethanolic extract of cocoa pericarp at 100 mg mL^{-1} with zones of inhibition between 10.98 and 12.21 mm against *Salmonella*, *E. coli, S. aureus* and *B. cereus*. The inhibitory effect can be influenced by the type of phenolic compounds (Orihuela, 2016) that acts to a greater or lesser degree on the cell wall of bacteria, resulting in the loss of cell structure and the death of the microorganism. Another explanation for the inhibitory effect may be the diffusion

Table 2. Inhibition of growth of *Salmonella* spp. by phenolic compounds extracted from the pericarp of cocoa.

Phenolic compounds in solution (%)	Diameter of inhibition zone (mm)
0.5	7.78±1.15 a
1.0	10.67±1.32 ab
1.5	11.78±1.86 b

Mean value and standard deviations of measurements made with nine replicates. Different letters denote significant differences ($P<0.05$).

of hydrophilic compounds through the channel proteins of the outer membrane that surrounds the cell wall of gram-negative bacteria (Santos *et al*., 2014) This cell wall may contribute to the entry of phenolic compounds, which may have resulted in the observed cell death.

The measurement of bacterial growth curve (Figure 1) shows an increase in CFU mL^{-1} for the control sample along storage time. A complete inhibition of *Salmonella* spp. was observed after 2 h in the presence of phenolic compounds at 1 and 1.5%. The concentration of 0.5% showed a complete inhibition of *Salmonella* after 6 h. Phenolic compounds presented a dose-dependent antibacterial activity (Khan *et al*., 2021). Puupponen *et al*. (2001) showed that berry extracts at concentrations between 1 and 5 mg mL^{-1} were strong inhibitors of *Salm. enteric*. The inhibitory effect over time may be due to affectations of the bacteria cell wall because of the presence of the phenolic group (Puupponen *et al*., 2001). However, diffusion of phenolic compounds through the cell wall may allow an initial growth of bacteria (Figure 1) (Khan *et al*., 2021).

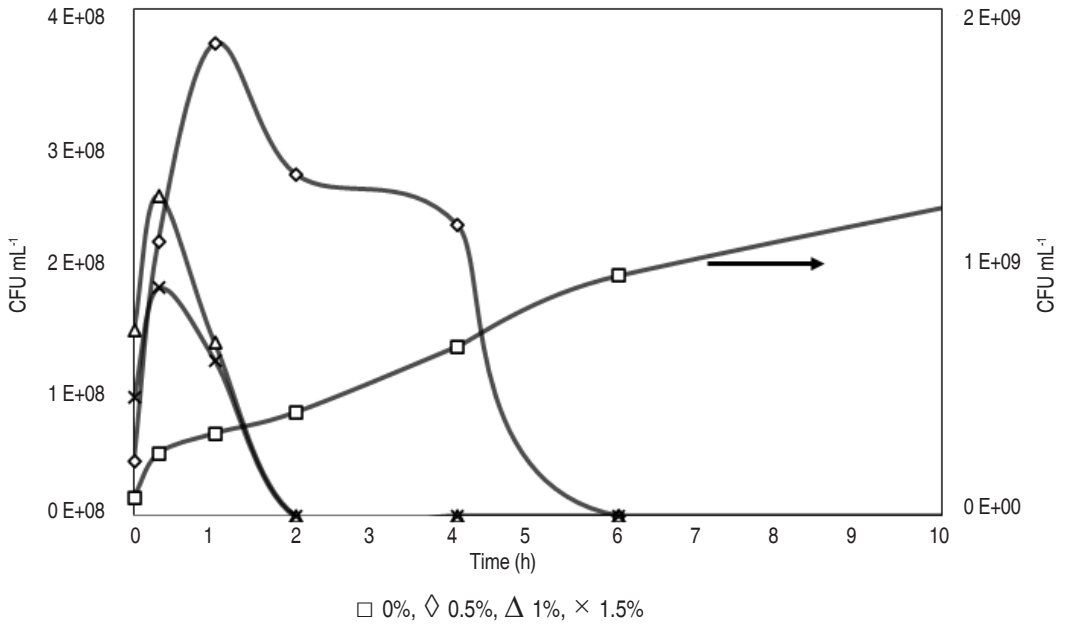

Figure 1. Curve of *Salmonella* growth in the presence of solutions of phenolic compounds.

The control sample (cheese with no immersion) showed presence of *Salmonella* spp. According to Ecuadorian regulations, NTE INEN 1529-15: 2013 (INEN, 2013), *Salmonella* should not be present in cheese. Cheese samples previously immersed in phenolic compound solutions at 1% showed an absence of *Salmonella* at 0 day of storage. In fact, Figure 1 shows no presence of *Salmonella* in cheese treated with phenolic compounds at 0.5% after 2 h of storage.

Sensory analyzes

Results of the duo-trio test showed that 15 of 16 panelists did not find difference between cheese previously immersed in a solution of phenolic compounds and the control sample. Texture analysis displayed that penetration force of control sample was 1.02 N, which was lower than cheese treated with phenolic compounds, showing values between 1.92 and 1.99 N (P<0.05). Instrumental texture of cheese does not have a single dominant characteristic and exhibit at least two primary characteristics (e.g. chewiness and cohesiveness) (Meullenet *et al*., 1998).

Figure 2 shows the responses of the panelists to the five-point hedonic test about cheese treated with phenolic compounds. Firmness and odor obtained the highest ratings among the evaluated characteristics, since 56.3% of the panelists classified them as “I like it”. For the flavor, 37.5% of the panelists gave a rating of “I like it” and an equal percentage for the color of the

cheese with the label of "I neither like nor dislike". The lower color rating could be due to the fact that phenolic compounds developed a yellow color in cheese, color that did not change along storage.

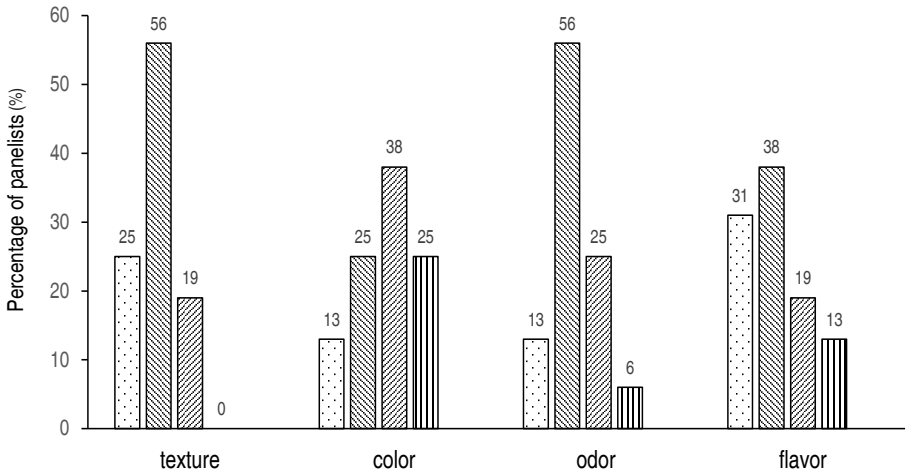

Figure 2. Sensory analyzes. Percentage of panelists and their evaluation of firmness, color, odor and flavor on cheese using a five-point hedonic scale, with scores ranging from "I like it a lot" ⬚ "I like it" ▩ , "I neither like nor dislike" ▨ , "I dislike it" ▥ "I dislike it a lot" □ .

Based on present results, it is difficult to conclude if color had influence either in odor or flavor. Moreover, visual and auditory appreciations can modify the flavor of food but are not intrinsic to it (Auvray and Spence, 2008). Koch and Koch (2008) stated that "In fact, it may be that color has nothing to do with the taste of food or drink". Meanwhile, Bayarri *et al.* (2001) suggested that "the possible influence of color on flavor perception is under discussion and no clear conclusions have been attained yet".

CONCLUSIONS

The solutions of phenolic compounds at concentrations of 1 and 1.5% showed the highest *in vitro* inhibition of *Salmonella* spp., requiring 2 h to achieve a complete inhibition of the bacteria.

Sensory analysis showed there was no difference between the cheese treated with phenolic compounds and a control cheese. Firmness and odor had high ratings whereas color had the lowest rating among the evaluated attributes.

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Chemical characterization, polyphenol content and antioxidant capacity of two pitahaya ecotypes (*Hylocereus* spp.)

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Caracterización química, contenido de polifenoles y capacidad antioxidante de dos ecotipos de pitahaya (*Hylocereus* spp.)

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Keywords:
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ABSTRACT

Pitahaya has originated worldwide interest due to its content of bioactive compounds with proven beneficial effects on health, acting as antioxidants against free radicals. This study aimed to evaluate the nutraceutical potential of the peel and pulp of the red (*Hylocereus monacanthus*) and yellow (*Hylocereus megalanthus)* pitahaya ecotypes for nutritional formulation purposes. Two pitahaya ecotypes were analyzed, obtaining a methanolic extract of the peel and edible part to perform the proximal chemical analysis, the phytochemical screening, and determine antioxidant activity by the DPPH, ABTS, and IC_{50} methods. Flavonoids, tannins, quinones, among other bioactive compounds, were identified. Yellow pitahaya presented higher content of polyphenols and higher antioxidant activity by the ABTS method, while the average inhibition percentage for both ecotypes was 93% by DPPH method. IC_{50} was higher for the edible part of red pitahaya with 1.68 mg mL^{-1}. Both ecotypes have a high content of polyphenols and a high antioxidant capacity, which agree with those found in different studies such as those of Colombia, Brazil and Korea, being as high or even higher than most varieties of citrus fruits in Peru. Future studies should consider the inclusion of other metabolites and bioactive substances such as betalains due to their antioxidant activity. Both pitahaya ecotypes are rich in antioxidants, bioactive compounds, have low energy density, and may be suitable for food prescriptions as a functional ingredient in food industry.

Palabras clave:
ABTS
Capacidad antioxidante
Compuestos bioactivos
DPPH
Alimentos funcionales
Polifenoles.

RESUMEN

La pitahaya ha suscitado el interés mundial debido a su contenido de compuestos bioactivos con comprobados efectos benéficos para la salud, actuando como antioxidantes frente a los radicales libres. El objetivo de este estudio fue evaluar el potencial nutracéutico de la cáscara y pulpa de los ecotipos pitahaya roja (*Hylocereus monacanthus*) y amarilla (*Hylocereus megalanthus)*, con fines de formulación nutricional. Se analizaron dichos ecotipos de pitahaya, obteniéndose un extracto metanólico de la cáscara y parte comestible de ambos ecotipos a fin de realizar el análisis químico proximal, la marcha fitoquímica, y determinar actividad antioxidante por los métodos DPPH, ABTS e IC_{50}. Se identificaron flavonoides, taninos, quinonas, entre otros compuestos bioactivos. La pitahaya amarilla presentó mayor contenido de polifenoles y mayor actividad antioxidante por el método ABTS, mientras que el porcentaje de inhibición promedio para ambos ecotipos fue del 93% por el método DPPH. El IC_{50} fue mayor para la pulpa de pitahaya roja con 1,68 mg mL^{-1}. Ambos ecotipos presentan un alto contenido de polifenoles y una alta capacidad antioxidante, los cuales concuerdan con los encontrados en distintos estudios como los de Colombia, Brazil y Corea, siendo tan alta o incluso superior a la de la mayoría de las variedades de cítricos en Perú. Futuros estudios deberían considerar incluir a otros metabolitos y sustancias bioactivas como las betalainas debido a su actividad antioxidante. Ambos ecotipos de pitahaya son ricos en antioxidantes, compuestos bioactivos, y de bajo aporte calórico, recomendándose su uso en prescripciones alimentarias y en la industria de alimentos como ingrediente funcional.

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Natural antioxidants are substances that can be part of our diet, capable of preventing the adverse effects of reactive oxygen species (ROS) -free radicals-, by inhibiting or interrupting the reactions in which they participate (Olugbami *et al*., 2014). ROS are produced during cell metabolism or by exposure to oxidizing compounds. These free radicals are related to non-communicable diseases and pathologies, such as cancer and cellular aging. A high amount of ROS leads to significant oxidation of various macromolecules, causing damage to cells and tissues (Seyidoglu and Aydin, 2016).

Polyphenols are antioxidant compounds of plant origin, which include flavonoids, phenolic acids, tannins, and lignans, among others, that show a protective effect against the harmful impacts of free radicals. These phenolic compounds can be found in a varied diet, rich in fruits and vegetables, abundant in essential nutrients such as vitamins, minerals, and antioxidants necessary for the body (Seyidoglu and Aydin, 2016). A diet rich in polyphenols, based on the daily consumption of fruits and vegetables has a beneficial effect on the prevention of non-communicable diseases (NCDs) (Williamson, 2017).

Pitahaya or dragon fruit is an exotic, non-climacteric fruit (Ortiz-Hernández *et al*., 2012), but it may behave as climacteric when collected at a high maturation state (Vásquez-Castillo *et al*., 2016). It shows various functional/nutraceutical characteristics (Joshi and Prabhakar, 2020), having antioxidant, antiproliferative, anti-inflammatory, chemopreventive and antidiabetic properties (Joshi and Prabhakar, 2020; Kim *et al*., 2011). These properties are explained by its high content of polyphenols and secondary metabolites such as steroids, triterpenes, tannins, and flavonoids (Ibrahim *et al*., 2018). Pitahaya species (*Hylocereus spp*.) are tropical fruits native to Mexico, Central, and South America. However, it is currently produced in many Asian countries, Australia, Israel, and the USA (Verona-Ruiz *et al*., 2020). Pitahaya fruit's pulp is white, red or fuchsia with edible black seeds has a gelatinous consistency and a sweet taste (Ibrahim *et al*., 2018). Regarding its nutritional value, the fruit is rich in vitamins (mainly vitamin C), minerals (especially magnesium, potassium and phosphorus), antioxidants and fiber, but their levels differ among varieties or species (Ibrahim *et al*., 2018; Verona-Ruiz *et al*., 2020). Researchers in Korea, focusing on the antioxidant and antiproliferative properties of pitahaya, found marked differences in the polyphenol and flavonoid contents of red and white pitahaya pulp and peel. Red pitahaya peel and white pitahaya peel contained similar polyphenols and flavonoids levels, while red pitahaya pulp contained more polyphenols and flavonoids than white pitahaya pulp (Kim *et al*., 2011).

Despite being popular as a health food in many countries (Ibrahim *et al*., 2018), its production and consumption are not widespread in Peru (Ramos, 2017). Research in this country about local pitahaya species as functional foods is scarce, despite the great global interest that this fruit has arisen lately (Verona-Ruiz *et al*., 2020). Many ecotypes -differentiated and locally adapted varieties- are found among pitahaya species (Ortiz-Hernández *et al*., 2012); thus, it is expected to find differences in their chemical composition, nutritional value and antioxidant capacity. In addition, there is a research gap on the evaluation of the nutraceutical potential of the *Hylocereus megalanthus* "yellow pitahaya" and *Hylocereus monacanthus* "red pitahaya" species.

In this context, this research aimed to chemically characterize the fruit, determine the polyphenol content and evaluate the antioxidant capacity of both pitahaya ecotypes for nutritional formulation purposes.

MATERIALS AND METHODS

The protocol for this research proyect was submitted to the Ethics and Research Committee of the Faculty of Health Sciences at *Universidad Peruana de Ciencias Aplicadas* , Lima Peru. They exempted the protocol from further revision and its execution was approved based on the documents FCS/203-09-18 , FCS/CEI 210-09-19, and FCS/CEI 024-02-20.

Physicochemical characterization, total phenolic content, and ABTS radical scavenging capacity assays were carried out at *La Molina Calidad Total Laboratorios - UNALM*. Phytochemical screening, DPPH radical scavenging capacity and the IC50 assays were performed at the *Instituto de Investigación de Bioquímica y Biología Molecular de la Universidad Nacional Agraria La Molina*.

Plant materials

The samples of *Hylocereus megalanthus* "yellow pitahaya" and *Hylocereus monacanthus* "red pitahaya" were obtained from *Mercado Modelo de Frutas*, Lima, Peru, between Jan 2019- Jan 2020. The yellow pitahaya ecotype was selected in its 5 stage of maturity, indicated by the peel yellow color, with slightly greenish nipple tips, based on a visual maturity scale for yellow pitahaya (ICONTEC, 1996). The red pitahaya ecotype was selected in its full maturity stage, indicated by the peel red-purple color in 75-100% of the fruit (Osuna-Enciso *et al*., 2011). Fruits that showed bruises and deterioration were excluded. Botanical identification of the fruits as *Hylocereus monacanthus* (Hort. Ex Lem) Britton & Rose (red pitahaya) and *Hylocereus megalanthus* (K. Schum. Ex Vaupel) Ralf Bauer (yellow pitahaya) was carried out in the Natural History Museum of the *Universidad Nacional Mayor de San Marcos* (UNMSM).

After washing and drying, the weight of each fruit was recorded. Then, the peel and the pulp were weighed separately. The edible part (pulp and seeds) was homogenized using a mortar, evenly distributed on Petri dishes and frozen at -20 °C for 48 h. Afterwards, the samples were lyophilized at -60 °C for 2 days, obtaining the material for the extraction process. Later, the peels were cut into 2 mm slices and dried using a vacuum oven at 40 °C until there was no difference in weight. The dried peels were ground with a blender and sieved using a 300-µm sieve (Standard Mesh N° 50), obtaining the pitahaya peel flour ready for the extraction step.

For the proximal chemical analysis, the edible part of the fruits (pulp and seeds) was separated from the peel, placed in separated aluminum trays, frozen at -20 °C, and then lyophilized for 48 h. This material was used for the determination of the chemical composition of both pitahaya ecotypes.

Reagents

Analytical grade chemicals were used in all the assays and analyses. 2,2-diphenyl-1-picrylhydrazy (DPPH), gallic acid, 6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Folin-Ciocalteu reagent and methanol reagent and HPLC grade, were obtained from Sigma-Aldrich (St. Louis, MO, USA). Chloroform, potassium persulfate, sodium carbonate, Shinoda, Mayer, Dragendorff,Lieberman Burchard, Borntrager, Gelatine, and FeCl3, Rosenheim, Kedde and Ninhydrin reagents were purchased in Merck (Merck KGaA, 64271 Darmstadt , Germany).

Extraction procedure

The extraction technique was based on the method reported by Lock de Ugaz (1994) with modifications. First, 10 g of the lyophilized homogenate of the edible part and the peel flour were macerated separately in 100 mL methanol at room temperature for 7 days, stirring for about 2 min daily. Then, the mixture was filtered using Whatman paper # 4 and the methanolic extract was obtained (100 mL). The methanolic extract -obtained as described above- was used for the phytochemical screening, determination of the DPPH radical scavenging capacity and the IC_{50} assay.

A second methanolic extract was prepared to determine the ABTS radical scavenging capacity and the total phenolic content (Folin-Ciocalteu assay). Thus, 25 g of the lyophilized homogenate of the edible part and of the peel flour were weighed and homogenized with 25 mL of methanol (80%), constantly stirring to obtain a uniform consistency. Then, the mixture was transferred into a 50 mL centrifuge Falcon tube and macerated for 20 to 24 h at 4 °C. After that time, the sample was concentrated by centrifuging at 4000 rpm ((KENDRO Labofuge 400R) for 30 min, and then the extract was filtered using Whatman paper # 4. The supernatant was transferred to 1.5 mL Eppendorf tubes, avoiding the light. The samples were stored at -18 and -20 °C until the antioxidant capacity (ABTS) analysis was performed.

Physicochemical characterization

To determine physicochemical characteristics, 400 g of each fresh pitahaya ecotype was used. Crude fiber (NTP 205.003:1980 Revised in 2011) (INACAL, 2011), ash (AOAC 9030.05), moisture (AOAC 925.10), fat (AOAC 922.06), protein (AOAC 978.04), (AOAC International, 2016), carbohydrate (by difference), energy provided by proteins, carbohydrates, and fat, and total energy content (Collazos *et al*., 1993) were quantified. The results from each determination were reported as a single value.

Phytochemical screening

The methodology proposed by Lock de Ugaz (1994) was followed to determine phytochemicals present in pitahaya

pulp and peel. The test was performed in triplicate for each extract. Figure 1 shows the flow diagram with the steps for this analysis. The methanolic extract was partitioned into five fractions. These five fractions were evaluated by qualitative reactions to screen for specific phytochemicals, namely: tannins, amino acids and flavonoids (fraction A), steroids and quinones (fraction B), cardenolides, steroids and alkaloids (fraction C), leucoanthocyanidins, cardenolides, steroids and alkaloids (fraction D), and flavonoids and leucoanthocyanidins (fraction E). The color intensity of the precipitate formation was used as an analytical response to these tests.

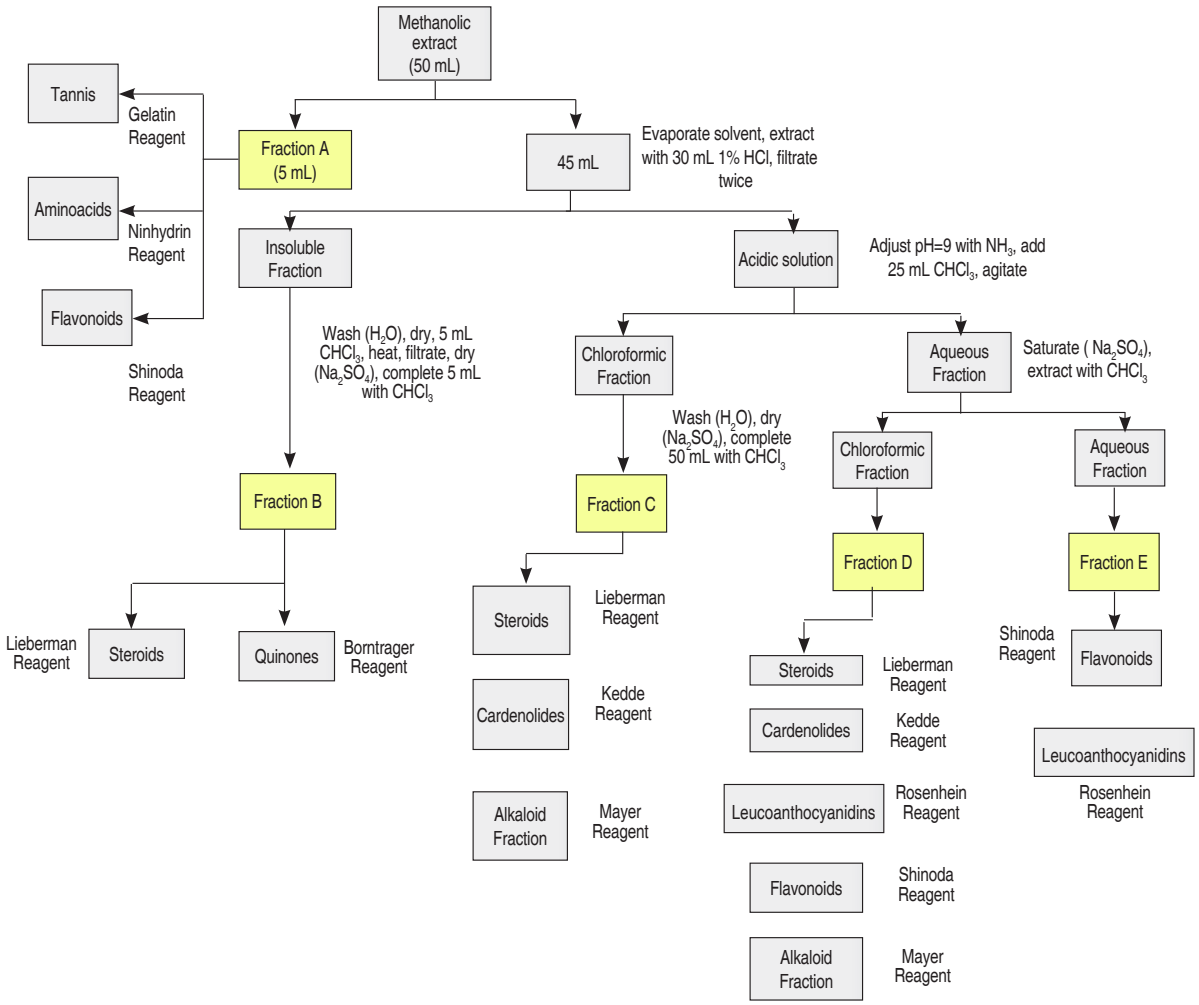

Figure 1. Steps for the phytochemical screening based on the methanolic extracts of yellow and red pitahaya.

Total phenolic content

The determination of total phenolic compounds or polyphenols in pitahaya pulp and peel was performed using a modified Folin-Ciocalteu method (Singleton and Rossi Jr, 1965). For the calibration curve, 0.1 mL aliquots of 10, 20, 40, 60, 80 mg mL^{-1} gallic acid standard stock solution (SIGMA) (10 mg mL^{-1}) were mixed with 8.5 mL of distilled water, followed by the addition of 1.0 mL of sodium carbonate (Na_2CO_3) (20% v:v). Mixtures were incubated for 5 min at 20 °C, and then 0.5 mL Folin–Ciocalteu reagent was added. Mixtures were stirred vigorously and then incubated - under constant agitation- for 30 min in darkness at 20 °C. Absorbance was measured using a spectrophotometer (Spectrum Pharo 300 Merck) at 760 nm. The same procedure was applied to test the pitahaya samples, replacing the gallic acid solution for the methanolic extracts of the samples. The total phenolic content of pitahaya extracts was calculated as

mg gallic acid equivalent (GAE)100 g^{-1} of dry sample and determined from the standard curve of gallic acid and reported as a single value.

DPPH radical scavenging capacity

DPPH radical scavenging capacity was performed in triplicate for each extract using Blois's method (Blois, 1958). A 0.3 M DPPH methanolic solution was prepared. Then, 2 mL of the pitahaya extracts (5% v/v) were added to 0.8 mL of the DPPH solution. Samples were incubated for 30 min at 20 °C. Gallic acid was used as a positive control at 31.3 µg mL^{-1}.

The decrease in absorbance of pitahaya test mixtures (due to quenching of DPPH free radicals) was determined at 517 nm, and the percentage of inhibition was calculated according to the equation:

$$\%\text{inhibition}=\left(\frac{\text{Ac}-(\text{Am}-\text{Abm})}{\text{Ac}}\right)\times 100$$

Where Ac is the absorbance of the reagent blank (DPPH+methanol), Am is the absorbance of the sample+DPPH, and Abm is the absorbance of the sample blank (sample+methanol).

IC_{50}

IC_{50} is a parameter widely used to measure and compare the antioxidant activity of test samples. For this study, the IC_{50} value is the concentration of the pitahaya test mixture required to quench 50% of the initial DPPH radicals (Ordoñez-Gómez *et al.*, 2018).

IC_{50} was obtained from the linear regression between the percentage of inhibition (which represents the antioxidant activity of the samples) in the ordinate versus the concentration of the samples (µg mL^{-1}) in the abscissa.

ABTS radical scavenging capacity

ABTS method (µmol Trolox eq g^{-1}) was used to determine the hydrophilic antioxidant capacity (Arnao *et al.*, 2001). The assay is based on the ability of radical scavenging compounds to reduce the blue-green radical cation (ABTS • +) to a non-colored form.

The extent of discoloration is calculated relative to the Trolox antioxidant standard. Reagent A was prepared with ABTS at a concentration of 7.84 mg mL^{-1} in distilled water. Reagent B was prepared with potassium persulfate at a concentration of 1.32 mg mL^{-1} in distilled water; both solutions were stored in the dark at 20 °C. The chromogenic radical (ABTS2+) stock solution was prepared, mixing equal volumes (1:1) of reagents A and B. The mixture was allowed to react for 12 h in the dark at 20 °C. Then, 1 mL of the ABTS stock solution was taken and diluted with 65 mL of methanol (80%). The absorbance of the prepared solution was read at 734 nm. It was corrected by adding methanol (80%) or stock solution. The reading was taken again at the same absorbance until it was within the range 1.1±0.02.

A standard Trolox curve was made, by preparing a series of Trolox standard solutions that contains different Trolox concentrations and a constant volume of ABTS stock solution, using methanol (80% v:v) as diluent.

To determine the radical scavenging capacity of the pitahaya samples, 150 µL of the sample was mixed with 2850 µL of the radical ABTS solution. A mixture of the standard solution and methanol was used as a blank. The reaction took place at 20 °C for 30 min, and the absorbance was measured at 734 nm in a Spectroquam UV/BIS Pharo 300 spectrophotometer. Finally, the hydrophilic antioxidant capacity quantified was expressed in µmol Trolox eq g^{-1} of sample, and reported as a single value.

Statistical analyzes

DPPH antioxidant activity results were expressed as mean±standard deviation of the three repetitions. The results were compared by means of the Wilcoxon test for two-tailed paired samples, with statistical significance determined at $P<0.05$. Statistical analyzes were carried out using STATA v.15.The results for the physicochemical characterization, total phenolic contents, and ABTS radical scavenging capacity assays were not available in triplicate.

RESULTS AND DISCUSSION

Physicochemical characterization

Table 1 shows the physicochemical characterization of *Hylocereus monacanthus* (red ecotype) and *Hylocereus megalanthus* (yellow ecotype) based on the proximate analysis results. The pulp of *Hylocereus megalanthus* showed the highest protein content (2.2%). The peel of both ecotypes had a higher percentage of moisture and crude

fiber than the edible part. *Hylocereus monacanthus* showed a slightly higher percentage of crude fiber (pulp: 2.3%, peel: 0.9%) than *Hylocereus megalanthus* (pulp: 2.0%, peel: 0.8%). These values are lower than the crude fiber values reported for *Hylocereus polyrhizus* (11.35 %) (Cordeiro *et al*., 2015). The fat content varied from 0.1 to 0.6% in the edible part of the red and yellow ecotypes, respectively. In all cases, the energy provided by carbohydrates exceeded 80%. Thus, based on the results of the present study, local ecoytypes of yellow and red pitahaya had a carbohydrate content (between 10-19% carbohydrates) similar to those in apples, pears, peaches, sweet granadilla, and guava (Ministerio de Salud del Perú, 2017). The energy content values of the edible part for *Hylocereus megalanthus* and *Hylocereus monacanthus* were different (85.4 kcal $100g^{-1}$ of sample and 55.3 kcal 100 g^{-1} of sample, respectively), being the latter value comparable with the values of the edible part reported by researchers in Brazil (Jeronimo and Costa Orsine, 2015) for *Hylocereus undatus* (53.68 kcal 100 g^{-1} sample), and those reported for pears and apples (Ministerio de Salud del Perú, 2017). The average energy content of the edible part (70.35 kcal 100 g^{-1} sample) is comparable to those in grapes, figs and cherimoya, being lower than those in banana and lucuma (Ministerio de Salud del Perú, 2017). In general, the low energy content of pitahaya, particularly the red ecotype, makes it appropriate for low-calorie diets (Jeronimo and Costa Orsine, 2015) . The values for percentage of moisture, proteins, lipids, and carbohydrates for *Hylocereus monacanthus* (85.7%, 1.2%, 0.1%, and 12.4%, respectively) and *Hylocereus megalanthus* (79%, 2.2%, 0.6%, and 17.8%, respectively) in the present study are very similar to those reported for *Hylocereus undatus* (Jeronimo and Costa Orsine, 2015). According to Verona-Ruiz *et al*. (2020), *Hylocereus megalanthus* has a higher percentage of soluble solids and is sweeter than *Hylocereus monacanthus*, which correlates with the percentage of carbohydrates of both species in this study.

The average (peel and pulp) protein content and fat content for both ecotypes are similar to those reported by Verona-Ruiz *et al*. (2020) for *Hylocereus megalanthus* and *Hylocereus undatus*; however, the yellow ecotype in the present study showed higher values. Researchers in Ecuador reported that *Hylocereus megalanthus* seeds are a good source of omega 6 fatty acids, mainly linoleic acid (69.98%) (Altuna *et al*., 2018). Scarce information regarding *Hylocereus monacanthus* was found in the literature. More studies are needed that focus on the fatty acid composition of the seeds of local pitahaya ecotypes, as they could be used as a raw material to extract healthy oils with functional properties.

Phytochemical screening

Table 2 shows the results of the phytochemical screening of the methanolic extracts of the peel and the pulp of *Hylocereus monacanthus* and *Hylocereus megalanthus*

Table 1. Physicochemical characteristics of yellow and red pitahaya

Physicochemical characteristics	Yellow ecotype[a]		Red ecotype[b]	
	Peel	Pulp	Peel	Pulp
Carbohydrates (g $100g^{-1}$ sample)	11.7	17.8	6.8	12.4
Total Energy (kcal $100g^{-1}$ sample)	54.1	85.4	30.8	55.3
% kcal from carbohydrates	86.5	83.4	88.3	89.7
% kcal from fat	1.7	6.3	0.0	1.6
% kcal from protein	11.8	10.3	11.7	8.7
Protein (g $100g^{-1}$ sample) (factor: 6.25)	1.6	2.2	0.9	1.2
Fat (g $100g^{-1}$ sample)	0.1	0.6	0.0	0.1
Moisture (g $100g^{-1}$ sample)	84.4	79	90	85.7
Ash (g $100g^{-1}$ sample)	2.2	0.4	2.3	0.6
Crude fiber (g $100g^{-1}$ sample)	2.0	0.8	2.3	0.9

[a]*Hylocereus megalanthus*, [b]*Hylocereus monacanthus.*

The presence of tannins, steroids, flavonoids, amino acids, cardenolides and leukoanthocyanidins, and traces of alkaloids was qualitatively determined for both *Hylocereus megalanthus* and *Hylocereus monacanthus*. For the yellow ecotype, the peel and pulp showed more presence of triterpenoids, while leukoanthocyanidins and cardenolides were found mainly in the peel. For the red ecotype, the peel and pulp contained more cardenolides and flavonoids. Compared to the phytochemical screening results for a local ecotype of *Hylocereus undathus* (Figueroa and Mollinedo, 2017), both studies had positive results for flavonoids and negative results for anthraquinones. Likewise, alkaloids were positively detected in *Hylocereus undathus;* however, only traces of them were identified in the samples in this study. These differences in the qualitative identification of phytochemicals in pitahaya may be due to the use of different solvents for the extraction, the variability between species, and the geographical origin of the samples. Pitahaya extracts rich in bioactive compounds have been studied due to their therapeutic properties. Flavonoids, tannins, and terpenoids are reported to have antimicrobial properties, while triterpenoids and steroids possess anticancer activity (Ibrahim *et al*., 2018). Terpenoids also show anti-diabetic properties (Joshi and Prabhakar, 2020).

On the other hand, polyphenols, flavonoids (including leukoanthocyanidins), alkaloids, amino acids, and steroids in *Hylocereus spp.* could be responsible for the hepatoprotective properties of the fruit (Ibrahim *et al*., 2018), while cardenolides are well-known bioactive compounds, showing anticancer and cardiotonic properties (Verma *et al*., 2016). Future research should include betacyanins determination, especially for red pitahaya due to their antioxidant properties (Joshi and Prabhakar, 2020).

Table 2. Qualitative analysis of the methanolic extracts of yellow and red ecotypes of pitahaya.

Fraction	Reagent	Secondary metabolite	Red pitahaya[a]		Yellow pitahaya[b]	
			Peel	Edible part	Peel	Edible part
A	NINHYDRIN	Aminoacids	-	+	+ + +	+ + +
	SHINODA	Flavonoids	+	+	+	-
	GELATIN	Tannins	+ +	+ + +	+ + +	+ + +
	$FeCl_3$	Tannins	+ +	+ +	+ + +	+ + +
B	BORNTRAGER	Anthraquinones	-	-	-	-
	LIEBERMAN BURCHARD	Steroids (S) Triterpenoids (T)	S (++)	S (++)	S(+++), T (++)	S (+++), T (+++)
C	KEDDE	Cardenolides	-	-	++	-
	LIEBERMAN BURCHARD	Steroids (S) Triterpenoids (T)	-	-	S (+)	-
	MAYER	Alkaloids	-	-	-	-
D	SHINODA	Flavonoids	-	+	-	-
	ROSENHEIN		-	-	-	-
	KEDDE	Cardenolides	+ + +	+ + +	-	-
	LIEBERMAN BURCHARD	Steroids (S) Triterpenoids (T)	S (+)	-	S (++)	S (+/-)
	MAYER	Alkaloids	-	-	(+/-)	-
E	SHINODA	Flavonoids	+	+	(+/-)	-
	ROSENHEIM	Leucoanthocyanidins	(+/-)	(+/-)	+ + +	(+/-)

[a]*Hylocereus monacanthus*, [b]*Hylocereus megalanthus*, * (-): Negative, (+): Mildly positive, (++): Moderately positive, (+++): Markedly positive, (+/-): Traces.

Total phenolic content

Table 3 shows the total polyphenols content in the methanolic extracts of the red and yellow ecotypes of pitahaya determined by the modified Folin-Ciocalteu method (Singleton and Rossi Jr, 1965).

The peel of *Hylocereus monacanthus* showed a higher total phenolic content (0.68 mg GAE g^{-1} of sample), in contrast to the value obtained in the peel of *Hylocereus megalanthus* (0.43 mg GAE g^{-1} of sample). When comparing the phenolic content in the pulp, *Hylocereus megalanthus* showed a higher value (0.48 mg GAE g^{-1} of sample) than *Hylocereus megalanthus* (0.32 mg GAE g^{-1} of sample). The polyphenols content in the methanolic extracts of the pulp of the yellow pitahaya is about 12% higher than that of the peel; meanwhile, the polyphenols content of the red pitahaya peel is about twice the amount found in the pulp.

Table 3. Total phenolic content of yellow and red ecotypes of pitahaya

Values expressed on	Total phenolic content			
	Yellow pitahaya[a]		Red pitahaya[b]	
	Peel	Edible part	Peel	Edible part
Dry basis[a] (mg GAE g^{-1} sample)	0.43	0.48	0.68	0.32

[a]*Hylocereus megalanthus*, [b]*Hylocereus monacanthus*.

Researchers in Colombia (Daza *et al*., 2014) assessed the total phenolic content in the ethanolic extract of the pulp, peel, and seeds of *Cereus triangularis* (yellow pitahaya). They reported the sample as yellow pitahaya but *Cereus triangularis* is actually a synonym of *Hylocereus trigonus*. This duplicity is explained by the challenges in the classification of pitahaya plants. They were classified initially under the genus *Cereus*, but the genus *Hylocereus* (synonym of *Selenicereus*) is currently used (The Plant List, 2013). Further, morphological and genetic heterogeneity in pitahaya by hybridization among species and varieties caused taxonomical confusion to identify them at the species level (Abirami *et al*., 2021).

Daza *et al*. (2014) reported 102±1.2, 77.6±0.4 and 202.7±1.1 mg GAE g^{-1} of dry sample, of peel, pulp and seeds, respectively. After comparing their results with the values in the present study, there are considerable differences in the phenolic contents for pulp (102 GAE g^{-1} of dry sample versus 0.48 mg GAE g^{-1} of dry yellow pitahaya pulp, and 0.32 mg GAE g^{-1} of dry red pitahaya pulp); these values are 212 to 316-fold higher than those in the present study. The same applies to results of the peel (77.6 mg GAE g^{-1} of dry sample versus 0.43 mg GAE g^{-1} of yellow pitahaya peel, and 0.68 mg GAE g^{-1} of red pitahaya peel), presenting values 115 to 182-fold higher than those in this study. It is not clear whether these differences could be explained by the methodology (variations in the duration of the extraction procedure and the use of a different solvent) (Daza *et al*., 2014), the species and maturation stage, and/or the geographical origin of the cultivar (Ibrahim *et al*., 2018; Som *et al*., 2019). In a recent study on a red-pulp pitahaya species in Australia (Suleria *et al*., 2020), researchers reported values of total phenolic content for the ethanolic peel extracts (0.45±0.12 mg GAE g^{-1} of sample), and they are similar to the results of this study (0.43 mg GAE g^{-1} of yellow pitahaya peel, and 0.68 mg GAE g^{-1} of red pitahaya peel).

Antioxidant activity

ABTS radical scavenging capacity

A high antioxidant activity was found by the ABTS method in both ecotypes; nonetheless, the yellow pitahaya ecotype presented the highest values in both the peel and the pulp, 731.68 and 579.46 µmol Trolox eq g^{-1} of sample respectively, as shown in Table 4.

The higher antioxidant capacity in the pulp compared to peel found in the red pitahaya ecotype is consistent with the results reported by researchers in Malaysia (Mohd Adzim Khalili *et al*., 2012) for the methanolic extracts of the peel and pulp of red pitahaya (*Hylocereus* sp.), based on the ABTS method.

Table 4. Radical scavenging capacity of yellow and red ecotypes of pitahaya

Values expressed on	ABTS Radical scavenging capacity			
	Yellow pitahaya[a]		Red pitahaya[b]	
	Peel	Edible part	Peel	Edible part
Dry basis (µmol Trolox eq g^{-1} sample)	731.68	579.46	364.50	565.62

[a]*Hylocereus megalanthus*, [b]*Hylocereus monacanthus*.

The pulp is more relevant for nutritional purposes, and for both ecotypes, the values of antioxidant activity in the pulp were very similar (579.46 and 565.62 µmol Trolox eq g^{-1} of sample, for yellow pitahaya and red pitahaya, respectively) and can be considered to have a high antioxidant capacity.

For the yellow pitahaya ecotype, the antioxidant activity of the peel was 26.7% higher than the pulp antioxidant activity. It is likely that the peel of *Hylocereus megalanthus* had more antioxidant compounds than the pulp; in fact, leucoanthocyanidins were qualitatively detected in the peel but not in the pulp. On the contrary, for the red pitahaya ecotype (*Hylocereus monocanthus*) was found that the peel (364.5 µmol Trolox eq g^{-1} of sample) showed 36% less antioxidant activity than the pulp (565.6 µmol Trolox eq g^{-1} of sample).

Colombian researchers determined antioxidant capacity by the ABTS method in the ethanolic extracts of the peel, pulp, and seeds of yellow pitahaya (*Hylocereus megalanthus* Haw), finding a higher antioxidant capacity in the peel compared to the pulp (without seeds); nevertheless, the seeds showed the highest antioxidant capacity (Torres-Grisales *et al*., 2017). The edible part -the pulp with the seeds- was analysed in this study; however, the peel of *Hylocereus megalanthus* still showed a higher antioxidant activity than the pulp and seeds together.

DPPH radical scavenging capacity

As shown in Table 5, the pulp and peel methanolic extracts of both ecotypes presented values of 93% of DPPH radical inhibition, with no significant difference (P>0.05) when comparing the values of peel vs. pulp for red and yellow pitahaya.

Table 5. DPPH radical scavenging capacity and IC50 in the yellow and red pitahaya ecotypes.

Sample	DPPH radical scavenging capacity (%) Mean±standard deviation	IC_{50} (mg mL^{-1})
YPP[a]	93.31±0.71	2.8
YPF[a]	93.14±3.70	1.68
RPP[b]	93.16±1.48	2.53
RPF[b]	93.62±3.04	2.67

YPP=yellow pitahaya peel, YPF=yellow pitahaya pulp, RPP=red pitahaya peel, RPF=red pitahaya pulp, [a]*Hylocereus megalanthus*, [b]*Hylocereus monacanthus*.

The DPPH test results in this study are higher than the results reported by researchers in South Korea (Kim *et al*., 2011), for the methanolic extracts of the peel and edible part of the red pitahaya (56.8±5.6% and 33.2±1.8%) and white pitahaya (68.1±2.8% and 23.8±3.3%) respectively. On the other hand, Colombian researchers (Torres-Grisales *et al*., 2017) reported an 8% lower antioxidant capacity (85.0±0.2%) in the ethanolic extract of yellow pitahaya pulp (*Hylocereus megalanthus* Haw). The difference between their results and those in this study for yellow pitahaya could be explained by the inclusion of the seeds in the edible part, use of a different solvent for the extraction and probably by genus and species variations.

The high radical scavenging capacity of the samples in the present study could be due not only to the presence of phenolic compounds but also to other metabolites present such as betalains and their derivatives (in the case of red pitahaya) (Kim *et al*., 2011).

IC_{50}

Results were also expressed as IC_{50} (mg mL^{-1}) (Table 5), which correspond to the amount of extract required to reduce DPPH radical by 50%; thus, the lower the IC_{50}, the higher the antioxidant capacity of the extract (Olugbami *et al*., 2014).

The IC_{50} values for the peel and edible part of the pitahaya samples in this study were 2.80 mg mL^{-1} and 1.68 mg mL^{-1} for *Hylocereus megalanthus* and 2.53 mg mL^{-1} and 2.67 mg mL^{-1} for *Hylocereus monacanthus*. The antioxidant activity of pitahaya samples in this study was slightly lower, except for the yellow pitahaya pulp, that showed a higher antioxidant capacity in contrast to aguaymanto, which was obtained from four different areas in Peru (1.86, 2.04, 2.24, and 2.36 mg mL^{-1}) (Teixeira et al., 2016).

On the other hand, a study carried out in Peru (Ordoñez-Gómez *et al*., 2018), the methanolic extracts of various citrus fruits presented, in most cases, higher IC_{50} values and lower antioxidant capacity than those found in the pitahaya ecotypes samples of this study.

Thus, the results of the present study confirm the potent antioxidant capacity of the yellow and red local ecotypes of pitahaya, which is as high or even higher than the antioxidant capacity in most citrus varieties in Peru. The dissemination of these findings may be helpful to promote the consumption of local pitahaya ecotypes, their prescription in people diets; and their utilization as raw materials in food processing due to their nutraceutical properties.

Betalains were not included in the phytochemical screening of this study. Nevertheless, they should be included in future research in order to complement these results. Statistical analysis was only applied to the DPPH radical scavenging capacity assay, since the results for all the other analyses were not available in triplicate; this was due to a methodological limitation of the study. Finally, the stage of maturity of the fruits for each ecotype was not the same; however, it is an important variable to standardize to obtain accurate results when comparing characteristics of both species.

CONCLUSIONS

Local red and yellow pitahaya ecotypes show a high nutraceutical potential and can be used in dietary prescriptions. The low carbohydrate content (12.4-17.8%) and the low energy content (55.3–85.4%) of the pulp make both species, particularly the red pitahaya, a good option for inclusion on and low sugar diets.

The high antioxidant capacity of the local ecotypes of *Hylocereus megalanthus* and *Hylocereus monacanthus* is explained by their high content of total polyphenols. Both species show similar IC_{50} values to those reported for other locally-produced fruits with high antioxidant capacity. The presence of other bio-active compounds in the yellow and red pitahaya extracts, such as tannins, steroids, flavonoids, amino acids, cardenolides, leukoanthocyanidins, and triterpenoids, indicate a high nutraceutical potential. Future research could focus on the quantitive determination of these bio-active molecules to establish the nutraceutical potential of these fruits more accurately, including betalains.

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Physical, physiological, physicochemical and nutritional characterization of pumpkin (*Cucurbita maxima*) in postharvest stage cultivated in Antioquia-Colombia

Caracterización física, fisiológica, fisicoquímica y nutricional de la auyama (*Cucurbita maxima*) en la etapa de postcosecha cultivada en Antioquia-Colombia

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Keywords:
Color coordinates
Firmness
Respiratory rate
Total carotenoids

ABSTRACT

Pumpkin (*Cucurbita maxima*), also called squash is a mass consumption fruit used in agro-industrial transformations to obtain new food products. This study aimed to evaluate the physical, physiological and physicochemical properties during a storage period of 42 days and the nutritional compounds of the freshly harvested fruit fractions. According to the CIEL*a*b* space, both the pulp and peel did not present significant changes in L* and a* values during the evaluated storage time; however, in the peel, a change in the b* values was evidenced. In addition, there was a decrease in the firmness of 10.8% in the fruit with peel and in the pulp, it was of 19.8% was observed, with a whole fruit weight loss of 2.33% and an average respiration rate of 6.9 mg CO_2 $kg^{-1}h^{-1}$. According to physicochemical characteristics evaluated in the pulp, the values of pH, percentage of humidity, acidity, water activity and total soluble solids had no statistically significant changes occurred during the storage time. At the nutritional level, pumpkin is a good source of minerals, with a high concentration of potassium in all its fractions, and also has in total carotenoids (4.11±1.6 mg of β-carotene g^{-1} oven dry (o.d) in pulp and 6.24±2.7 mg of β-carotene g^{-1} (o.d) in peel). It was possible to conclude that the pumpkin has a low respiration rate, maintaining its physicochemical characteristics suitable for consumption throughout the evaluation period, presenting suitable conditions.

Palabras clave:
Coordenadas de color
Firmeza
Tasa de respiración
Carotenoides totales

RESUMEN

La calabaza (*Cucurbita maxima*), también llamada auyama es una fruta de consumo masivo, utilizada en transformaciones agroindustriales para la obtención de nuevos productos alimenticios. Este estudio tuvo como objetivo evaluar las propiedades físicas, fisiológicas y fisicoquímica durante a un periodo de almacenamiento de 42 días y nutricionales de las fracciones del fruto recién cosechado. Para las coordenadas del color CIEL*a*b*, los valores de L* y a* tanto para la pulpa y cáscara no presentaron cambios significativos durante el tiempo de almacenamiento evaluado, pero sí presentó cambios en los valores de b* en la cáscara del fruto. Además, se observó una disminución en la firmeza-fuerza de 10,8% en el fruto con cáscara y en la pulpa de 19,8%, con una pérdida peso de fruto entero del 2,33% y una tasa de respiración promedio de 6,9 mg CO_2 kg^{-1} h^{-1}. De acuerdo a las características fisicoquímicas evaluadas en la pulpa del fruto, pH, porcentaje de humedad, acidez, actividad de agua y sólidos solubles totales no se presentaron cambios estadísticamente significativos durante el tiempo de almacenamiento. A nivel nutricional la auyama es una buena fuente de minerales, destacando la alta concentración de potasio en todas sus fracciones, y también en carotenoides totales (4,11 mg de β-caroteno g^{-1} (b.s.) en la pulpa y 6,24 mg de β-caroteno g^{-1} base seca (b.s.) en la cáscara). El fruto vegetal de la auyama bajo condiciones normales de almacenamiento presenta una baja tasa de respiración manteniendo sus características fisicoquímicas aptas para el consumo durante todo el periodo de evaluación, presentando condiciones adecuadas para el desarrollo de productos agroindustriales que permitan darle valor agregado durante el período de evaluación.

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Pumpkin (*Cucurbita sp.)* is a mass consumption fruit in Colombia and throughout the world. In this country, it occupies a planted area of 6820 ha, and a production that in 2017 was of 92180 t and a yield of 13.51 t ha-1. For the year 2018, in the department of Antioquia, the harvested area was 100 ha, with a production of 1200 t and a yield of 12.0 t ha^{-1}, being the municipality of Dabeiba the largest producer with 65 ha cultivated (Agronet, 2018). Its importance in the diet has generated interest in improving it genetically, achieving better yields, and in the quality of the pulp (Valdés *et al*., 2014).

The *Cucurbita sp*. species has diverse genera and abundant cultivars, with variable shape, size, and color, in addition to different levels of resistance to diseases. It is widely cultivated in South America and the United States (Rodríguez *et al*., 2018). Pumpkin corresponds to a crop of American origin developed under varied environmental and climatic conditions, existing from 100 to 3000. Currently, 118 genera and 825 species have been found around the world (Sohail *et al.,* 2018).

Pumpkin, a transitory dicotyledonous fruit, has cylindrical or penta-angular stems, with petioles of 12 to 30 cm; the leaves are circular, heart-shaped, with dimensions of about 20 cm long and 30 cm wide; the yellow flowers are bell-shaped, with five lobes and up to 12 cm long. The peduncle is strong with, a rounded penta-angular base and a large apex. The fruits are round, flattened, oval, oblong or pear-shaped, with varied ribs, between 15 and 60 cm in diameter, and with weights between 2 and 45 kg. It is characterized by its climbing or creeping habit, presence of tendrils, unisexual and showy flowers, entomophilic pollination, annual or perennial vegetative cycle, herbaceous, and usually monoecious plants, but there are also andromonoic and dioecious (Rodríguez *et al*., 2018). Its pulp is intensely yellow, orange, pale green, or white, and the hollow center contains loose pulpy fibers and numerous oval, flat, white to brown seeds with fine skin, irregular margins, and a fleshy grain (Caseres *et al*., 2010).

Pumpkin corresponds to one of the most consumed fruit since, it is important in food, medicine, as a raw material for agribusiness it is used for the manufacture of oils, and dehydrated products, and also for the extraction of phytochemicals compounds such as carotenoids. It has a high volume of production, around 20 to 30 kg $plant^{-1}$, and its cultivation is associated with small and medium farmers from many warm and temperate areas of the world (Rodríguez *et al*., 2018).

Pumpkin is a weather-tolerant crop. The fruits are subject to changes in the postharvest stage, such as an increase in enzymatic activity and a decrease in dry matter. However, they are commercially stored outdoors and at room temperature. Some investigations have reported an increase in total soluble solids, a decrease in acidity, beta carotenes and ascorbic acid during storage in environmental conditions of 29±2 °C and 83±7% relative humidity (Nansikombi *et al*., 2019). These losses reduce the income of medium and small producers, and they occur due to poor postharvest handling, lack of collection centers, inappropriate transportation, poor packaging and storage (Asohofrucol, 2012).

The aim of this study was to evaluate the physical, physiological, physicochemical, and nutritional characteristics of postharvest pumpkins grown in the municipality of Dabeiba, Antioquia, Colombia, where the phenotypic conditions of the crops adapted to this area make them have specific properties that can be used for the agro-industrial transformation and the development of the region.

MATERIALS AND METHODS

Fruit samples

Pumpkin fruits (*Cucurbita maxima*) were harvested in the municipality of Dabeiba (Antioquia/Colombia), with a physiological or harvest maturity index of 99±17, corresponding to the ratio of total soluble solids on the percentage of acidity at 0 day of postharvest. Figure 1 shows the appearance of the fruit and its inner aspect. The pumpkin was selected according to physical aspects, especially green color, shape and weight. A total of 42 experimental units were used, consisting of a completely random design. The experimental phase was developed in the Fruit and Vegetable Laboratory of the Universidad Nacional de Colombia Medellín campus under environmental conditions of 65±10% RH and 23±4°C.

Physical characteristics

The color determination was measured with a sphere spectrophotometer (model SP60, X-RITE Inc., MI, USA), with an aperture of 4 mm, illuminant D65, and a 10°

Figure 1. Aspect of pumpkin fruit (*Cucurbita maxima*) used in the study.

observer as a reference. From the reflection spectra, the color coordinates of the CIEL*a*b* system were obtained, where L* is an indicator of luminosity, a* represents the chromaticity green (-) to red (+) and b* represents blue (-) to yellow (+) chromaticity. For the epidermis and fresh pulp color, the measurements were made at three reference points on the external surface of the fruit, for a total of six experimental units per day, during 42 days, for days 0, 7, 14, 21, 28, 35 and 42 of postharvest (Thole *et al.*, 2020). The browning index (BI) was calculated using CIEL*a*b* coordinates by Equations 1 and 2 (Maskan, 2001):

$$BI = \frac{(X - 0.31)\ x\ 100}{0.17} \quad (1)$$

X is the ratio of the color coordinates L*, a*, b* as follows:

$$X = \frac{(a^* + 1.7L^*)}{(5.645L^* + a - 3.01b^*)} \quad (2)$$

Weight, volume, equatorial and polar diameter were measured both for the whole fresh fruit. For each experimental unit, polar diameter measurements were made from the base to the apex of the fruit and the equatorial diameter using a digital Vernier caliper (resolution of 0.01mm), according to methodology proposed by Rodríguez (2018). The pycnometer method was used for the volume.

Physiological characteristics

Respiration rate was evaluated by measuring the carbonic gas production of the fruit by means of the respirometer technique, using the Petenkoffer chemical method. This value was calculated by the use of stoichiometry and expressed in mg of $CO_2\ kg^{-1}\ h^{-1}$. For the physiological weight loss expressed as a percentage, 6 fruits were stored at 65±10% RH and 23±4 °C, and were followed up during the evaluation period of 42 days, assessing the daily weight and comparing it with the weight of the initial day (0 day).

For the firmness measurement, unidirectional compression tests were applied using a cylindrical stainless-steel probe of 5 mm diameter, under a loading rate of 2 mm s^{-1}, with a penetration depth of 10 mm. The equipment used consisted of a TA-XT2i® texture analyzer and the Texture Expert Exceed software, Version 2.64 Stable Micro Systems (Labaky *et al.*, 2020). The measurements were made in the equatorial region of the fruit, recording the resistance to penetration during 42 days of storage.

Physicochemical characteristics of the pumpkin fruit during storage

The acidity value was obtained by means of potentiometric titration, where the results were expressed as a percentage of citric acid (Equation 3). The pH was determined with a Schott model CG-840B equipment at 25 °C. The evaluation of the total soluble solids (TSS) was carried out using the Leica auto ABBE refractometer with a scale of 0-32% at 25 °C, and the results were expressed as degrees Brix (°Bx). The moisture percentage was determined with an infrared lamp equipment (Sartorius, MA150). In addition, the water activity (a_w) was measured with a 25 °C dew point hygrometer (Aqua LAB Decagon series 3TE) (Marquez and Valenzuela, 2012).

$$\text{Acidity (\%)} = \left(\frac{V_{NaOH(mL)} \times N_{NaOH\left(\frac{meq}{mL}\right)} \times \left(\frac{0.064g}{meq}\right)}{W_{\text{sample weight(mL o g)}}} \right) \times 100 \quad (3)$$

$V_{NaOH(mL)}$ = Spent volume of NaOH; $N_{NaOH\left(\frac{meq}{mL}\right)}$ = Normality of NaOH

Nutritional composition of the pulp, seed and peel

The proximal analysis in each part of the fruit (pulp, seed and peel) was performed, by processing a total number of six fruits. The ash content was determined by means of the gravimetric method in muffle at 500-550 °C until constant

weight according to AOAC-923.03 (2005). The fat content was determined by means of the Soxhlet extraction method using, petroleum ether as a solvent (AOAC-920.39, 1990), the total dietary fiber (AOAC-985.29/90), the nitrogen content was obtained by volumetric method (Kjeldahl method) (AOAC-955.04/90), the carbohydrate content in the fruit by difference according to % CHO = 100 – (% Moisture + %Ashes + %Proteins + %Fat + %Total Dietary Fiber) expressed in g 100^{-1} g^{-1} edible part wet base (w.b). The content of some minerals such as Ca, Mg, Na, Fe, Cu, Mn, and Zn was measured by atomic absorption spectrophotometry according to NTC 5151 (2003). The quantification of total carotenoids was determined by spectrophotometry of the solution at 450 nm and Sigma-Aldrich St. Louis, MO, USA β-carotene was used as standard, according to the modified methodology of Biswas *et al.* (2011). Three extractions were performed for each repetition of the treatment, expressed in mg of β- carotene g^{-1} oven dry (o.d).

RESULTS AND DISCUSSION

Physical characteristics

Figure 2 shows the results of the CIEL*a*b* color coordinates with L*=56.08±3.49; a*=25.39±2.18 and b*=58.67±5.41 as average values for the pulp for 42 days of storage, while the peel presented average coordinates of L*=33.50±3.28; a*=-1.81±1.76 and b*=5.21±3.88. This result denotes that the pulp is located in the universe of orange tones, while the peel presented green and blue tones, similar results were found by Saini *et al.* (2015).

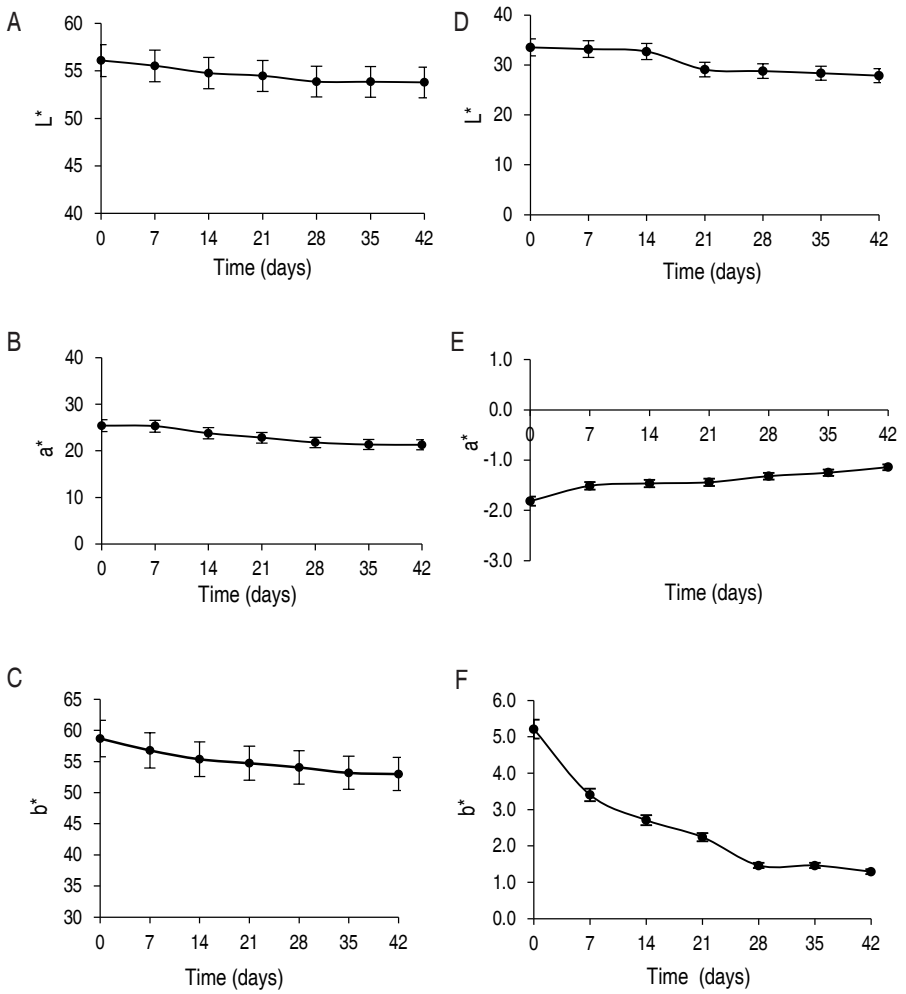

Figure 2. CIEL*a* b* color coordinates for pumpkin pulp (*Cucurbita maxima*) (A, B and C) and for peel (D, E and F), for 42 days. L*: CIE color space coordinate degree of luminosity, a*: CIE color space coordinate degree of green-red and b*: CIE color space coordinate degree of blue-yellow. The symbols represent the mean and the vertical bars the standard error for n=6.

The color coordinate L* corresponds to the luminosity for both pulp and peel, where this characteristic does not show significant differences during the 42 days of storage regarding the 0 day of postharvest. However, in other fruits, significant changes have been found in their postharvest stage for luminosity in both pulp and peel, due to enzymatic type reactions developed by the presence of the group of polyphenol oxidase enzymes, that in the presence of phenolic substrates and oxygen trigger a series of reactions, affecting the luminosity of the fruit (Piedra, 2017).

In the pulp, the a* and b* values tend to orange tones, typical of carotenoid-type pigments, most likely β-Carotenes, precursor molecules of vitamin A. This behavior was consistent during the evaluation period, affirming that the pigments responsible for this tonality did not present significant differences with respect to the 0 day of postharvest, which agrees with other authors (Jaeger *et al*., 2012). For the peel, the b* coordinate, corresponding to chlorophyll pigments of type b associated with the dark green color, showed a significant and continuous decrease throughout the postharvest period, while the a* coordinate showed significant differences during the postharvest period. Consequently, the dark green of the fruits decreased, and this is associated with the decrease in the concentration of chlorophyll b, due to the enzymatic activity of chlorophylases hydrolyzing this molecule to chlorophyllide and phytol (Vargas-Madriz *et al*., 2019).

Figure 3 shows the browning index (BI) for pumpkin pulp and peel, which is most likely associated with the activity of polyphenol oxidase enzymes in the presence of phenolic substrates and oxygen (Marquez and Valenzuela, 2012). For both pulp and peel during the evaluation period, there were no significant differences during the evaluation period. However, the BI for the pulp was significantly higher than that of the peel, a situation that may be due to the higher enzymatic activity and the higher concentration of carotenoid-type pigments present in this fraction of the fruit (Uscanga *et al*., 2019).

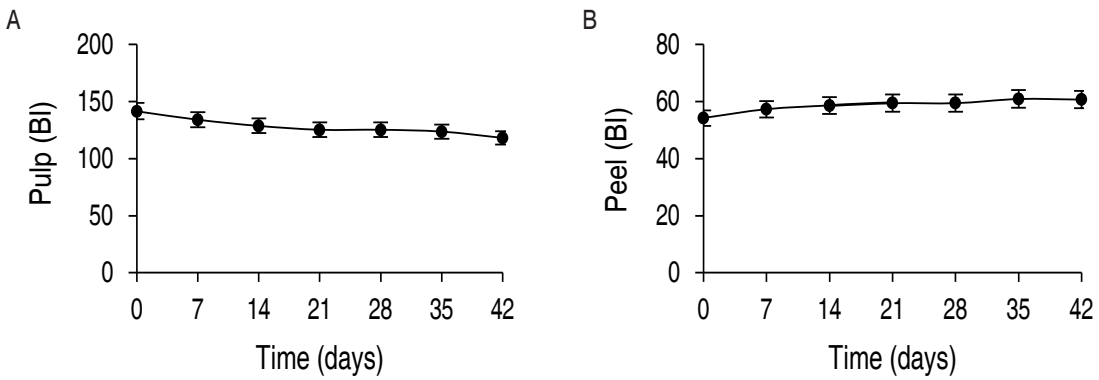

Figure 3. Browning index of pumpkin pulp (A) and peel (B) (*Cucurbita maxima*), stored for 42 days at 65± 10 % RH and 23± 4 °C. The symbols represent the mean and the vertical bars the standard error for n=6.

Table 1 shows the characteristic dimensions of the pumpkin at 0 day of evaluation, indicating that the description of the morphology of the fruits are elliptical, due to the fact that the equatorial diameter is greater than the polar diameter. These results are similar to those reported by Caseres *et al*. (2010).

Table 1. Characteristic dimensions of the entire pumpkin fruit (*Cucurbita maxima*)

Physical description	Average±SD
Weight (g)	2782±0.3
Volume (mL)	2250±0.5
Equatorial Diameter (cm)	21.10±0.1
Polar Diameter (cm)	15.30±0.1

Physiological characteristics

In Figure 4A, the maximum respiratory values were detected between days 0 and 8, probably because of the increase in mitochondrial activity and the great availability of carboxylates as substrate, combined with the degradation of starch, by enzymatic action and the formation of some low molecular weight carbohydrates (Marquez and Valenzuela, 2012). From day 8 to day 20, an accelerated decrease in CO_2 production was observed until an asymptotic behavior was evidenced over time, which is related to the states of physiological maturity as maturity (Suarez *et al.,* 2016). The maximum respiration value was 11 mg CO_2 kg^{-1} h^{-1}, typical of non-climacteric fruits, which is consistent with that reported by other investigations (Marquez and Valenzuela, 2012).

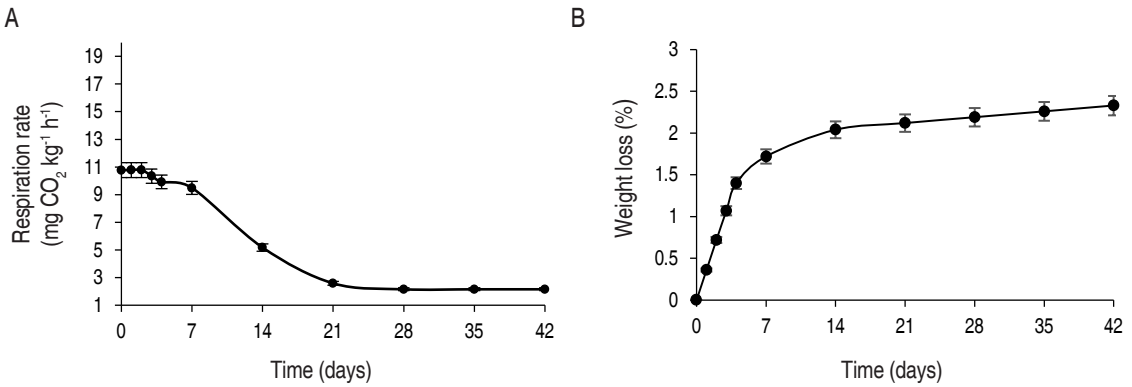

Figure 4. Respiration rate (A) and weight loss (B) of accumulated weight (%) of pumpkin (*Cucurbita maxima*) during a period of 42 days, n=6

Regarding the physiological weight loss presented in Figure 4B, it was possible to identify a continuous weight loss behavior during the entire postharvest stage. The period with the greatest quantity is that between days 0 and 14 of evaluation: the highest mass transfer (water) took place between the interior of the fruit and the external conditions of the storage environment until the hygroscopic equilibrium was reached, finding an average total loss of 2.33±0.05%. This aspect is favorable for this fruit, since it could present a low degree of deterioration during the storage period, similar to study performed by Suarez *et al.* (2016) using cucumber (*Cucumis sativus* L).

Figure 5 presents the variation of the firmness of pumpkin with peel and without peel during the storage period. The firmness of the pumpkin presented variability during the storage time. For the day 0, it was found that the whole fruit with peel presented an average penetration force of 93.29±5.85 N and for fruit without peel, that value is 80.62±6.52 N, showing a higher resistance to penetration in fruits with peel. This is due to materials such as cellulose, hemicellulose, lignin and other high molecular weight polysaccharides that make up the peel (Bemfeito *et al*., 2020). In the pulp, a slightly more accelerated decreasing behavior was found than that in the peel, showing a

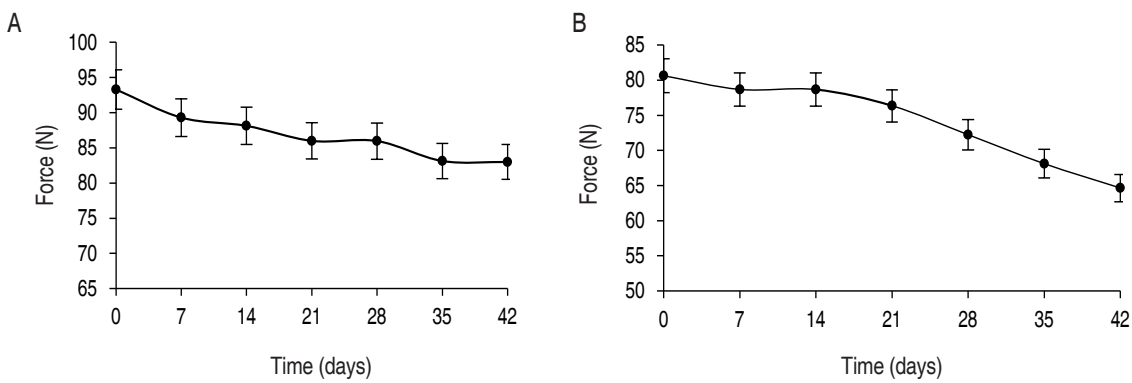

Figure 5. Firmness force for pumpkin fruits (*Cucurbita maxima*) with peel (A) and without peel (pulp) (B) stored during 42 days. The symbols represent the mean and the vertical bars the standard error for n=6.

significant difference between 0 day and 42 days, with average values in the fruit with peel of 83±0.64 N and 64.63±3.65 N without peel. It is, probably related to changes at the cell wall level because of the hydrolysis of pectic compounds by the action of the Pectin Methyl Esterase (PME), polygalacturonase and cellulase enzymes, which, in turn, decompose high molecular weight polymers, such as cellulose and hemicellulose, and obtaining the loss of cellular turgor, as a final result (Africano-Pérez *et al.*, 2016).

Physical-chemical characteristics of the pumpkin fruit during storage

Figure 6 shows the variation of the main physical-chemical characteristics that are related to the quality of pumpkin fruits. These results show not statistically significant differences regarding the 0 day of postharvest. Values obtained for the physical-chemical characteristics are similar to those reported by other investigations (Ziaul *et al.*, 2019).

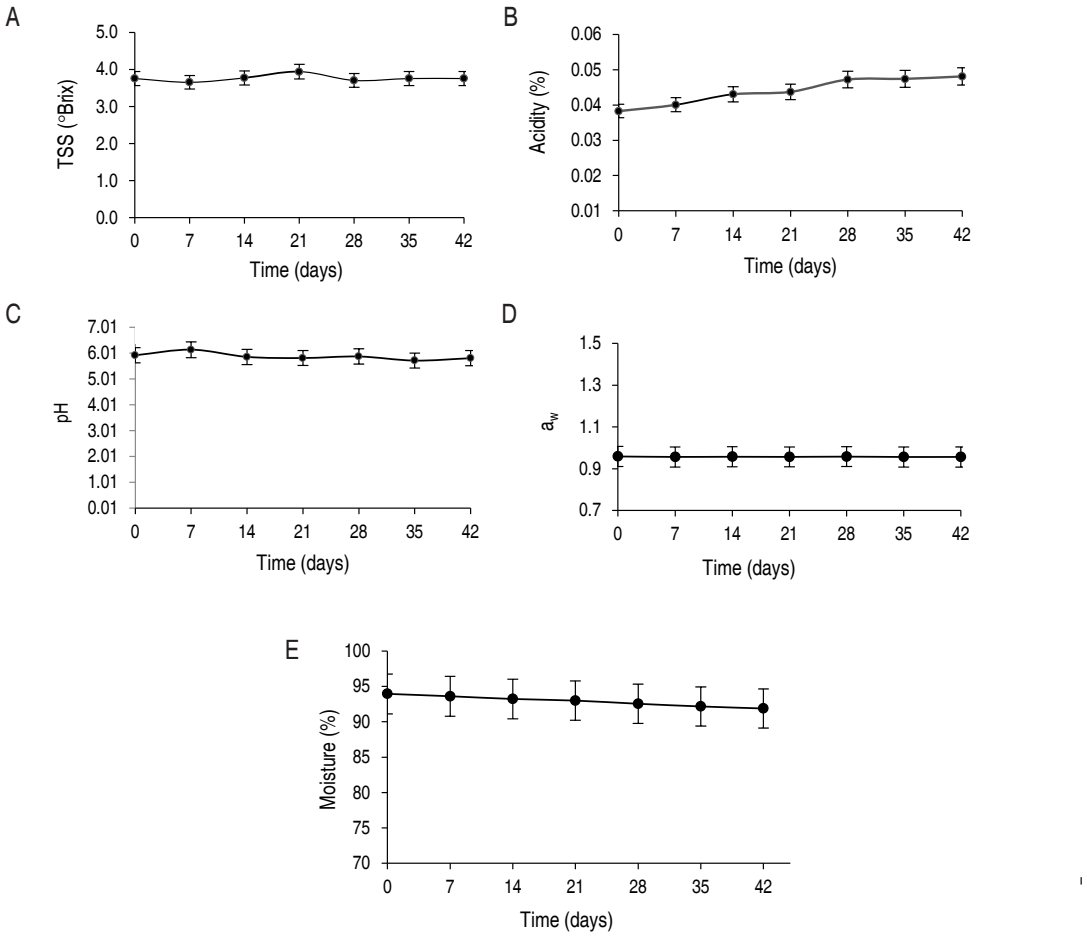

Figure 6. Total soluble solids (A), percentage of acidity (B), pH (C), water activity a_w (D) and percentage of moisture (E), for pumpkin pulp (*Cucurbita maxima*) during a storage period of 42 days, n= 6.

At 0 day, the pumpkin pulp showed acidity values of 0.038±0.003%, expressed as citric acid, pH of 5.92±0.29, TSS of 3.76±0.73 °Bx, a_w of 0.96±0.004 and a moisture content of 92.63±2.19%. Any of the samples evaluated had significant changes over time, showing a high moisture content and water activity, which refers to free or available water within the food (Ramírez and Villa, 2015). The fruit is protected thanks to the high level of firmness of the peel and therefore, presents natural barriers to the attack of microorganisms, probably due to some phytochemical substances found in the shell and present defense against microorganisms, especially

against fungi and yeasts. However, the plant must be protected from physical alterations caused by impacts or presence of mechanical forces during the handling and transportation of the product (Marquez and Valenzuela, 2012).

Nutritional composition of the pulp, seed and peel

The nutritional composition in each of the parts of the pumpkin fruit is shown in Table 2. As in many other foods of plant origin, the nutritional content depends on the variety, the climatic conditions where they are grown, the state of maturity and the agronomic handling of the plantation. In the *C.maxima* pulp, a moisture content (>84%) and low concentrations of carbohydrates (13.3%), protein (0.11%), fat (0.04%), fiber (0.11%) and ash (0.10%) were reported by Mi *et al*. (2012). In terms of minerals, the content of potassium was predominant among all the elements analyzed in the pumpkin pulp, seed and peel. According to Kulczynski and Gramza (2019), there is a region/climate correlation effect on this mineral in pumpkins. Some authors report significant concentrations of β-carotene and α-carotene in pumpkin pulp (Rodríguez *et al*., 2018; Valdés *et al*., 2014). Also report β-carotene concentrations in *C.pepo* and *C.mochata* between 4.58±2.27 and 2.92±1.44 mg 100^{-1} g^{-1} o.d, respectively (Kulczynski and Gramza, 2019). In some species, 171 to 461 μg g^{-1} o.d of the fresh product is found mostly in the fruit peel (Mi *et al*., 2012).

Table 2. Nutritional composition of the pulp, seed and peel of the pumpkin (*Cucurbita maxima*)

Composition	Pulp	Seed	Peel
Moisture (g $100^{-1}g^{-1}$ w.b)	92.70±0.34	55.60±0.65	86.01±0.56
Protein (g 100^{-1} g w.b)	0.68±0.02	16.07±0.18	2.00±0.05
Fat (g 100^{-1} g^{-1} w.b)	0.173±0.02	13.32±0.45	0.43±0.01
Ashes (g 100^{-1} g^{-1} w.b)	0.64±0.01	2.14±0.23	0.83±0.02
Carbohydrates (g 100^{-1} g^{-1} w.b)	3.81±0.12	0.21±0.01	4.74±0.05
Fiber would not diet (g 100^{-1} g^{-1} w.b)	2.00±0.03	12.65±0.12	5.97±0.15
P (% of total ash)	0.032±0.01	0.71±0.8	0.35±0.04
Ca (% of total ash)	0.27±0.03	0.11±0.09	0.33±0.01
Mg (% of total ash)	0.14±0.01	0.40±0.08	0.25±0.01
K (% of total ash)	3.31±0.05	1.02±0.09	1.77±0.42
Fe (ppm)	26.00±0.02	75.00±0.01	97.00±0.001
Mn(ppm)	4.00±0.01	43.00±0.01	27.00±0.01
Zn (ppm)	10.0±0.02	46.00±0.02	19.00±0.002
Total Carotenoids (mg of β- carotene g^{-1} o.d)	4.11±1.6	0.57±0.02	6.24±2.77

CONCLUSIONS

Pumpkin fruits in this study had a low respiration rate, preserving the physiological and physicochemical characteristics throughout the 42 days evaluated. The total weight loss of the pumpkin fruits during the evaluation period was 2.33%, and the peel fruit firmness was 10.8%, an aspect that provides quality commercial to the product, making it suitable for human consumption and agro-industrial transformation.

According to the nutritional composition evaluated, pumpkin fruit adapted to the climatic conditions of the Dabeiba-Colombia region is considered a good source of total carotenoids and potassium in all of their fractions, and they have low concentrations of carbohydrates and fat in the pulp.

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Postharvest characterization of seven arracacha cultivars (*Arracacia xanthorrhiza* Bancroft)

UNIVERSIDAD NACIONAL DE COLOMBIA

Caracterización poscosecha de siete cultivares de arracacha (*Arracacia xanthorrhiza* Bancroft)

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ABSTRACT

Keywords:
Firmness
Respiratory rate
Starch
Storage
Tuberous roots

Arracacha is used by farming families as a fundamental crop for food security because of its caloric content. In Colombia, there are diverse cultivars that have been scarcely studied. The postharvest quality and starch content of seven arracacha cultivars were characterized. A completely randomized design was used with seven treatments, consisting of the cultivars 'yema de huevo', 'paliverde', 'palirrusia', 'yucatana', 'blanca de tarro', 'palinegra' and 'amarilla de tarro'. The results showed that the pH, the total soluble solids, and the maturity ratio increased for all cultivars except for 'palinegra' during the first 8 days after harvest. The total titratable acidity decreased for 'amarilla de tarro'; in the rest of cultivars, there was a slight increase over time. The respiratory rate and firmness increased in all cultivars until day 12, with higher values for 'palinegra'. Starch content, respiratory rate, and firmness decreased, while L* increased. The loss of mass had the highest values in the first 3 days of storage. The color index and the L* and b* parameters increased over time; therefore, increases in luminosity and yellow colorations were observed. 'Paliverde' showed the highest starch content, being the cultivar less suitable for industries.

RESUMEN

Palabras clave:
Firmeza
Intensidad respiratoria
Almidón
Almacenamiento
Raíces tuberosas

La arracacha está considerada dentro de un esquema de agricultura familiar como un cultivo fundamental para la seguridad alimentaria por su aporte energético. En Colombia existen una gran diversidad de cultivares que han sido poco estudiados. Por lo anterior, se caracterizó la calidad poscosecha y el contenido de almidón de siete cultivares de arracacha. Se empleó un diseño completamente al azar con siete tratamientos, conformados por los cultivares 'yema de huevo', 'paliverde', 'palirrusia', 'yucatana', 'blanca de tarro', 'palinegra' y 'amarilla de tarro'. Los resultados mostraron que el pH, los sólidos solubles totales, y la relación de madurez aumentaron para todos los cultivares excepto para el cultivar 'palinegra' durante los primeros ocho días después de la cosecha. La acidez total titulable disminuyó para 'amarilla de tarro' y en los demás cultivares se presentó un ligero aumento en el tiempo. La intensidad respiratoria y la firmeza aumentaron en todos los cultivares hasta el día 12, con mayores valores para 'palinegra'. El contenido de almidón, la intensidad respiratoria y la firmeza disminuyeron, mientras L* aumentó. La pérdida de masa presentó los mayores valores en los primeros 3 días de almacenamiento. El índice de color y los parámetros L* y b* aumentaron a través del tiempo, por lo que se apreciaron incrementos en la luminosidad y en las coloraciones amarillas. 'Paliverde' mostró los mayores contenidos de almidón, por lo que sería el cultivar menos apto para las industrias.

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In Colombia, arracacha has significant socioeconomic importance (Muñoz *et al*., 2015), and its production has increased 33.5% from 2013 to 2018, with 109,986 t with a participation of 15 departments (Agronet, 2020). An 83% of arracacha production in Colombia is concentrated in the departments of Tolima, Norte de Santander, and Boyacá, with a production of 67,586, 12,179, and 11,649 t, respectively (Agronet, 2020), which generate employment and stimulate family economies in the region (Muñoz *et al*., 2015).

The tuberous root is the most important part of arracacha since it is the commercial and edible portion of the plant (Rojas and Barreto, 2016). Arracacha can be grouped into three classes according to the color of the root epidermis: white, yellow, and purple (Muñoz *et al*., 2015). Arracacha is one of the most pleasant and nutritious native foods (Jiménez, 2005) because of its easy digestion of starches and high content of calcium, phosphorous, iron, niacin, vitamin A, ascorbic acid, proteins, fiber, and carbohydrates (Pacheco *et al*., 2020). In addition to the significant source of starch, it has ideal characteristics for agribusiness such as low gelatinization temperature, gelatinization enthalpy, a tendency to retrograde, high maximum apparent viscosity, and swelling capacity at moderate temperatures (60 °C). It has a soft and elastic gel, with high paste clarity (Castanha *et al*., 2018).

Physical, chemical, rheological, and sensory properties determine the quality of the roots and are used for selecting transformation techniques for fried chips, frozen foods, snacks, and other products (Vitti *et al*., 2003). Therefore, characterizing the properties of different arracacha cultivars during the post-harvest period is necessary to facilitate the agro-industrial implementation of these species, take advantage of their genetic diversity and encourage the productive sector to plant the cultivars that have the greatest potential for commercialization, fresh consumption, and/or agro-industrial transformation.

The limited research has hampered the implementation of good management practices in the production and postharvest process, which may have contributed to the incursion of these species in the agro-industrial sector and a decrease of constant losses of fresh products, as well as the characteristics of this cultivar. The postharvest period has not yet been evaluated and characterized for this plant; therefore, this research aimed to characterize the physical-chemical properties in the postharvest period of seven arracacha cultivars in order to provide a tool for farmers to select cultivars based on market needs.

MATERIALS AND METHODS

This research was carried out at the Plant Physiology laboratory of the Universidad Pedagógica y Tecnológica de Colombia (Boyacá, Colombia)". The arracacha roots used in the research were harvested in the municipalities of Ramiriquí, Boyacá, Turmequé, and Nuevo Colón, in the department of Boyacá.

At the harvesting stage, roots with a complete peel that was perfectly adhered to the pulp, with a diameter greater than 4 cm, whose shape was elongated, conical, and without secondary or deformed roots, were selected. The roots that presented damage, mechanical and/or attack of pests and diseases, were discarded. The roots were classified by cultivar, according to the size of the strain, the number of reserving roots, the predominant color of the root, and the presence of a purple ring (Alvarado and Ochoa, 2010). After classification, the material was stored in 20×20×7 cm extruded polystyrene foam containers.

A completely randomized design was used with 7 treatments that corresponded to different arracacha cultivars: 'yema de huevo' (YH), 'paliverde' (PV), 'palirrusia' (PR), 'yucatana' (YTN), 'blanca de tarro' (BT), 'palinegra' (PN) and 'amarilla de tarro' (AT). Each treatment had 4 replications, for a total of 28 experiment units (EU); each EU had 18 roots, harvested between 10 and 12 months after planting.

The response variables were measured every three days. The mass loss (ML) was determined using an Adam® PGW2502e 0.01 g precision electronic balance (Adam Equipment Inc, Oxford). The total soluble solids (TSS) were measured by a Hanna HI 96803 refractometer with a scale from 0 to 85% (Hanna Instruments, Spain). The pH was measured in 25 mL of arracacha juice with a Metrohm 744 digital potentiometer (Metrohm AG, Switzerland). The total titratable acidity

(TTA) was quantified following the methodology used by Rozo-Romero *et al*. (2015). The maturity ratio (MR) was expressed as the TSS/TTA ratio. The color index (CI) was calculated with Equation 1 by CIELab parameters L*, a*, and b*, which were measured using a Konica Minolta CR-20 colorimeter (Konica Minolta, Japan). Two readings were taken for each root at two equidistant points.

$$CI = \frac{1000a^*}{L^* b^*} \quad (1)$$

The firmness was determined by a PCE-FM 200 digital penetrometer (PCE Ibérica SL, Albacete, Spain) by averaging two measurements in the equatorial root zone with a 6 mm tip. The respiratory rate (RR) was calculated by placing the roots in sealed 2 L SEE BC-2000 chambers (Vernier Software & Technology, OR, USA) connected to a VER CO2-BTA infrared sensor (Vernier Software & Technology, OR, USA) and a Labquest2 interface (Vernier Software & Technology, OR, USA) for 5 min, expressed in mg of CO_2 kg^{-1} h^{-1}. The starch percentage was determined according to Hernández-Medina *et al*. (2008) methodology adapted for arracacha.

A cross-sectional and longitudinal analysis of variance (ANOVA) was carried out to establish the possible statistical differences between the treatments and between times. Afterward, the Shapiro-Wilk normality test and the Levene variance homogeneity test were used for the residuals. The averages were compared with the Tukey test ($P \leq 0.05$), and a multiple linear correlation was done between the response variables. SAS® v. 9.2e was used (SAS Institute Inc., Cary, NC, USA). A cluster analysis was performed by Ward's method of minimum variance to determine the level of similarity of the postharvest physical-chemical characteristics between the evaluated plant cultivars.

RESULTS AND DISCUSSION

pH

This parameter showed significant differences between treatments in each measurement, but not between times, with values ranging between 5.2 and 6.9 (Table 1). The pH tended to increase during the first 8 days after harvest (dah) and then decreased towards 12 dah. The AT cultivar had the highest values (6.8), followed by the PN, PV, and YH cultivars with values of 6.47, while the lowest values were obtained for YTN, PR, and BT with average values of 5.69 at 8 dah. The BT cultivar often showed the lowest pH values when compared to the other cultivars evaluated.

The AT cultivar had a higher pH than BT. This latter had a longer postharvest life (17 dah), similar to what was observed by Vargas *et al*. (2017) who stated that a higher pH value indicates faster maturation and shorter shelf life. On the other hand, García and Pacheco (2008) found a pH of 6.60 and 6.65 for 'yellow' and 'white' cultivars, respectively, while Carmo and Leonel (2012) obtained values of 6.57 for the 'yellow' cultivar.

Total soluble solids (TSS)

The TSS ranged from 3.25 to 13 °Brix. There were statistical differences in the TSS values between the cultivars and times (Figure 1A and B). All cultivars showed an increase in TSS up to 8 dah and a subsequent decrease until 12 dah. The AT cultivar showed the highest TSS value for all samples (11.4 °Brix), followed by the PN cultivar (9.2 °Brix). The lowest TSS value was recorded for the PR, YTN, and BT cultivars, with a value of 6.5 °Brix at 8 dah.

Arracacha roots have 11% sucrose, 3.5% fructose, and 3.7% glucose (Pacheco *et al*., 2020), which once synthesized, are degraded to sucrose in the cytosol of the leaves and finally exported to tissues such as roots. This may explain the initial TSS content, with subsequent degradation of starch to glucose and a corresponding increase in TSS that occurs in the postharvest period (Yahia *et al*., 2019). A subsequent decrease in TSS, before the loss of commercial quality, may be related to changes in the RR and, therefore, in the oxidative degradation of sugars (Alós *et al*., 2019), accompanied by tissue plasmolysis (Moreno *et al*., 2013). Furthermore, high consumption of substrates during respiration could explain a rapid decrease in TSS (Saltveit, 2019). Barrera *et al.* (2004) found the TSS ranged from 6.1 to 9.60 in arracacha, while Buso *et al.* (2014) obtained values between 5.5 and 10.8 in arracacha coated with chitosan.

Total titratable acidity (TTA)

Statistical differences were seen in the TTA for the arracacha cultivars evaluated in each measurement,

except at 8 dah. The TTA was ranged from 0.07 to 0.25%. This latter was observed in the AT cultivar (Table 1), which had the highest value with an average of 0.18%, followed by the BT cultivar with 0.14%. The lowest value was registered in the PN, YH, and PV cultivars with 0.10% at 10 dah. The TTA did not present statistically significant differences between the times for the seven arracacha cultivars. Nevertheless, a slight increase was observed up to 8 and 10 dah. The AT cultivar decreased in all measurements and 56% less TTA at the end of postharvest was recorded. A decrease in TTA is mainly attributed to the use of organic acids as respiratory substrates (Vallarino and Osorio, 2019) or the conversion to sugars through gluconeogenesis (Alós *et al*., 2019). Ruiz (2011) determined that the TTA in arracacha was 0.74%, which was higher than the highest value found in all seven arracacha cultivars in this study (0.25%).

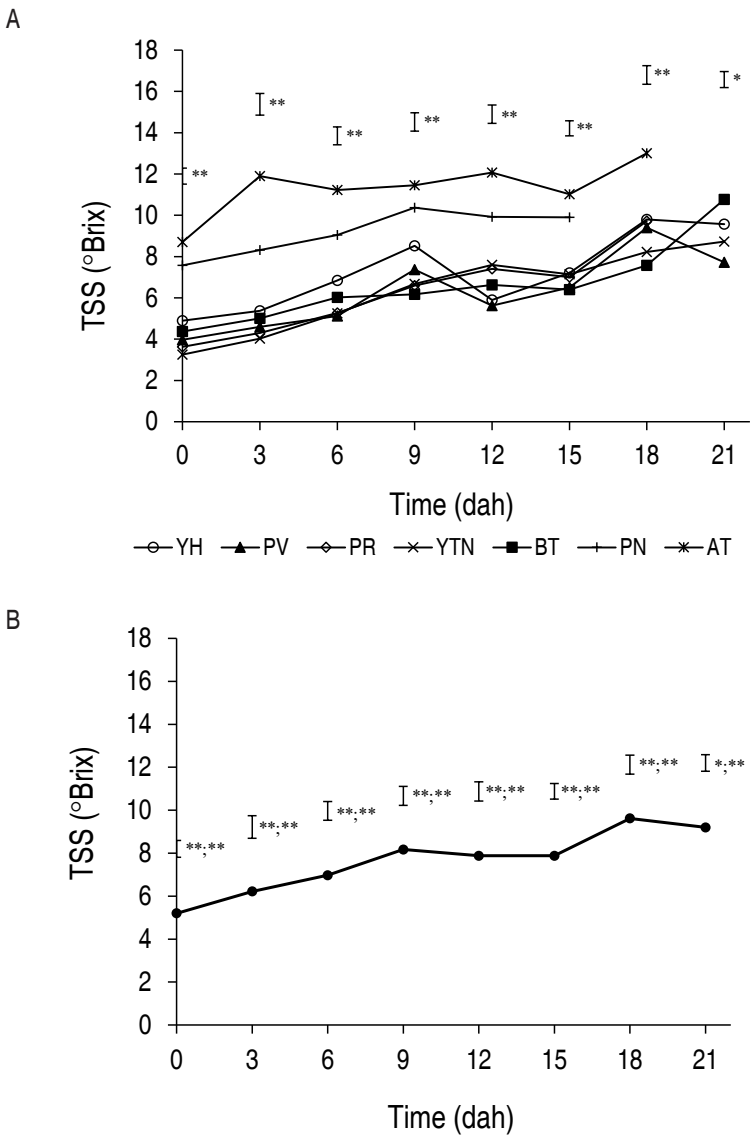

Figure 1. TSS A. in the different arracacha cultivars. B. average. YH: yema de huevo. PR: palirrusia. BT: blanca de tarro. AT: amarilla de tarro. PV: paliverde. YTN: yucatana. Vertical bars indicate the standard error (n=28). * and ** indicate significant effect according to the Tukey test for $P \leq 0.05$ and $P \leq 0.01$, respectively, between treatments before the semicolon and between times after the semicolon.

Table 1. pH, TTA, and MR parameters evaluated on roots of different arracacha cultivars during the postharvest period.

		Treatments						
Parameter	**dah**	**YH**	**PV**	**PR**	**YTN**	**BT**	**PN**	**AT**
pH	0	5.5±0.16^{B,d}	6.19±0.11^{B,b}	5.38±0.07^{B,c}	5.39±0.14^{B,a}	5.33±0.05^{B,c}	6.45±0.26^{AB,a}	6.65±0.1^{A,a}
	3	5.74±0.2^{B,d}	6.41±0.08^{B,b}	5.57±0.04^{B,bc}	5.59±0.14^{B,a}	5.54±0.05^{B,c}	6.26±0.21^{B,a}	6.85±0.06^{A,a}
	6	6.41±0.08^{B,abc}	6.18±0.06^{B,b}	5.58±0.07^{B,bc}	5.86±0.06^{B,a}	5.62±0.004^{B,c}	6.14±0.21^{B,a}	6.92±0.07^{A,a}
	9	6.54±0.06^{B,abc}	6.47±0.02^{B,b}	5.66±0.09^{C,bc}	5.89±0.05^{C,a}	5.52±0.05^{C,c}	6.4±0.15^{B,a}	6.94±0.02^{A,a}
	12	6.11±0.08^{BCD,cd}	6.4±0.07^{B,b}	5.86±0.15^{CD,ab}	5.62±0.08^{D,a}	5.63±0.1^{D,c}	6.35±0.19^{BC,a}	6.79±0.02^{A,a}
	15	6.11±0.11^{BC,bcd}	5.99±0.07^{BC,b}	5.59±0.18^{C,bc}	5.53±0.06^{C,a}	5.22±0.11^{C,c}	6.34±0.25^{D,a}	6.8±0.02^{A,a}
	18	6.23±0.12^{B,bcd}	6.39±0.06^{AB,b}	6.07±0.03^{B,a}	6.28±0.2^{B,a}	5.89±0.05^{B,b}	---	6.72±0.1^{A,a}
	21	6.48±0.1^{B,ab}	6.93±0.04^{A,a}	---	5.87±0.11^{C,a}	6.09±0.02^{BC,a}	---	---
TTA (%)	0	0.07±0.004^{B,a}	0.08±0.007^{B,a}	0.11±0.017^{B,a}	0.08±0.008^{B,c}	0.11±0.009^{B,d}	0.11±0.011^{B,a}	0.23±0.011^{A,ab}
	3	0.09±0.017^{B,a}	0.08±0.001^{B,a}	0.12±0.017^{B,a}	0.1±0.005^{B,bc}	0.12±0.009^{B,cd}	0.12±0.012^{B,a}	0.25±0.019^{A,a}
	6	0.07±0.018^{B,a}	0.08±0.005^{B,a}	0.13±0.02^{AB,a}	0.12±0.007^{AB,abc}	0.14±0.014^{AB,bcd}	0.14±0.027^{AB,a}	0.21±0.04^{A,abc}
	9	0.14±0.008^{A,a}	0.11±0.011^{A,a}	0.16±0.04^{A,a}	0.12±0.021^{A,abc}	0.14±0.007^{A,bcd}	0.09±0.007^{A,a}	0.14±0.016^{A,bc}
	12	0.1±0.012^{A,a}	0.1±0.027^{A,a}	0.1±0.015^{A,a}	0.2±0.048^{A,a}	0.14±0.016^{A,bcd}	0.11±0.008^{A,a}	0.16±0.013^{A,abc}
	15	0.1±0.015^{B,a}	0.12±0.014^{AB,a}	0.16±0.027^{AB,a}	0.15±0.007^{AB,abc}	0.17±0.009^{A,ab}	0.12±0.018^{AB,a}	0.14±0.011^{AB,c}
	18	0.13±0.026^{AB,a}	0.12±0.009^{AB,a}	0.09±0.009^{B,a}	0.15±0.009^{AB,abc}	0.16±0.008^{A,bc}	---	0.14±0.009^{AB,bc}
	21	0.1±0.017^{B,a}	0.07±0.008^{B,a}	---	0.18±0.019^{A,ab}	0.21±0.003^{A,a}	---	---
MR	0	67.1±3.78^{AB,a}	49.38±4.69A^{BC,b}	33.84±5.86^{C,b}	41.71±6.27^{BC,a}	41.15±4.47^{BC,a}	70.57±10.1^{A,a}	37.68±3^{C,b}
	3	57.1±9.13^{AB,a}	54.41±3.41^{AB,b}	35.45±6.86^{B,b}	39.46±2.99^{B,a}	40.15±1.48^{B,a}	66.85±7.75^{A,a}	47.98±6.02^{AB,ab}
	6	93.81±25.74^{A,a}	60.9±3.45^{AB,b}	40.86±12.15A^{B,b}	43.86±4.5A^{B,a}	42.32±3.55^{B,a}	64.54±18.81^{AB,a}	52.63±18.37^{AB,ab}
	9	61.99±7.43^{B,a}	66.9±8.88^{B,ab}	40.39±6.04^{B,b}	57.04±21.91^{B,a}	44.63±1.94^{B,a}	120.34±8.01^{A,a}	81.59±6.83^{AB,a}
	12	58.48±9.25A^{B,a}	53.86±20.15^{AB,b}	71.76±14.54^{AB,ab}	37.29±6.78^{B,a}	48.32±5.22^{AB,a}	92.71±8.12^{A,a}	75.2±5.45^{AB,ab}
	15	74.15±12.91A^{B,a}	55.88±8.21^{AB,b}	42.66±11.82^{AB,b}	46.22±3.33^{AB,a}	37.18±2.85^{B,a}	81.89±13.33^{A,a}	80.88±11.46^{AB,a}
	18	76±11.64^{ABC,a}	79.88±8.67A^{BC,ab}	105.86±13.03^{A,a}	56.64±4.3^{BC,a}	48.23±4.12^{C,a}	---	91.86±8.17^{AB,a}
	21	91.74±8.04^{A,a}	112.82±11.94^{A,a}	---	48.64±7.99^{B,a}	52.21±1.54^{B,a}	---	---

Means of 4 replicates ± standard error. YH: yema de huevo, PV: paliverde, PR: palirrusia, YTN: yucatana, BT: blanca de tarro, PN: palinegra and AT: amarilla de tarro. TTA: total titratable acidity, MR: maturity ratio. Mean values in the rows with different uppercase letters have statistically significant differences ($P \leq 0.05$) between treatments. Mean values in the columns with different lowercase letters have statistically significant differences ($P \leq 0.05$) between times. ---: loss of consumer quality.

Maturity ratio (MR)

The MR increased in the final measurements in all arracacha cultivars. Statistical differences between cultivars were observed for all measurements, except at 10 dah (Table 1). At 10 and 12 dah, there was a decrease in the MR. Moreover, there were statistical differences for the MR over time. The PN and AT cultivars showed higher MR values (86.5 and 76.8, respectively). The YTN and BT cultivars had lower MR values with averages of 45.2 and 43.3, respectively.

In tuberous roots, an increase in the MR means a better flavor because of an increase in TSS (R^2=0.4857), which indicates better organoleptic characteristics (Figueiredo *et al*., 2011). Once tuberous roots are harvested, the formation of α-amylase enzymes begins, which together with β-amylases degrade starch, generating sweet flavors in the cultivars (Alós *et al*., 2019). García and Pacheco (2008), based on a quantification of TSS and TTA in arracacha, found average values of MR for white and yellow morphotypes of 84.78 and 83.72, respectively, like those found for PN and YH.

Firmness

The firmness showed significant statistical differences between cultivars and times, except at 10 dah. The initial values ranged from 9.1 to 16.7 N and subsequently, they increased (Table 2). The firmness decreased in the arracacha cultivars at 8, 10, and 12 dah. The PR cultivar displayed increased firmness over time from 12.4 to 44.9 N, which was the highest one, followed by the PV and YTN cultivars, with an increase from 10.1 to 35.3 N and 11.1 to 33.98 N, respectively.

Table 2. Firmness and respiratory rate of different arracacha cultivars during the postharvest period.

Parameter	dah	Treatments						
		YH	PV	PR	YTN	BT	PN	AT
Firmness (N)	0	9.12±0.21^{C,c}	10.11±0.27^{C,e}	12.46±0.37^{BC,e}	11.18±0.12^{C,e}	11.13±0.34^{C,f}	16.71±0.66^{A,c}	15.73±1.71^{AB,a}
	3	10.78±0.67^{C,c}	14.15±1.12^{BC,de}	15.73±1.34^{BC,de}	16.53±1.17^{BC,de}	16.58±0.61^{BC,e}	23.53±1.17^{AB,b}	17.3±2.61^{A,a}
	6	12.43±1.33^{B,c}	18.58±2.27^{AB,cde}	19.06±2.21^{AB,cde}	22.58±2.05^{A,cde}	22.08±1.1^{A,d}	25.32±1.23^{A,ab}	19.65±2.84^{AB,a}
	9	24.73±3.57^{AB,ab}	29.08±1.59^{A,ab}	31.28±1.39^{A,d}	27.68±2.52^{AB,bc}	22.55±0.78^{AB,cd}	29.2±1.26^{A,a}	18.38±1.88^{B,a}
	12	23.8±2.34^{A,ab}	24.53±1.08^{A,bc}	27.18±1.93^{A,bc}	29.7±1.11^{A,bc}	26.46±1.46^{A,dc}	25.2±1.01^{A,ab}	24.7±1.36^{A,a}
	15	30.25±2.5^{BC,ab}	35.33±2.39^{AB,a}	44.95±3.4^{A,a}	33.98±1.44^{B,ab}	30.33±0.88^{BC,ab}	21.3±1.83^{C,bc}	20.87±3.04^{C,a}
	18	23.75±0.74^{A,ab}	26.73±1.81^{A,abc}	24.28±1.51^{A,bcd}	24.9±1.5^{A,c}	23.38±0.41^{A,cd}	---	22.03±1.72^{A,a}
	21	18.35±1.99^{B,bc}	22.55±3.36^{B,bcd}	---	38±1.78^{A,a}	33.25±1.03^{A,a}	---	---
RR ($mg.CO_2.kg^{-1}.h^{-1}$)	0	16.85±2.3^{A,ab}	17.44±1.21^{A,a}	18.83±1.14^{A,ab}	17.52±3.28^{A,abc}	18.75±1.63^{A,a}	19.09±3.32^{A,a}	15.22±3.81^{A,b}
	3	17.42±0.47^{BC,ab}	16.56±1.36^{C,a}	19.31±1.77^{BC,ab}	14.16±2.12^{C,abc}	18.35±0.51^{BC,a}	25.4±3.71^{B,a}	34.49±0.72^{A,a}
	6	18.12±2.23^{AB,ab}	15.75±3.5^{AB,a}	20.73±3.6^{AB,ab}	10.16±1.4^{B,c}	17.41±1.42^{AB,a}	26.58±2.74^{A,a}	25.05±3.67^{A,ab}
	9	26.83±3.34^{A,a}	26.78±5.52^{A,a}	25.45±3.06^{A,ab}	15.71±2.38^{A,abc}	17.22±0.84^{A,a}	27.06±4.85^{A,a}	28.96±4.58^{A,ab}
	12	16.64±1.44^{B,b}	15.29±2.04^{B,a}	31.44±4.51^{AB,a}	20.55±0.63^{AB,ab}	19.09±1.15^{AB,a}	36.13±7.34^{A,a}	26.25±4.92^{AB,ab}
	15	16.32±1.82^{B,b}	15.83±0.48^{B,a}	30.66±2.46^{A,ab}	17.72±2.22^{B,abc}	15.34±3.46^{B,a}	21.58±3.92^{AB,a}	22.95±1.99^{AB,ab}
	18	19.95±2.34^{ABC,ab}	16.16±1.63^{BC,a}	18.14±1.34^{ABC,b}	23.1±1.93^{AB,a}	14.62±0.15^{C,a}	---	23.6±1.25^{A,ab}
	21	15.75±2.14^{A,b}	16.77±2.83^{A,a}	---	13.62±0.73^{A,bc}	20.81±1.58^{A,a}	---	---

Means of 4 replicates ± standard error. YH: yema de huevo, PV: paliverde, PR: palirrusia, YTN: yucatana, BT: blanca de tarro, PN: palinegra and AT: amarilla de tarro. RR: respiratory rate. Mean values in the rows with different uppercase letters are significantly different ($P \leq 0.05$) between treatments. Mean values in the columns with different lowercase letters are significantly different ($P \leq 0.05$) between times. ---: loss of consumer quality.

An increase in firmness occurs because of the formation of hard consistencies related to the synthesis of lignin from soluble carbohydrates (Yahia *et al*., 2019), similar results were reported by Rainoso (2010) who found that lignin increased from 1.54 to 2.38% in the first 9 days postharvest in arracacha, while a decrease in firmness is probably due to the degradation of pectins as a result of the increased activity of polygalacturonase, generating softness in plant material (Nunes, 2010), comparable with the results obtained for the AT and PN cultivars at 8 dah and the other cultivars at 10 and 12 dah. Ruiz (2011) found that the firmness of arracacha was 37.16 N, like PR, PV, and YTN.

Starch

The starch content presented statistical differences between cultivars and times from the first day of storage and up to 15 dah. The initial starch values ranged from 12.5 to 23.8% (Figure 2). The starch content tended to decrease until 10 and 12 dah, after that there was a slight increase in some evaluated cultivars. PV cultivar had the highest starch content (16.7%), followed by PR and YTN cultivars (13.5%) at 10 dah, while the lowest values were obtained for PN and AT with 11.2%. Starch contents of 19.53 and 11.1% have been found in arracacha by Souza (2013) and Alayo (2015), respectively, similar to those seen for AT.

The increase in starch contents at 10 dah can be attributed to the fact that, at the last postharvest stage, the loss of water from the cultivar is high and the concentration of starch rises despite the amount

is lower than at the beginning of the postharvest stage, known as the dilution effect; another less probable theory for the increase in starch in the postharvest stage involves the reconversion of sugars into starch, promoted by the enzyme starch synthase (Yahia *et al*., 2019).

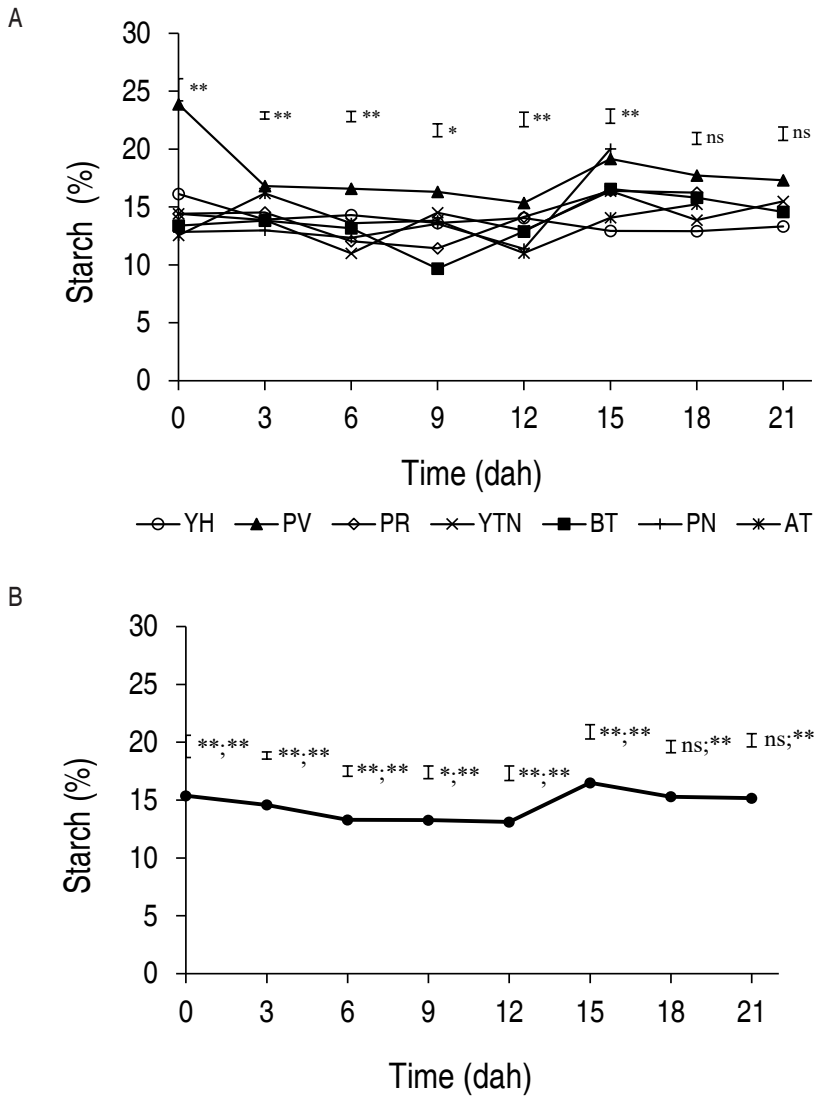

Figure 2. Starch A. in the different arracacha cultivars. B. average. YH: yema de huevo. PR: palirrusia. BT: blanca de tarro. AT: amarilla de tarro. PV: paliverde. YTN: yucatana. Vertical bars indicate the standard error (n=28). * and ** indicates significant effect according to the Tukey test for $P \leq 0.05$ and $P \leq 0.01$, respectively, between treatments before the semicolon and between times after the semicolon.

Respiratory rate (RR)

Statistical differences between the cultivars and times were observed (3, 5, 10, 12, and 15 dah). Initially, the RR ranged between 15.2 and 19.09 mg of CO_2 kg^{-1} h^{-1}, then had a peak at 8 and 10 dah, and subsequently decreased (Table 2). The highest RR was observed for PN at 10 dah (36.13 mg of CO_2 kg^{-1} h^{-1}), followed by AT and PR cultivars (25.87 and 24.98 mg of CO_2 kg^{-1} h^{-1}, respectively).

An increase in RR is correlated with an increase in the activity of ethylene (Iqbal *et al*., 2017), caused by the stress suffered by roots when are extracted from the plant

at the harvest stage since this increases energy demand (Munné-Bosch *et al.,* 2018) and the starch and sugar reserves are used in metabolic processes such as tissue softening, pigmentation and volatile synthesis (Saltveit, 2019), which explains the dynamics of the PN, AT and PR cultivars with a higher RR and shorter postharvest life. Henz *et al.* (2005) found that, in undamaged arracacha roots, the RR was 15.3 and 3.8 mg of CO_2 kg^{-1} h^{-1} at room temperature and refrigeration, respectively. Similarly, Rainoso (2010) stated that the RR of arracacha roots increases until the fifth and seventh day and subsequently stabilizes and/or begins to decrease. Nunes *et al.* (2010) found that RR in arracacha ranged from 8.48 to 44.25 mg of CO_2 kg^{-1} h^{-1}, similar to the values for PN and AT of this study.

Mass loss (ML)

Statistically significant differences between times and treatments were observed for all measurements. ML increased significantly up to 3 dah in the BT, PR, and YTN cultivars (Figure 3A and B), similar to that was observed in *Daucus carota* (Araujo *et al*., 2004). ML in all seven cultivars for the measurements at 15 and 17 dah was about 66%. Cultivars with higher ML values were BT (81.7%) and PR (75.7%), followed by PV and YTN cultivars with 66.1%. Lower ML values were for AT and PN with 41.6 and 46.3%, respectively.

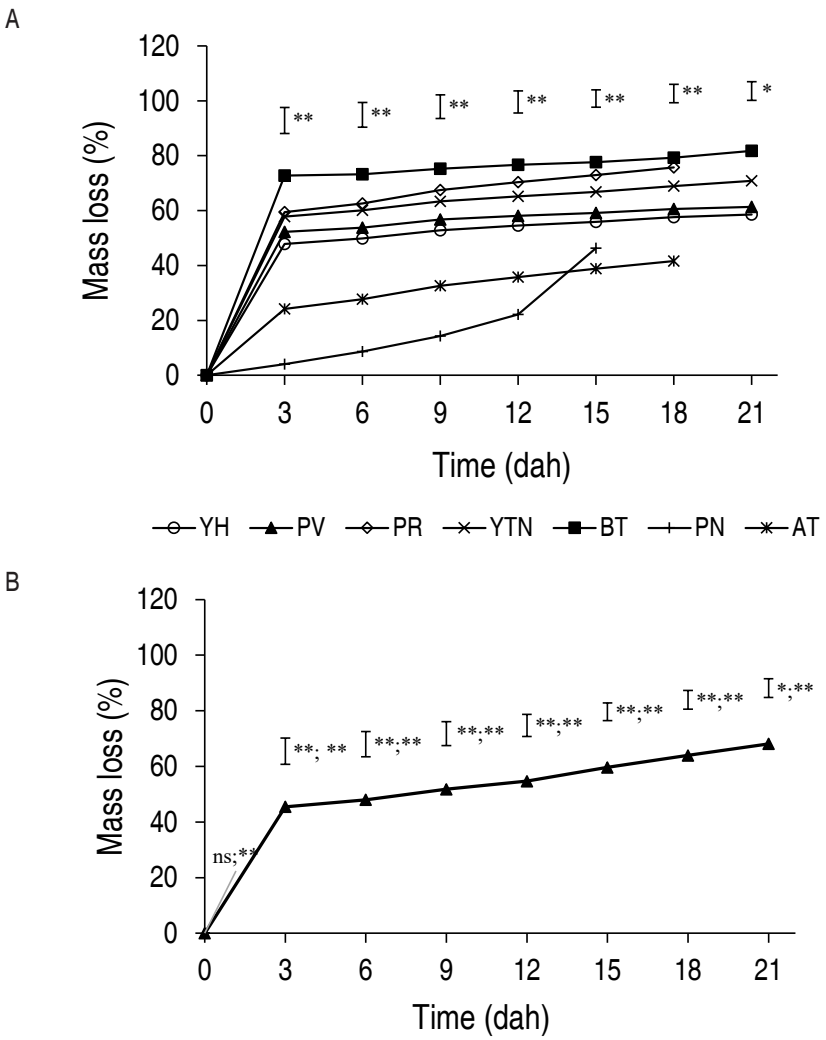

Figure 3. Mass loss A. in the different arracacha cultivars, B. average. YH: yema de huevo. PR: palirrusia. BT: blanca de tarro. AT: amarilla de tarro. PV: paliverde. YTN: yucatana. Vertical bars indicate the standard error (n=28). * and ** indicates significant effect according to the Tukey test for $P \leq 0.05$ and $P \leq 0.01$, respectively, between treatments before the semicolon and between times after the semicolon.

The highest firmness values and the lowest the ML are in arracacha (R^2=0.5018 from correlation analysis), possibly because these roots have more efficient structures that prevent dehydration. According to Link *et al.* (2004), weak cell walls and membranes allow water to escape through respiration at a higher speed, which depends on the pressure of the water vapor, the evaporation surface, and the resistance to the diffusivity of the product to the environment. A water loss of 7% is the maximum commercially allowed for roots (Díaz-Pérez, 2019).

CIEL*a*b* space

Parameter L*, statistical differences between cultivars and times were observed (except at 18 and 21 dah). It was observed that L* tended to increase in all cultivars up to 15 dah and then decreased, except in AT, which presented a decrease of 21.5% (Table 3). L* increased for PV and YTN cultivars by 14 and 12%, respectively, followed by BT (9.7%), YH (9.3%), PN (5.10%), and PR (4.99%).

The cultivars presented lighter colorations over time, except for AT cultivar, in which the coloration darkened during storage. An increase in luminosity is due to the loss of water (Vitti *et al*., 2003) and also the increase in yellow shades (Jha *et al*., 2006), which is correlated with the increase of b* in arracacha roots (R^2=0.6196). Carmo and Leonel (2012) found that in 'yellow' cultivar, L* ranged from 70.6 to 76.5, while García and Pacheco (2008) found values of 73.14 and 77.89 for 'white' and 'yellow' cultivars, similar to what was observed in BT, YTN, and PR.

Parameter a*, statistically significant differences were observed between the cultivars for a* value, except at 12 and 15 dah. The initial values ranged between 5.9 and 10.03, and at the end of the period were between 8.15 and 10.5 (Table 3). There were significant differences over time, the a* values had a slightly increasing trend after 8 dah. Besides, this parameter presented positive values, which indicated the absence of green colorations. Salas (2018) stated that β-carotene is the predominant pigment in arracacha and that, at the postharvest stage, it can increase its concentration as a result of the dilution effect that is generated by the loss of water from the material. On the other hand, Carmo and Leonel (2012) found a* values that ranged from 0.27 to 3.71 for "yellow" arracachas, lower values than those found in this research.

Parameter b*, statistically significant differences between the cultivars and times were observed for all measurements, except at 18 dah. The initial and final b* values ranged from 11.43 to 26.03 and from 18.95 to 26.8, respectively (Table 3). b* value decreased until 5 and 8 dah and then increased, except for AT and PN cultivars, which decreased constantly. At the postharvest, PV and BT presented increases of 39.7% and 28.9%, respectively, followed by the YTN cultivar (21.7%), YH (19%), and PR (7.9%). b* had positive values in all cultivars, indicating the absence of blue tones and a predominance of yellow ones; the latter is associated with the synthesis of carotenoids that increase during the first 15 dah. b* values have been found to range from 34.3 to 42.6 (Carmo and Leonel, 2012), higher values than the 'white' materials (21.91) evaluated by García and Pacheco (2008).

Color index (CI)

The CI had statistically significant differences between the cultivars, but not between times. The initial values ranged between 3.73 and 15.34, and the final values were between 5.18 and 9.22 (Table 3). After 8 dah, the CI remained constant. The YH and PV cultivars presented the highest CI with 15.46 and 21.31, respectively, while BT showed the lowest CI with 3.13. Differences in the CI can be attributed to different levels of carotenoids, phenolic compounds, and enzymatic activity of polyphenol oxidase in each cultivar (Enríquez *et al*., 2020), while a decrease in CI probably occurs as a result of the instability of the carotenoids, as they are easily degraded thanks to the oxidative processes, light, and pH (Meléndez *et al*., 2004).

Cluster analysis

According to the cluster analysis, the cultivars were classified into two large groups (Figure 4). The first group includes the PN and AT cultivars, which had similar characteristics, pH and TSS values were higher than those of the second group, which is formed by the YH, PV, PR, YTN, and BT cultivars. A subgroup was created for the YTN and BT cultivars, which had similar firmness values (11.17 and 11.12 N, respectively), and showed a difference with the rest of the cultivars. Within the second group, the YH cultivar had the highest RR.

Table 3. Color parameters evaluated on roots of different arracacha cultivars during the postharvest period.

Parameter	dah	Treatments						
		YH	PV	PR	YTN	BT	PN	AT
L*	0	65.58±1.07^{A,a}	51.63±3.31^{BC,ab}	49.63±3.38^{BC,a}	57.08±1.75^{ABC,ab}	60.3±1.61^{AB,ab}	49.05±2.86^{C,a}	58.18±1.54^{ABC,a}
	3	67.68±1.28^{A,a}	56.28±5.64^{ABC,ab}	50.73±2.48^{BC,a}	58±1.68^{ABC,ab}	59.89±1.2^{AB,abc}	47.3±2.09^{C,a}	55.8±1.35^{ABC,ab}
	6	53.65±1.59^{A,b}	47.63±2.15^{AB,b}	43.15±2.19^{B,a}	49.93±2.51^{AB,b}	53.1±0.87^{A,bc}	53.35±0.54^{A,a}	55.45±2.28^{A,ab}
	9	68.63±1.33^{A,a}	57.9±5.19^{AB,ab}	51.3±1.97^{B,a}	59.43±2.01^{AB,ab}	59.75±1.39^{AB,abc}	51.45±1.2^{B,a}	51.73±0.73^{B,abc}
	12	69.93±2.36^{A,a}	58.9±3.03^{B,a}	47.45±3.33^{C,a}	55.78±2.67^{BC,ab}	52.73±1.06^{BC,c}	52.25±1.76^{BC,a}	52.18±1.67^{BC,ab}
	15	71.25±0.6^{A,a}	65.35±0.83^{AB,ab}	54.33±1.7^{C,a}	62.98±2.74^{B,a}	65.5±1.14^{AB,a}	51.55±0.9^{C,a}	51.53±0.99^{C,bc}
	18	72.45±2.48^{A,a}	61.65±2.31^{BC,ab}	52.1±3.42^{CD,a}	61.7±2.56B^{C,a}	64.23±1.97^{AB,a}	---	45.63±0.6^{D,c}
	21	71.65±1.7^{A,a}	59.2±2.26^{B,ab}	---	64.25±1.98^{AB,a}	66.15±2.66^{AB,a}	---	---
a*	0	5.9±0.55^{B,c}	9.05±0.85^{AB,b}	10.03±0.45^{A,ab}	7.63±0.88^{AB,a}	6.63±0.49^{AB,c}	9.35±1.26^{AB,a}	9.25±0.71^{AB,a}
	3	6.03±0.56^{B,c}	8.28±0.88^{AB,b}	9.68±0.4^{A,ab}	7.5±0.76^{AB,a}	7.15±0.36^{AB,c}	9.7±0.47^{A,a}	7.35±0.3^{AB,a}
	6	17.13±1.41^{A,a}	13.58±1.29^{AB,a}	5.43±0.7^{CD,c}	3.28±1.88^{D,b}	2.95±1.02^{D,d}	9±0.56B^{C,a}	8.6±0.33^{BC,a}
	9	6.63±0.61^{B,bc}	7.55±0.8^{AB,b}	8.08±0.51^{AB,b}	7.1±0.5^{AB,ab}	6.9±0.3^{B,c}	9.48±0.35^{A,a}	8.35±0.57^{AB,a}
	12	8.73±0.72^{A,bc}	10.53±0.87^{A,ab}	10.23±0.52^{A,ab}	8.83±0.47^{A,a}	8.53±0.37^{A,bc}	9.68±0.46^{A,a}	8.4±0.78^{A,a}
	15	8.78±0.79^{A,bc}	10.73±0.8^{A,ab}	10.83±0.23^{A,a}	9.55±0.8^{A,a}	11.01±0.3^{A,a}	8.88±0.26^{A,a}	8.63±0.7^{A,a}
	18	8.93±0.45^{AB,bc}	10.83±0.55^{A,ab}	10.5±0.37^{A,a}	9.53±0.59^{AB,a}	10.48±0.63^{A,ab}	---	8.15±0.21^{B,a}
	21	9.83±0.41^{A,b}	9.4±0.51^{A,b}	---	9.95±0.33^{A,a}	10.05±0.32^{A,ab}	---	---
b*	0	24.15±0.76^{A,cd}	11.43±1.56^{B,c}	18.85±1.55^{AB,ab}	19.85±0.27^{A,bc}	19.08±0.28^{AB,bc}	25.95±3.71^{A,a}	26.03±1.95^{A,a}
	3	24.78±0.35^{A,bcd}	24.73±1.23^{A,a}	18.55±1.2^{B,ab}	19.88±0.27^{AB,bc}	20.29±0.26^{AB,bc}	24.31±2.12^{A,a}	21.65±0.58^{AB,ab}
	6	20.65±0.69^{AB,d}	13.38±1.53^{C,bc}	14.3±1.4^{C,b}	16.55±0.53^{BC,c}	17.73±0.33^{ABC,c}	22.7±1.07^{A,a}	21.5±1.83^{AB,ab}
	9	23.78±0.55^{A,cd}	17.43±3.25^{BC,abc}	15.2±0.89^{C,b}	17.85±0.39^{ABC,c}	17.95±0.37^{ABC,c}	21.88±1.1^{AB,a}	22.37±0.3^{AB,ab}
	12	30.75±0.98^{A,a}	18.98±0.38^{C,abc}	20.6±0.73B^{C,a}	22.83±0.63^{B,ab}	21.85±0.17^{BC,b}	22.55±0.76^{BC,a}	20.2±1.34^{BC,ab}
	15	28.98±0.67^{A,abc}	21.2±0.36^{C,ab}	21.38±0.65^{C,a}	24.55±1.76^{DC,a}	26.93±0.14^{AB,a}	20.33±0.86^{C,a}	20.8±1.17^{C,ab}
	18	29.8±1.29^{A,ab}	18.18±1.83^{D,abc}	20.48±0.82^{BCD,a}	25.2±1.57^{ABC,a}	26.38±0.12^{AB,a}	---	19.38±1.9^{CD,b}
	21	26.48±2.42A,^{abc}	18.95±2.56^{A,abc}	---	25.35±0.68^{A,a}	26.83±1.71^{A,a}	---	---
CI	0	3.73±0.39^{B,b}	15.34±3.75^{A,ab}	10.72±0.96^{AB,a}	6.73±0.94^{B,a}	5.76±0.62^{B,ab}	7.35±1.77^{B,a}	6.11±0.2^{B,b}
	3	3.59±0.35^{C,b}	5.95±1.01^{BC,b}	10.28±0.76^{A,a}	6.51±0.74^{BC,a}	5.88±0.37^{BC,ab}	8.44±1.5^{AB,a}	6.08±0.31^{BC,b}
	6	15.46±1.73^{AB,a}	21.31±5.3^{A,a}	8.79±0.33^{BC,a}	3.96±2.5^{C,a}	3.13±1.15^{C,b}	7.43±0.28^{BC,a}	7.21±0.9^{BC,ab}
	9	4.06±0.37^{C,b}	7.48±1.72^{AB,b}	10.36±0.84^{A,a}	6.69±0.74^{ABC,a}	6.43±0.46^{BC,a}	8.42±0.18^{AB,a}	7.22±0.46^{ABC,ab}
	12	4.06±0.3^{C,b}	9.42±0.45^{AB,b}	10.46±0.95^{A,a}	6.93±0.27^{B,a}	7.4±0.42^{B,a}	8.21±0.53^{AB,a}	7.97±0.69^{D,ab}
	15	4.25±0.32^{C,b}	7.74±0.76^{AB,b}	9.32±0.37^{A,a}	6.18±0.4^{B,a}	6.24±0.3^{B,a}	8.47±0.29^{A,a}	8.05±0.2^{AB,ab}
	18	4.13±0.47^{B,b}	9.66±0.95^{A,b}	9.84±0.74^{A,a}	6.13±0.29^{B,a}	6.18±0.49^{B,a}	---	9.22±1.07^{A,a}
	21	5.18±0.91^{A,b}	8.38±1.93^{A,b}	---	6.11±0.48^{A,a}	5.66±0.74A,^{ab}	---	---

Means of 4 replicates ± standard error. YH: yema de huevo, PV: paliverde, PR: palirrusia, YTN: yucatana, BT: blanca de tarro, PN: palinegra and AT: amarilla de tarro. CI: color index. Mean values in the rows with different uppercase letters are significantly different ($P \leq 0.05$) between treatments. Mean values in the columns with different lowercase letters are significantly different ($P \leq 0.05$) between times. ---: loss of consumer quality.

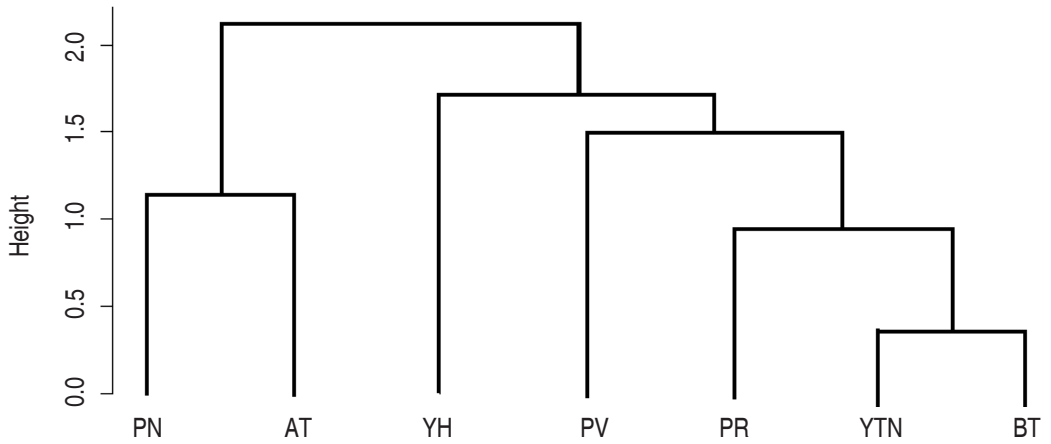

Figure 4. Dendrogram of arracacha cultivars. YH: yema de huevo, PV: paliverde, PR: palirrusia, YTN: yucatana, PN: palinegra and AT: amarilla de tarro.

CONCLUSION

The pH and TSS tended to increase in all cultivars, except for PN. The highest pH and TSS values were recorded in the AT, PN, and PV cultivars. The firmness of the arracacha roots increased at the postharvest stage up to 10 dah and the PN, AT and PR cultivars presented the highest value. The highest ML in the arracacha roots occurred at the first 3 dah. PV showed the highest starch content. After the postharvest stage, arracacha increases yellow tones and luminosity; the latter is inversely proportional to the starch content. In future studies, the application of maturity retardants is recommended in order to prolong the storage life.

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Short article

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Effect of *Lactobacillus acidophilus* added to a starch coating related to the microbiological contamination, quality and acceptability of fresh cheese

Efecto de *Lactobacillus acidophilus* añadido a un revestimiento de almidón en relación a la contaminación microbiológica, calidad y aceptabilidad de queso fresco

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ABSTRACT

The fresh cheese produced in the province of Manabí is an Ecuadorian artisan cheese. The processing conditions commonly do not comply Ecuadorian regulations, resulting in the presence of pathogenic microorganisms such as *Salmonella*. The high number of cases of Salmonellosis in the province of Manabí justifies the need to identify and control the possible sources of this pathogenic microorganism. In the present work, the effect of the addition of *Lactobacillus acidophilus* to fresh cheese was studied, by immersing it in a starch solution with $1x10^8$ CFU mL^{-1} of *L. acidophilus* with further storage for 30 days at 4 °C. The pH, acidity, weight loss, instrumental firmness, number of CFU of mesophilic aerobic bacteria and acceptability of fresh cheese were analyzed. At the same time, a duo-trio analysis was carried out, followed by a satisfaction analysis with the participation of semi-trained panelists. The presence of *L. acidophilus* reduced the pH and acidity in the fresh cheese in relation to the control sample. Satisfaction results, on a five-point hedonic scale, which ranged from 1 (I dislike it very much) to 5 (I like it very much), showed that the cheese treated with *L. acidophilus* and the control sample received a rating between "I neither like nor dislike" and "I like it moderately", with values of 3.63 for the treated sample and 3.50 for the control. The application of *L. acidophilus* did not affect the organoleptic acceptability of cheese and produced less changes in pH, acidity and weight loss during storage for 30 days at 4 °C in relation to the control sample.

Keywords:
Coated cheese
Inhibition
Salmonella
Satisfaction test
Sensory analysis

RESUMEN

El queso fresco producido en la provincia de Manabí es un queso artesanal ecuatoriano, cuyas condiciones de procesamiento comúnmente no cumplen con la normativa ecuatoriana, dando como resultado la presencia de microorganismos patógenos como la *Salmonella*. El alto número de casos de Salmonelosis en la provincia de Manabí justifica la necesidad de identificar y controlar las posibles fuentes de este microorganismo patógeno. En el presente trabajo se estudió el efecto de la adición de *Lactobacillus acidophilus* al queso fresco, mediante la inmersión de este en una solución de almidón con $1x10^8$ UFC mL^{-1} de *L. acidophilus* y su posterior almacenamiento por 30 días a 4 °C, durante los cuales se analizó el pH, acidez, pérdida de peso, firmeza instrumental, número de UFC de bacterias aerobias mesófilas y satisfacción del queso fresco. Al mismo tiempo se realizó un análisis dúo-trío y seguidamente uno de satisfacción con la participación de panelistas semientrenados. La presencia de *Lactobacillus acidophilus* redujo el pH y acidez en el queso fresco en relación con la muestra control. Los resultados de satisfacción, en una escala hedónica de cinco puntos, que varió desde 1 (me desagrada mucho) a 5 (me agrada mucho), mostraron que el queso tratado con *L. acidophilus* y la muestra control recibieron una calificación entre "ni me gusta ni me disgusta" y "me gusta moderadamente", con valores de 3.63 para la muestra tratada y 3.50 para el control. La aplicación de *L. acidophilus* no afectó el nivel de agrado de los panelistas por el queso fresco y produjo menores cambios de pH, acidez y pérdida de peso durante su almacenamiento por 30 días a 4 °C en relación a la muestra control.

Palabras clave:
Queso recubierto
Inhibición
Salmonella
Prueba de satisfacción
Análisis sensorial

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Fresh cheese produced in Manabí province is an Ecuadorian artisan cheese with a moisture of 37% (Minga and Pérez, 2019), with good acceptance among consumers, especially in the province of Manabí (Ecuadorian coast). Previous studies have shown that fresh cheese processing does not fulfill Ecuadorian regulations due to the presence of pathogenic microorganisms such as *Salmonella* (Zambrano, 2014). Additionally, during the commercialization the cold chain is affected in some points, since the cheese is exhibited at 25 °C, which increases the microbiological load affecting the health of consumers (Lobacz, 2020). Salmonellosis cases in the province of Manabí, Ecuador are around 600 annually (Ministerio de Salud Pública, 2015-2018). The presence of *Salmonella* and other pathogenic microorganisms has been detected in cheeses from Mexico (Plumb *et al*., 2019) and Egypt (El-Baz *et al*., 2017) among others.

Several authors have studied the control of pathogenic microorganisms in different varieties of cheese such as the control of *E. coli, S. enterica, B. cereus, S. scuiri* and *P. aeruginosa* using lactic acid bacteria (LAB) (Al-Gamal *et al*., 2019); the inoculation of *L. rhamnosus* in semi-hard goat cheese to inhibit the growth of pathogenic bacteria (Rodrigues *et al*., 2015) and the use of *L. acidophilus* to control the development of mesophilic aerobic bacteria and *Salmonella* in fresh cheese (Santacruz and Castro, 2018).

Coating is a layer that works as barrier against moisture, gases, loss of aroma and flavors of food. At the same time, it can act as carriers of antimicrobial microorganisms or substances (Guimarães *et al*., 2018). In fact, coatings have been used in cheese preservation, reducing water loss and spoilage damage (Costa *et al*., 2018). Moreover, the effect of coatings on several types of cheeses have been studied previously such as Gouda (Göksen *et al*., 2020); Port Salut (Ollé *et al*., 2014); Kashar (Kavas *et al*., 2015) among others.

The present work is a complementary study of a previous work where the growth of mesophilic aerobic bacteria and *Salmonella* in fresh cheese could be inhibited using *L. acidophilus.* The effect of a cassava starch + *L. acidophilus* as a coating on fresh cheese was studied, evaluating its acidity, pH, weight loss, firmness and counting of mesophilic aerobic bacteria during 30 days of storage at 4 °C. Additionally, the difference of satisfaction among panelists was evaluated for coated and uncoated cheeses.

MATERIALS Y METHODS

Strains of *L. acidophilus* were obtained from Chr. Hansen A/S (Denmark). The fresh cheese was purchased in stores located in the center of the city of Manta, Ecuador.

Preparation of coated cheese

The fresh cheese was coated with a starch + *L. acidophilus* (SL) film. A Cassava starch solution 1% (w/v) was prepared according to Santacruz *et al.* (2015), with slight modifications. The starch solution was heated with constant stirring from room temperature (approximately 25 °C) to 90 °C, keeping this temperature for 5 min. Afterwards, the mixture was homogenized with an ultraturrax (Polytron, Switzerland) at 11,000 rpm for 4 min. Free *L. acidophilus* was added to the starch solution guaranteeing a starch coating with an absorbance between 0.08 and 0.1, measured at 625 nm (Medina *et al*., 2005). This absorbance guaranteed an equivalent of $1x10^8$ CFU mL^{-1} of *L. acidophilus* of starch solution.

The fresh cheese was immersed in the previous solution for 5 min, dried at room temperature and finally transferred to plastic bags before storage for 30 days at 4 °C. Every day during storage, cheese was taken out from refrigerator and kept at room temperature for 8 h, then placed again into the refrigerator. This procedure allowed to simulate commercialization conditions.

Physical, chemical and microbiological analyses of fresh cheese

Cheese samples previously coated with a SL film were analyzed after 0, 10, 20 and 30 days of storage at 4 °C. Moisture content, titratable acidity, pH, and firmness of the cheese were examined. Moisture content was determined according to method 15.259 (AOAC, 1984), titratable acidity with method 16.276 (AOAC, 1984), pH was measured using a potentiometer (HANNA, Germany), firmness was measured as the maximum force (N) required to penetrate cheese cubes with a side length of 5 cm, using a texturometer (SHIMADZU EZ-XL, Japan). For the penetration test, a 2 mm diameter penetration probe was used at a speed of 20 mm s^{-1}, with a penetration

depth of 15 mm (Santacruz, 2021). The determination of mesophilic aerobic bacteria was carried out according to Castro *et al.,* (2014). Uncoated cheese was used as a control sample for all analyzes.

Sensory analysis

Sensory analyses were done at 0 day of storage only, with cheese containing no *Salmonella*. This avoided health problems to panelists due to the growth of mesophilic aerobic bacteria in cheese during storage. Presence of *Salmonella* was analyzed by the Ecuadorian regulation for microbiological food control (INEN, 2015). For this stage, eight semi-trained panelists were involved in both tests (UPAEP, 2014). The panelists were previously chosen based on their ability to identify basic flavors (Sharif *et al*., 2017). First, a duo-trio test was carried out in two sessions, which aimed to determine if there was a difference between the sample coated with SL and the control sample. In this case, the number of correct responses was determined for a probability level of 5%. Afterwards, a satisfaction test was performed using a 5-point verbal hedonic scale (Wadhwani and McMahon, 2012), with a scale ranging from "I like it a lot" (5) to "I dislike it a lot" (1).

Statistical analyses

Comparisons of control sample with coated cheese with a SL film were analyzed by means of an IBM SPSS Statistics software, using a one-way ANOVA (significance level of 5%). All measurements were made in triplicate.

RESULTS AND DISCUSSION

The presence of *L. acidophilus* in cheese caused a lower pH and acidity compared to the control sample after 30 days of storage. Similar results were obtained by Mozuriene *et al.* (2016). The lower presence of mesophilic aerobic bacteria due to the inhibitory action of *L. acidophilus* may trigger a lower production of acidic metabolites and thus a lower acidity. Additionally, the increase of *L. acidophilus* may be responsible of a higher production of organic acids that act as buffer, controlling the increase of pH during storage. Regarding the weight loss, coated cheese showed a lower loss compared to control sample. The coating may act as a barrier against moisture loss.

Results of microbiological analyzes showed that control cheese had a higher quantity of mesophilic aerobic bacteria than the sample coated with *L. acidophilus* (P<0.05, Table 1). Santacruz and Castro (2018) showed that viability of *L. acidophilus* decreased along storage, however the viable cells inhibited the development of mesophilic aerobic (Mozuriene *et al*., 2016) and *Salmonella*. Coatings were able to control the release of the antimicrobial agents on the cheese surface improving the microbiological quality of the cheese (Santacruz and Castro, 2018; Krishnan *et al*., 2015) and *Salmonella* spp. in fresh cheese. Previous studies showed the inhibition of *E. coli*, *L. monocytogenes* and *S. enteritidis* by LAB (Winkowski *et al*., 1993; Lord, 2002). The presence of LAB could inhibit microorganisms through the production of bacteriocins (Aymerich *et al*., 2000).

Table 1. pH, acidity, weight loss, firmness and mesophilic aerobic bacteria counting in cheese stored at 4 °C during 30 days.

		Days of storage			
		0	10	20	30
pH	LAB[1]	5.95±0.07	6.57±0.09	7.00±0.28	7.10±0.08
	Control	5.98±0.08	7.31±0.11	7.46±0.21	7.62±0.09
Acidity	LAB[1]	0.17±0.01	0.18±0.02	0.21±0.01	0.26±0.01
	Control	0.17±0.00	0.23±0.02	0.32±0.03	0.62±0.04
Weight loss (%)	LAB[1]	0.00±0.00	1.69±0.28	2.77±0.35	4.74±0.26
	Control	0.00±0.00	4.57±1.18	8.72±1.21	11.81±0.34
Firmness(N)	LAB[1]	0.87±0.02	1.34±0.01	1.34±0.10	1.12±0.14
	Control	0.89±0.02	1.17±0.01	1.16±0.01	0.97±0.11
Mesophilic aerobic bacteria (Log CFU g^{-1})	LAB[1]	8.60±0.01	4.10±0.06	6.10±0.08	6.40±0.11
	Control	8.60±0.02	9.20±0.09	10.23±0.07	10.80±0.08

n=3. Results were expressed as means±SD (standard deviation). [1]LAB: fresh cheese coated with a SL film.

Sensory analysis

Results of the duo-trio test showed that the number of correct responses was 12 of 16, which showed a difference between the cheese samples coated with *L. acidophilus* compared to control sample (Wittig, 2001). Another aspect to evaluate was the sensorial acceptation by the panelists. The results showed that there was no difference in panelists satisfaction between coated and control sample ($P<0.05$). Both received a rating between "I neither like nor dislike" and "I like it moderately", with values of 3.63 and 3.50 for coated and control sample, respectively. Previous works showed similar results; in fact, LAB strains increased the acceptability and shelf-life of unripe curd cheese (Mozuriene *et al*., 2016). Coelho *et al.* (2014) found no significant differences in overall sensory evaluation of fresh cheese. Even after the addition of *L. acidophilus,* the semi-hard goat cheese showed better sensory scores in the acceptance test (Fernandes *et al*., 2012). The SL film could be used as an agent to control the growth of mesophilic aerobic bacteria in fresh cheese with no changes on the satisfaction among consumers.

CONCLUSIONS

The use of a cassava starch +*L. acidophilus* film inhibited the development of mesophilic aerobic in fresh cheese showing no difference in panelists satisfaction between coated and control sample. SL film-coated cheese could act as a barrier against moisture loss and may be a promising option to control mesophilic aerobic bacteria in fresh cheese during storage under refrigeration conditions with no changes in consumers satisfaction.

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POLÍTICA EDITORIAL

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

La Revista Facultad Nacional de Agronomía Medellín (RFNA), es una publicación de la Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia - Sede Medellín. Esta orientada a profesores, investigadores, estudiantes, extensionistas y a todos aquellos profesionales que crean conocimiento y articulan la ciencia y la tecnología para hacer más productivo el campo a nivel empresarial y de economía campesina.

La Revista recibe y publica, sin ningún costo, artículos en idioma inglés de investigación, revisión, reseñas, cartas al editor y editoriales.

La periodicidad de la Revista es cuatrimestral, con circulación nacional e internacional y tiene como objetivo divulgar artículos escritos en inglés, originales, inéditos y arbitrados (peer review) de carácter científico que respondan a preguntas específicas y que proporcionen soporte y pruebas a una hipótesis, en aspectos relacionados con las Ciencias Agronómicas, Zootecnia, Ciencias Forestales e Ingeniería Agrícola y de Alimentos y otras afines que contribuyan a la solución de los limitantes del agro en el trópico.

Teniendo en cuenta los criterios considerados por Colciencias, la revista acoge documentos de las siguientes tipologías:

Artículos de investigación científica y tecnológica: Documento que presenta, de manera detallada, los resultados originales de proyectos terminados de investigación. La estructura generalmente utilizada contiene cuatro partes fundamentales: Introducción, metodología (materiales y métodos), resultados y discusión, y conclusiones. La extensión máxima debe ser de 5200 palabras, excluyendo figuras, tablas, referencias. El mínimo de referencias bibliográficas sugerido es de 10 y el máximo de 30. Este tipo de artículos es arbitrado e indexado.

Artículos de revisión: Documentos producto de una investigación terminada donde se analizan, sistematizan e integran los resultados de investigaciones publicadas o no publicadas, sobre un campo en ciencia o tecnología, con el fin de dar cuenta de los avances y las tendencias de desarrollo. Se caracteriza por presentar una cuidadosa revisión bibliográfica de por lo menos 50 referencias. La extensión máxima debe ser de 6000 palabras, excluyendo figuras, tablas, referencias. Este tipo de artículos es arbitrado e indexado.

Artículos cortos: Documento breve que presenta resultados originales preliminares o parciales de una investigación científica o tecnológica, que por lo general requieren de una pronta difusión. Para todos los casos el 60% de las citas debe provenir de artículos publicados en los últimos diez años.

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Unidades, abreviaturas y estilo

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Los números del uno al nueve se escriben en palabras, excepto cuando incluyen unidades de medida o se mencionan varios números. Ejemplo: “ocho tratamientos”, “3, 7 y 9 lecturas”, “15 kg”. Use cero antes del punto decimal. Para separar números en intervalos de uno o más años, use la letra “a”, y guión para temporadas de crecimiento. Ejemplo: Periodo 2002 a 2005; temporadas de crecimiento 1999-2000, 2000-2001.

Título y autores

El título del artículo no debe incluir abreviaturas y es obligatoria su respectiva traducción al idioma español. En lo posible, el título no debe exceder de 15 palabras y debe reflejar con precisión el contenido del documento. Cuando contenga nombres científicos de especies vegetales o animales, éstos se deben escribir con letra cursiva (itálica) en minúsculas, sólo con mayúsculas la primera letra del género y del clasificador. Debajo del título en inglés se escribe el nombre(s) y apellido(s) de los autores, sin sus respectivos títulos académicos, ni cargos laborales, en una línea horizontal y de acuerdo con su contribución en la investigación y/o preparación del artículo.

Como nota al pie de la primera página, se escribe el título de pregrado, el cargo laboral de los autores, el nombre y la ciudad de ubicación de la entidad a la cual prestan sus servicios o del patrocinador para la realización del trabajo y su respectiva dirección de correo electrónico, indicando el autor de correspondencia. Además, se debe adjuntar un resumen de la hoja de vida de los autores, donde se mencionen los artículos publicados en otras revistas.

Resumen, abstract y palabras claves

El resumen no debe exceder de 250 palabras escritas en un único párrafo. Se debe escribir en inglés y español. Debe contener en forma breve la justificación, los objetivos, los métodos utilizados, los resultados obtenidos más relevantes y las conclusiones. Es obligatorio acompañar el resumen con un máximo de seis palabras clave distintas a las utilizadas en el título. Se aceptan como palabras clave no sólo las palabras simples, sino también términos compuestos hasta de tres palabras. Deben ir escritas en minúsculas y separadas por comas.

Introducción

Puede tener o no título. Define el problema e informa sobre el estado del arte respecto al tema principal del artículo; además, señala las razones que justifican la investigación y plantea los objetivos de la misma. Es obligatorio acompañar los nombres vulgares con el nombre(s) científico(s) y la abreviatura(s) del clasificador en la primera mención dentro del texto. No se deben mencionar marcas de productos, sino su nombre genérico o químico

Materiales y métodos

En este apartado se deben describir en forma clara, concisa y secuencial, los materiales (vegetales, animales, implementos agrícolas o de laboratorio) utilizados en el desarrollo del trabajo; además, se mencionan los aspectos relacionados con la ubicación, preparación y ejecución de los experimentos. Se debe indicar el diseño seleccionado, las variables registradas, las transformaciones hechas a los datos, los modelos estadísticos usados y el nivel de significancia empleado. Evitar detallar procedimientos previamente publicados.

Resultados y discusión

Son la parte central del artículo, deben estar respaldados por métodos y análisis estadísticos apropiados. Se deben presentar de manera lógica, objetiva y secuencial mediante textos, tablas y figuras; estos dos últimos apoyos deben ser fáciles de leer, autoexplicativos y estar siempre citados en el texto. Las tablas se deben elaborar con pocas columnas y renglones. Se debe tener la precaución de incluir el nivel de significancia estadística representado por letras minúsculas del comienzo del alfabeto (a, b, c, d,...), un asterisco simple (*) para $P<0,05$, doble asterisco (**) para $P<0,01$ o triple asterisco (***) para $P<0,001$. Las investigaciones que no siguen un diseño estadístico, deben mostrar la información de manera descriptiva. Use subíndices para modificaciones, reserve superíndices para potencias o notas al pie en tablas y figuras.

La discusión: Se refiere al análisis e interpretación objetiva de los resultados, confrontándolos con los obtenidos en otras investigaciones, o con los hechos o teorías conocidos sobre el tema. Explica los resultados en particular cuando difieren de la hipótesis planteada. Destaca la aplicación práctica o teórica de los resultados obtenidos y

las limitaciones encontradas. Resalta la contribución que se hace a una determinada área del conocimiento y el aporte a la solución del problema que justifica la investigación. Finalmente, proporciona elementos que permitan proponer recomendaciones o lanzar nuevas hipótesis. No se deben hacer afirmaciones que van más allá de lo que los resultados pueden apoyar.

Conclusiones

Son las afirmaciones originadas a partir de los resultados obtenidos, deben ser coherentes con los objetivos planteados y la metodología empleada; además, expresar el aporte al conocimiento en el área temática estudiada y proponer directrices para nuevas investigaciones.

Agradecimientos

Si se considera necesario, se incluyen los agradecimientos o reconocimientos a personas, instituciones, fondos y becas de investigación, que hicieron contribuciones importantes en la concepción, financiación o realización de la investigación.

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- Se registra la fuente entre paréntesis, el cual debe incluir el apellido del autor y año, con coma entre autor y año. Ejemplo: (Pérez, 1995).

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En cada referencia para todos los autores cite primero el apellido, tener en cuenta que algunos autores hispanos citan sus dos apellidos, seguido de la inicial del nombre sin puntos, separando autores con coma y espacio.

Ejemplos:

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García Rodríguez JL, Giménez Suarez MC, Ortega Pérez E, Martín Ramos B, Calderón Guerrero C. 2014. Operaciones auxiliares en repoblaciones e infraestructuras forestales. Ediciones Paraninfo SA, Madrid. 208 p.

Capítulos de libros: Autor(es). Año. Título del capítulo. Páginas consultadas (pp. # - #). En: Apellidos e iniciales de los compiladores o editores (eds.). Título del libro. Edición. Casa editora, ciudad de su sede. Páginas totales (# p.). Ejemplo: Bernal H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

Bertoft E and Blennow A. 2016. Chapter 3 - Structure of potato starch. pp 57-73. In: Singh J and Kaur L. (eds.). Advances in potato chemistry and technology. Second edition. Academic Press, London. 752 p.

Artículos de revistas: Autor(es). Año. Título del artículo. Nombre completo de la revista volumen(número de fascículo): página inicial–página final. doi. Ejemplo: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Ponencias en memorias de congresos, seminarios, simposios: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. pp. 65-94. En: Memorias IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo, Bogotá.

High R. 2015. Plotting LSMEANS and Differences in Generalized Linear Models with GTL. In: 2015 Midwest SAS Users Group Conference Proceedings. Midwest SAS Users Group, Omaha. 9 p.

Tesis, trabajos de grado. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia. 78 p.

Adam M. 1992. The Impact of the Common Agricultural Policy on Agriculture in Greece (Master's thesis). Cambridge University. Cambridge, United Kingdom. 80 p.

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Suplemento de revista: Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Revista de Biología Tropical 52 Suppl. 2: 195-201.

Citas de internet: Autor(es). Año. Título del artículo. En: Nombre(s) de la publicación electrónica, de la página web, portal o página y su URL, páginas consultadas (pp. # - #) o páginas totales (# p.); fecha de consulta. Ejemplo: Arafat Y. 1996. Siembra de olivos en el desierto palestino. In: Agricultura Tropical, http://agrotropical.edunet.es. 25 p. consulta: noviembre 2003.

Patentes: Autor(es). Año. Título. País de la patente y número. Fuente. Ejemplo: Glenn RW. 1996. Liquid personal cleansing compositions which contain soluble oils and soluble synthetic surfactants. U.S. Patent No. 6194364. Retrieved from: https://patents.google.com/patent/US6194364B1/en

PUBLISHING POLICY

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

The Journal *Revista Facultad Nacional de Agronomía Medellín* (RFNA) is published by the Faculty of Agricultural Sciences of Universidad Nacional de Colombia – Medellín. It is aimed at professors, researchers and students in agronomy, animal, and forestry sciences, food and agricultural engineering, agricultural advisers and at all those professionals who create knowledge and articulate science and technology to make the field more productive at business and rural economy levels.

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INSTRUCTIONS TO AUTHORS

General guidelines

Papers must be sent b through the Open Journal System in the Universidad Nacional de Colombia journals web side http://www.revistas.unal.edu.co/, Will be considered only papers written in English. The four following formats must be submitted with the manuscript: (1) Editorial Criteria Checklist for Paper Submission; (2) Paper Publishing Authorization for the Revista Facultad Nacional de Agronomía Medellín, which accepts no simultaneous nomination of the article to other journals or editorial bodies, and the rights are given to the Journal for its release by the signature of all the manuscript's authors; (3) Personal information of each author; (4) Suggestion of possible peer reviewers. Publishing forms are: scientific and technological research articles, review articles, reflection articles, and short articles. Articles can be developed by professors and/or researchers at the Universidad Nacional de Colombia, or other related national or international institution, on Agricultural, Forestry, Food and Agricultural Engineering matters. Article extension must not exceed 5,200 words for research articles and 6,000 words for reviews. The manuscript must be letter-size sheets, line spacing double, continuous line number 12 point Times New Roman or Verdana font, 3 cm margin at the upper, 2 cm in the lower, 2.5 cm on the left and right side margins. Tables and figures (i.e, graphics, drawings, diagrams, flowcharts, photographs and maps) should be shown on separate sheets and numbered consecutively (Table 1 ... Table n, Figure 1…Figure n, etc.). Texts and tables should be submitted in MS-Word® word processor, original tables and diagrams of frequency (bar charts and pie charts) must be supplied in manuscript file and in its original MS-Excel®; other figures, such as photographs on paper and drawings, can be sent in original or scanned and sent in digital format compression JPG (or JPEG), preferably with a resolution of 600 x 600 dpi (300 dpi at least); original photographs are suggested to be sent as slides. As a general rule, tables and figures are only accepted in black and white. Color figures will be exceptionally accepted when strictly necessary and under discretion of the Editorial Board.

Units, abbreviations and style

International System of Units (SI), and those specific units of greater use by the scientific community must be used. When required must be used the exponential form. Example: kg ha^{-1}. The meaning of abbreviations should be cited in full when first mentioned in the manuscript. The writing style should be totally impersonal. Introduction, procedures and results should be written in grammatical past tense. Discussion should be written in grammatical present tense, avoiding the conjugation of verbs in first or third person singular or plural.

The numbers from 1 to 9 are written in words, except when they include units of measure or several numbers are listed. Example: “eight treatments”, “3,7 and 9 readings”, “15 kg”. Use zero before the decimal point. To separate numbers in intervals of one to two years, use the letter “a” and hyphen for growing seasons. Example period 2002 to 2005, growing seasons 1999-2000, 2000-2001.

Title and authors

The article should not include abbreviations and its translation into English is required. As far as possible, the title should not exceed 15 words and must accurately reflect the paper content. When the article contains scientific names of plants or animals, they should be written in italics in lower case, only the first letter of gender and classifier should be capital. Under the title in English the author or authors' name (s) and surname (s) is /are written, without academic degrees or job positions, in a horizontal line according to the contribution to research and / or preparation of the article.

As a footnote on the first page, write the title of undergraduate, authors' job positions, the name and city location of the entity to which they serve, or the sponsors for the research work and their respective email address. In addition, a summarized authors' résumé including reference to the articles published in other magazines should be attached.

Abstract and key words

The abstract should not exceed 250 words written in a single paragraph. It must be written in English and Spanish. It should contain in brief the justification, aims, methods used, the most relevant results, and conclusions. It is required to accompany the abstract with a maximum of six key words, translated into English, different from those used in the title. Single words as well as compound terms of up to three words are accepted as key words. They must be written in lowercase, separated by commas.

Introduction

It may or not have a title. It defines the problem and reports on the state of the art on the main subject of the article, it also points out the reasons for the research and sets out its aims. It is required to accompany common names with the corresponding scientific name (s) name and abbreviation (s) of the classifier at the first mention in the text. Brands must not be mentioned but the generic or chemical name.

Materials and methods

In this section, materials (crops, livestock, agricultural or laboratory implements) used in the development of work should be clearly, concisely and sequentially described. Aspects related to the location, preparation and execution of experiments should also be mentioned. The selected design, the recorded variables, the changes made to data, the statistical models used and the significance level used should be indicated. Authors must avoid detailing procedures previously published.

Results

They are the central part of the article and must be supported by appropriate statistical methods and analysis. They should be presented in a logical, objective and sequential way through texts, tables and figures; the latter two supports should be easy to read, self- explanatory and always quoted in the text. The tables should be composed by few columns and rows. Care should be taken to include the statistical significance level represented by lowercase letters of the beginning of the alphabet (a, b, c, d,…), a single asterisk (*) for $P<0.05$, double asterisk (**) for $P<0.01$ or triple asterisk (***) for $P<0.001$. Researches that do not follow a statistical design should display the information in a descriptive way. Use subscripts to modifications, reserve superscripts for potencials or footnotes in tables and figures.

Discussion

It refers to the analysis and objective interpretation of results, comparing them with those obtained in other research, or with known facts or theories on the subject. It explains the results, especially when they differ from the stated hypothesis. It emphasizes the practical or theoretical application of the obtained results and constraints encountered. Discussion also highlights the contribution that is made to a particular area of knowledge and to the solution of the problem that justifies the research. Finally, it provides elements that allow making recommendations or launching new hypotheses. Statements that go beyond what the results may support should be avoided.

Conclusions

Conclusions are assertions arising from the obtained results. They should be consistent with the objectives stated and the methodology used. They should also express the contribution to knowledge in the studied subject area and propose guidelines for further researches.

Acknowledgements

If necessary, acknowledgements or recognitions to individuals, institutions, funds and research grants that made important contributions in the design, financing or carrying out of the research are included.

Citing in-text format

- Citations in the text should be in parenthesis and include author's surname and year, with comma in-between. Example: (Pérez, 1995).

- If more than one date, they are separated by commas: Example: (Pérez, 1995, 1998, 2001).

- If there are two authors, they will be separated by the conjunction and. Example: (Gil and Ortega, 1993)

- If there are several works of an author published in the same year, they will be cited with a letter in alphabetical sequence of titles, adjacent to year. Example: (Gómez, 2000a, 2000b, 2000c)

- For citations with three or more authors, it is necessary to mention in the text the surname of the first author and replace the others by the Latin expression *et al.* (in italics), which means and others. All authors should be mentioned in the reference. Example: (García *et al.*, 2004)

- When the author is referenced within the text, only the year is enclosed in parentheses, and the comma that separates the author from the year is omitted. Example: (1) According to Castañeda (2000), ...; (2) In accordance with the results of Poveda *et al.* (2018), ...

- When an indirect source is cited, the information of the cited authors and the citing authors are placed. Example: (Magalhaes *et al.* (1979) state that ... (as cited in Gómez, 2004).

- Organizations are cited by their initials; in case they do not have their full name is used. Example: (1) (FAO, 2015), (2) (Ministerio de Agricultura y Ganadería, 2019)

References

Only bibliographical references cited in-text are listed in the references section. Lecture notes, articles in preparation, or any other publication with limited circulation are not accepted. Excessive self-citation should be avoided.

Bibliographic references are ordered alphabetically by first author's surname, without numbering and without indentation. To cite several publications of the same author, chronological increasing order must be followed. Alphabetical order of titles must be followed in case they are from the same year.

References should contain all the data allowing to its easy location. The titles of the papers, the surnames of the authors and the names of journals must be referenced and cited in their original language.

Examples:

For books: Author(s), Year. Book title, Edition, Publisher, Place of publication. Pages consulted (pp. #-#) or total pages.Example: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

García Rodríguez JL, Giménez Suarez MC, Ortega Pérez E, Martín Ramos B, Calderón Guerrero C. 2014. Operaciones auxiliares en repoblaciones e infraestructuras forestales. Ediciones Paraninfo SA, Madrid. 208 p.

For book chapters: Author(s). year. Chapter title. pages consulted (pp. # - #). In: Surnames and names of the editors or publishers (eds.). book title. Edition. Publisher, place of publication. total pages (# p.). Example: Bertoft E and Blennow A. 2016. Chapter 3 - Structure of potato starch. pp 57-73. In: Singh J and Kaur L. (eds.). Advances in potato chemistry and technology. Second edition. Academic Press, London. 752 p.

Beral H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

For journals: Author(s). year. Article title. journal full name volume(number): initial page-final page. Example: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Presentations in Memoirs of Congresses, seminars and symposia: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. pp. 65-94. En: Memorias IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo, Bogotá.

High R. 2015. Plotting LSMEANS and Differences in Generalized Linear Models with GTL. In: 2015 Midwest SAS Users Group Conference Proceedings. Midwest SAS Users Group, Omaha. 9 p.

Theses and dissertations: Adam M. 1992. The impact of the common agricultural policy on agriculture in Greece (Doctoral dissertation). Cambridge University. Cambridge, United Kingdom. 80 p.

Gómez C. 2004. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín. Colombia. 78 p.

Citation of a citation, list the secondary source in your reference list: Example: Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia. 78 p.

Journal Supplement: Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Journal of Tropical Biology 52 Suppl. 2: 195-201.

For internet citations: Author (s), year. Article. In: electronic publishing Name (s), the web page, portal or page name and its URL, pages consulted (pp. # - #) or total pages (# p.), date of consultation. Example: Arafat Y. 1996. Siembra de olivos en el desierto palestino. En: Tropical Agriculture, http://agrotropical.edunet.es. 25 p.; accessed: November 2003.

Patents: Author(s). Year. Title. Patent country and number. Retrieved from. Example: Glenn RW. 1996. Liquid personal cleansing compositions which contain soluble oils and soluble synthetic surfactants. U.S. Patent No. 6194364. Retrieved from: https://patents.google.com/patent/US6194364B1/en

ÉTICA EN LA PUBLICACIÓN CIENTÍFICA Y ACUERDO SOBRE POSIBLES MALAS PRÁCTICAS

La revista Facultad Nacional de Agronomía espera y verificará que los autores, revisores, editores y en general la comunidad académica y científica involucrada en nuestro proceso editorial, sigan estrictamente las normas éticas internacionales requeridas en el proceso de edición.

La revista Facultad Nacional de Agronomía sigue las normas éticas presentes en el COPE Best Practice Guidelines for Journal Editors y por el International Standars for Editors and Authors publicado por Committee on Publication Ethics.

Los autores deben evitar incurrir al plagio de la información. La revista define los siguientes lineamientos, criterios y recomendaciones sobre la ética en la publicación científica:

1. Criterios generales[1]

1.1. Los artículos deben contener suficiente detalle y referencias que permitan replicar o rebatir el estudio.

1.2.Declaraciones fraudulentas o deliberadamente inexactas constituyen un comportamiento poco ético.

1.3. Si el estudio incluye productos químicos, procedimientos o equipos que tienen cualquier riesgo inusual inherente a su uso, el autor debe identificar claramente estos en el artículo.

1.4. Si el estudio implica el uso de animales o de seres humanos, el autor debe asegurarse que el artículo contenga una declaración que haga explícito que se realizaron todos los procedimientos de conformidad con las leyes y directrices institucionales.

1.5. Se deben respetar los derechos de privacidad de los seres humanos.

2. Autoría[2]

Criterios:

2.1. Un "autor" es la persona que ha hecho una contribución intelectual significativa al artículo, por lo tanto, todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que los reúnan deben ser mencionados de forma explícita.

2.2. Se deben cumplir colectivamente tres criterios básicos para ser reconocido como autor:

a) Contribución sustancial a la concepción y diseño, adquisición de datos, análisis e interpretación del estudio.

b) Redacción o revisión del contenido intelectual.

c) Aprobación de la versión final.

2.3. El orden de la autoría debe ser una decisión conjunta de los coautores.

2.4. Las personas que participen en un estudio pero que no se ajusten a los criterios de autoría deben aparecer como "Colaboradores" o "Personas reconocidas".

2.5. Hay tres tipos de autorías que se consideran inaceptables: autores "fantasma", que contribuyen sustancialmente pero no son reconocidos (a menudo pagados por promotores comerciales); autores "invitados", que no hacen ninguna contribución discernible pero se nombran para aumentar las posibilidades de publicación; y autorías "honorarias", que se basan únicamente en una afiliación tenue con un estudio.

Recomendaciones:

2.6. Antes de iniciar la investigación se recomienda documentar la función y la forma como se reconocerá la autoría de cada investigador.

2.7. No se debe mentir sobre la participación de una persona en la investigación o publicación, si su contribución se considerada "sustancial" se justifica la autoría, bien sea como coautor o colaborador.

2.8. No se debe asignar una autoría sin contar con el consentimiento de la persona.

2.9. Todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que reúnan los requisitos deben aparecer como autores o contribuidores.

2.10. Algunos grupos colocan los autores por orden alfabético, a veces con una nota para explicar que todos los autores hicieron contribuciones iguales al estudio y la publicación.

3. Cambios en la autoría[3]

Criterios:

3.1. Hace referencia a la adición, supresión o reorganización de los nombres de autor en la autoría de un artículo aceptado.

3.2. Las peticiones de añadir o eliminar un autor, o para reorganizar los nombres de los autores, deben ser enviados por el autor correspondiente del artículo aceptado, y deben incluir:

a) La razón por la cual debe ser añadido o eliminado, o los nombres de los autores reorganizado.

b) La confirmación por escrito (e-mail) de todos los autores que están de acuerdo con la adición, supresión o reorganización. En el caso de adición o eliminación de los autores,esto incluye la confirmación de que el autor sea añadido o eliminado.

4. Conflicto de intereses[4]

Criterios:

4.1. Cuando un investigador o autor, editor tenga alguna opinión o interés financiero/personal que pueda afectar su objetividad o influir de manera inapropiada en sus actos, existe un posible conflicto de intereses. Este tipo de conflictos pueden ser reales o potenciales.

4.2. Los conflictos de intereses más evidentes son las relaciones financieras, como:

a) Directas: empleo, propiedad de acciones, becas, patentes.

b) Indirectas: honorarios, asesorías a organizaciones promotoras, la propiedad de fondos de inversión, testimonio experto pagado.

4.3. Los conflictos también pueden existir como resultado de relaciones personales, la competencia académica y la pasión intelectual. Por ejemplo, un investigador que tenga:

a) Algún tipo de interés personal en los resultados de la investigación.

b) Opiniones personales que están en conflicto directo con el tema que esté investigando.

Recomendaciones:

4.4. Revelar si se está en algún conflicto real o potencial de intereses que influya de forma inapropiada en los hallazgoso resultados del trabajo presentado, dentro de los tres (3) años de haber empezado el trabajo presentado que podría influir indebidamente (sesgo) el trabajo.

4.5. Revelar el papel de un promotor (o promotores) del estudio, si los hubiere, en el diseño del estudio, en la recopilación, análisis e interpretación de los datos, en la redacción del informe y en la decisión de presentar el documento para su publicación.

4.6. Los investigadores no deben entrar en acuerdos que interfieran con su acceso a todos los datos y su capacidad de analizarlos de forma independiente, y de preparar y publicar los manuscritos.

4.7. Al presentar un documento, se debe hacer una declaración (con el encabezamiento "Papel que ha tenido la fuente de financiación") en una sección separada del texto y colocarse antes de la sección "Referencias".

4.8. Algunos ejemplos de posibles conflictos de intereses que deben ser revelados, incluyen: empleo, consultoría, propiedad de acciones, honorarios, testimonio experto remunerado, las solicitudes de patentes / registros y subvenciones u otras financiaciones.

4.9. Todas las fuentes de apoyo financiero para el proyecto deben ser revelados.

4.10. Se debe describir el papel del patrocinador del estudio.

5. Publicación duplicada[5]

Criterios:

5.1. Los autores tienen la obligación de comprobar que su artículo sea basado en una investigación original (nunca publicada anteriormente). El envío o reenvío intencional de su trabajo para una publicación duplicada se considera un incumplimiento de la ética editorial.

5.2. Se produce una publicación duplicada o múltiple cuando dos o más artículos, sin hacerse referencias entre sí, comparten esencialmente las

mismas hipótesis, datos, puntos de discusión y/o conclusiones. Esto puede ocurrir en diferentes grados: Duplicación literal, duplicación parcial pero sustancial o incluso duplicación mediante parafraseo.
5.3. Uno de los principales motivos por los que la publicación duplicada de investigaciones originales se considera no ético es porque puede dar lugar a una "ponderación inadecuada o a un doble recuento involuntario" de los resultados de un estudio único, lo que distorsiona las pruebas disponibles.

Recomendaciones:

5.4. Los artículos enviados para su publicación deberán ser originales y no deberán haberse enviado a otra editorial. En el momento del envío, los autores deberán revelar los detalles de los artículos relacionados (también cuando estén en otro idioma), artículos similares en prensa y traducciones.
5.5. Aunque un artículo enviado esté siendo revisado y no conozca el estado, espere a que la editorial le diga algo antes de ponerse en contacto con otra revista, y sólo si la otra editorial no publicará el artículo.
5.6. Evite enviar un artículo previamente publicado a otra revista.
5.7. Evite enviar artículos que describan esencialmente la misma investigación a más de una revista.
5.8. Indique siempre los envíos anteriores (incluidas las presentaciones de reuniones y la inclusión de resultados en registros) que pudieran considerarse una publicación duplicada.
5.9. Evite escribir sobre su propia investigación en dos o más artículos desde diferentes ángulos o sobre diferentes aspectos de la investigación sin mencionar el artículo original.
5.10. Se considera manipulador crear varias publicaciones a raíz de la misma investigación.
5.11. Si desea enviar su artículo a una revista que se publica en un país diferente o en un idioma diferente, pregúntaselo a la editorial si se puede hacer esto.
5.12. En el momento del envío, indique todos los detalles de artículos relacionados en un idioma diferente y las traducciones existentes.

6. Reconocimiento de las fuentes

Criterios:

6.1. Los autores deben citar las publicaciones que han sido influyentes en la determinación de la naturaleza del trabajo presentado.
6.2. Información obtenida de forma privada, no debe ser usada sin explícito permiso escrito de la fuente.
6.3. La reutilización de las tablas y / o figuras requiere del permiso del autor y editor, y debe mencionarse de manera adecuada en la leyenda de la tabla o figura.
6.4. La información obtenida en el transcurso de servicios confidenciales, tales como manuscritos arbitrales o las solicitudes de subvención, no debe ser utilizada sin el permiso explícito y por escrito del autor de la obra involucrada en dichos servicios.

7. Fraude científico[6]

Criterios:

7.1. El fraude en la publicación científica hace referencia a la presentación de datos o conclusiones falsas que no fueron generados a través de un proceso riguroso de investigación.
7.2. Existen los siguientes tipos de fraude en la publicación de resultados de investigación:
a) Fabricación de datos. Inventar datos y resultados de investigación para después comunicarlos.
b) Falsificación de datos. La manipulación de materiales de investigación, imágenes, datos, equipo o procesos.
La falsificación incluye la modificación u omisión de datos o resultados de tal forma que la investigación no se representa de manera precisa. Una persona podría falsificar datos para adecuarla al resultado final deseado de un estudio.

Recomendaciones:

7.3. Antes de enviar un artículo, lea cuidadosamente las políticas editoriales y de datos de la revista.
7.4. Nunca modifique, cambie u omita datos de forma intencional. Esto incluye materiales de investigación, procesos, equipos, tablas, citas y referencias bibliográficas.
7.5. Tanto la fabricación como la falsificación de datos son formas de conducta incorrecta graves porque ambas resultan en publicaciones científicas que no reflejan con precisión la verdad observada.
7.6. El autor debe hacer una gestión adecuada de los datos que soportan la investigación, teniendo especial cuidado en la recopilación, producción, conservación, análisis y comunicación de los datos.
7.7. Mantenga registros minuciosos de los datos en bruto, los cuales deberán ser accesibles en caso de que un editor los solicite incluso después de publicado el artículo.

8. Plagio[7]

Criterios:

8.1. El plagio es una de las formas más comunes de conducta incorrecta en las publicaciones, sucede cuando uno de los autores hace pasar como propio el trabajo de otros sin permiso, mención o reconocimiento. El plagio se presenta bajo formas diferentes, desde la copia literal hasta el parafraseado del trabajo de otra persona, incluyendo: datos, ideas, conceptos, palabras y frases.
8.2. El plagio tiene diferentes niveles de gravedad, como por ejemplo:
a) Qué cantidad del trabajo de otra persona se tomó (varias líneas, párrafos, páginas, todo el artículo)
b) Qué es lo que se copió (resultados, métodos o sección de introducción).
8.3. El plagio en todas sus formas constituye una conducta no ética editorial y es inaceptable.
8.4. La copia literal solo es aceptable si indica la fuente e incluye el texto copiado entre comillas.

Recomendaciones:

8.5. Recuerde siempre que es esencial reconocer el trabajo de otros (incluidos el trabajo de su asesor o su propio trabajo previo) como parte del proceso.
8.6. No reproduzca un trabajo palabra por palabra, en su totalidad o en parte, sin permiso y mención de la fuente original.
8.7. Mantenga un registro de las fuentes que utiliza al investigar y dónde las utilizó en su artículo.
8.8. Asegúrese de reconocer completamente y citar de forma adecuada la fuente original en su artículo.
8.9. Incluso cuando haga referencia a la fuente, evite utilizar el trabajo de otras personas palabra por palabra salvo que lo haga entre comillas.
8.10. El parafraseado solo es aceptable si indica correctamente la fuente y se asegura de no cambiar el significado de la intención de la fuente.
8.11. Incluya entre comillas y cite todo el contenido que haya tomado de una fuente publicada anteriormente, incluso si lo está diciendo con sus propias palabras.

9. Fragmentación[8]

Criterios:

9.1. La fragmentación consiste en dividir o segmentar un estudio grande en dos o más publicaciones.
9.2. Como norma general, con tal de que los "fragmentos" de un estudio dividido compartan las mismas hipótesis, población y métodos, no se considera una práctica aceptable.
9.3. El mismo "fragmento" no se debe publicar nunca másde una vez. El motivo es que la fragmentación puede dar lugar a una distorsión de la literatura haciendo creer equivocadamente a los lectores que los datos presentados en cada fragmento (es decir, artículo de revista) se derivan de una muestra de sujetos diferente. Esto no solamente sesga la "base de datos científica", sino que crea repetición que hace perder el tiempo de los editores y revisores, que deben ocuparse de cada trabajo por separado. Además, se infla injustamente el número de referencias donde aparece citado el autor.

Recomendaciones:

9.4. Evite dividir inapropiadamente los datos de un solo estudio en dos o más trabajos.
9.5. Cuando presente un trabajo, sea transparente. Envíe copias de los manuscritos estrechamente relacionados al manuscrito en

cuestión. Esto incluye manuscritos publicados, enviados recientemente o ya aceptados.

10. Consentimiento informado

Criterios:

10.1. Los estudios sobre pacientes o voluntarios requieren la aprobación de un comité de ética.

10.2. El consentimiento informado debe estar debidamente documentado.

10.3. Los permisos y las liberaciones deben ser obtenidos, cuando un autor desea incluir detalles de caso u otra información personal o imágenes de los pacientes y cualquier otra persona.

10.4. Especial cuidado debe tenerse con la obtención del consentimiento respecto a los niños (en particular cuando un niño tiene necesidades especiales o problemas de aprendizaje), donde aparece la cabeza o la cara de una persona, o cuando se hace referencia al nombre de un individuo u otros datos personales.

11. Corrección de artículos publicados[9]

Criterio:

Cuando un autor descubre un error o inexactitud significativa en el trabajo publicado, es obligación del autor notificar de inmediato a la revista y cooperar en el proceso de corrección.

Referencias

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PUBLICATION ETHICS AND PUBLICATION MALPRACTICE STATEMENT

The journal Revista Facultad Nacional de Agronomia follows the COPE Code of Conduct and Best Practice Guidelines for Journal Editors and the International Standards For Editors and Authors, published by Committe on Publication Ethics.
The journal puts forth the following criteria and recommendations for ethical scientific publications:

1. General criteria[1]

1.1. Articles must contain sufficient details and references that allow the study to be replicable or refutable.
1.2. Fraudulent or deliberately inexact statements constitute unethical behavior.
1.3. If a study includes the use of chemical products, procedures, or equipment that presents an inherent risk, the author must state so in the article.
1.4. If the study involves the use of animals or human beings, the article must contain a clear statement that all of the procedures were carried out in strict compliance with laws and institutional directives.
1.5. The privacy of the human beings must be respected.

2. Authorship[2]

Criteria:
2.1. An "author" is a person that has made a significant intellectual contribution to an article; all of the individuals that are named as authors must fulfill the requirements for authorship and all of those individuals that do so must be explicitly named.
2.2. Three basic criteria must be met in order to be considered an author:
a) Substantial contribution to the study concept, design, and data collection, analysis and interpretation.
b) Revision of the intellectual content.
c) Approval of the final version.
2.3. The order of the author list must be a joint decision of the coauthors.
2.4. The individuals that participate in a study but that do not meet the criteria for authorship must be listed as an "Assistant" or "recognized person."
2.5. There are three types of unacceptable authorship: "ghost" authors, who make a substantial contribution but are not recognized (often paid by commercial promoters); "guest" authors, who do not make a discernable contribution but are named in order to increase the probability of publication; and "honorary" authors, who only have a tenuous connection to the study.
Recommendations:
2.6. Before starting the research, establish the function of each researcher and the manner in which they will be recognized.
2.7. It is not necessary to mention an individual's participation in a study or publication, but if their contribution is substantial, than authorship would be justified, either as an author or assistant.
2.8. Authorship cannot be bestowed on an individual without their consent.
2.9. All of the individuals that are named as authors must meet the requirements for authorship and all of those that meet the requirements must appear as authors or assistants.
2.10. Some groups list the authors alphabetically, sometimes with a notation that indicates that all of the authors contributed equally to the study and the publication.

3. Changes in the authorship[3]

Criteria:
3.1. Additions to, removals from, and reorganization of the author names in accepted articles must be noted.
3.2. Petitions to add to, remove from, or reorganize the authors must be sent by the corresponding author of the accepted articles and must include:
a) The reason for the addition, elimination, or reorganization.
b) A written statement (e-mail) from all of the authors that confirms their agreement with the addition, elimination, or reorganization. In the case of an addition or elimination, a confirmation is also required from the author to be added or removed.

4. Conflict of interest[4]

Criteria:
4.1. When a researcher or author has a financial/personal opinion or interest that could affect their objectivity or improperly influence their actions, there exists a possible conflict of interest. Conflicts can be actual or potential.
4.2. The most evident conflicts of interest are financial, such as:
a) Direct: employment, stocks, scholarships, patents.
b) Indirect: assistantship to promoting organizations, investment funds, paid expert testimony.
4.3. Conflicts can also arise from personal relationships, academic competition, and intellectual passion. For example, an author could have:
a) Some personal interest in the results of the research.
b) Personal opinions that are in direct conflict with the research topic.
Recommendations:
4.4. Disclose all conflicts of interest, actual or potential, that inappropriately influence the findings or results of a study, including any that arise within the three (3) years after the start of said study if they could unduly (bias) influence the study.
4.5. Disclose the role of any promoter (or promoters) in the study, if any, in the design, in the collection, analysis or interpretation of the data, in the document review, or in the decision to present the document for publication.
4.6. The researchers must not enter into agreements that interfere with their access to all of the data or with their ability to independently analyze the data or to prepare and publish the manuscript.
4.7. The document must contain a statement (with the heading "Role of the financial source") in a section that is separate from the text and before the References section.
4.8. Some examples of conflicts of interest that must be revealed include: employment, consulting, stocks, honorariums, paid expert testimony, patent requests or registration, and subsidies or other financing.
4.9. All of the sources of financial support for the project must be revealed.
4.10. The role of any study sponsors must be described.

5. Duplicate publication[5]

Criteria:
5.1. Authors have the obligation of proving that their article is based on original research (never before published). The intentional submission or resubmission of a manuscript for duplicate publication is considered a breach of editorial ethics.
5.2. A duplication publication, or multiple publication, results when two or more articles, without any reference to each other, essentially share the same hypothesis, data, discussion points, and/or conclusions. This can occur to different degrees: literal duplication, partial but substantial duplication or paraphrasal duplication.
5.3. One of the main reasons that duplicate publications are considered unethical is that they can result in the "inappropriate weighting or unwitting double counting" of results from just one study, which distorts the available evidence.
Recommendations:
5.4. Articles sent for publication must be original and not sent to other editors. When sent, the authors must reveal the details of related articles (even when in another language) and similar articles being printed or translated.

5.5. Even though a submitted article is being reviewed and the final decision is not known, wait to receive notification from the editors before contacting other journals and then only do so if the editors decline to publish the article.
5.6. Avoid submitting a previously published article to another journal.
5.7. Avoid submitting articles that essentially describe the same research to more than one journal.
5.8. Always indicate previous submissions (including presentations and recorded results) that could be considered duplicate results.
5.9. Avoid writing about your research in two or more articles from different angles or on different aspects of the research without mentioning the original article.
5.10. Creating various publications based on the same research is considered a type of manipulation.
5.11. If an author wishes to send an article to a journal that is published in a different country or a different language, ask for permission from the editors first.
5.12. When submitting an article, indicate all of the details of the article that were presented in a different language along with the relevant translations.

6. Acknowledging sources

Criteria:

6.1. Authors must cite the publications that had an influence on the determination of the nature of the offered study.
6.2. Privately obtained information cannot be used without the express written consent of the source.
6.3. Republishing tables or figures requires the permission of the author or editor, who must be appropriately cited in the table or figure legend.
6.4. Information obtained through confidential services, such as arbitration articles or subsidy applications, cannot be used without the express written consent of the author of the work involved in said services.

7. Scientific fraud[6]

Criteria:

7.1. Fraud in scientific publications refers to the presentation of false data or conclusions that were not obtained through a rigorous research process.
7.2. The following types of fraud exist for the publication of research results:
a) Fabricating data. Inventing research data and results for later dissemination.
b) Falsification of data. The manipulation of research material, images, data, equipment or processes. Falsification includes the modification or omission of data or results in such a way that the research is not represented in a precise manner. A person may falsify data in order to obtain the desired final results of a study.

Recommendations:

7.3. Before submitting an article, carefully read the editorial and data policies of the journal.
7.4. Never modify, change or omit data intentionally. This includes research material, processes, equipment, tables, citations, and bibliographical references.
7.5. Fabricating and falsifying data constitute grave misconduct because both result in scientific publications that do not precisely reflect the actual observations.
7.6. Authors must appropriately manage the data that supports the research, taking special care in the compilation, production, preservation, analysis and presentation of the data.
7.7. Maintain precise records of the raw data, which must be assessable in case the editors request them after publication of the article.

8. Plagiarism[7]

Criteria:

8.1. Plagiarism is one of the more common types of misconduct in publications; it occurs when an author passes the work of others off as their own without permission, citations, or acknowledgment. Plagiarism can occur in different forms, from literally copying to paraphrasing the work of another person, including data, ideas, concepts, paragraphs, and phrases.
8.2. Plagiarism has different degrees of severity; for example:
a) The quantity of work taken from another person (various lines, paragraphs, pages, or the entire article).
b) What is copied (results, methods, or introduction section).
8.3. Plagiarism, in all of its forms, constitutes unethical behavior and is unacceptable.
8.4. Literal copying is acceptable if the source is indicated and the text is placed in quotation marks.

Recommendations:

8.5. Always remember that it is vital to recognize the work of others (including the work of your assistants or your previous studies).
8.6. Do not reproduce the work of others word for word, in totality or partially, without the permission and recognition of the original source.
8.7. Maintain a record of the sources that are used in the research and where they are used in the article.
8.8. Be sure to accurately acknowledge and cite the original source in your article.
8.9. Even when referencing the source, avoid using the work of others word for word unless it is placed in quotations.
8.10. Paraphrasing is only acceptable if the source is correctly indicated and the source's intended meaning is not changed.
8.11. Use quotations, and cite all of the content that is taken from a previously published source even when using your own words.

9. Fragmentation[8]

Criteria:

9.1.Fragmentation occurs when a large study is divided or segmented into two or more publications.
9.2. As a general rule, as long as the "fragments" of a divided study share the same hypothesis, populations, and methods, this not considered an acceptable practice.
9.3. The same "fragment" can never be published more than one time. Fragmentation can result in distortion of the literature, creating the mistaken belief in readers that the data presented in each fragment (i.e. journal article) are derived from different subject samplings. This not only distorts the "scientific database", but creates repetition that results in a loss of time for editors and evaluators that must work on each article separately. Furthermore, the cited author receives an unfair increase in their number of references.

Recommendations:

9.4. Avoid inappropriately dividing the data of one study into two or more articles.
9.5. When presenting your work, be transparent. Send copies of the manuscripts that are closely related to the manuscript in question, including published, recently submitted and accepted manuscripts.

10. Informed consent

Criteria:

10.1. Studies on patients and volunteers require the approval of the ethics committee.
10.2. The informed consent must be duly documented.
10.3. Permission and waivers must be obtained when an author wishes to include details of a case or other personal information or images of the patients or any other person.
10.4. Special care should be taken when obtaining the consent

of children (especially when a child has special needs or learning disabilities) when their head or face is displayed or when reference is made to the name of an individual or other personal data.

11. Correction of published articles[9]

Criterion:

When an author discovers a significant inexactitude or error in a published article, they must immediately notify the journal and cooperate in the correction process.

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