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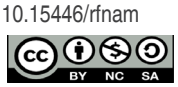

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El Comité Editorial dentro de sus políticas, envía los artículos a especialistas, con el fin de que sean revisados. Sus observaciones en adición a las que hacen los editores, contribuyen a la obtención de una publicación de reconocida calidad en el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

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Physiological and yield response to fertilization of short-cycle *Solanum tuberosum* cultivars in three high-Andean environments

Respuesta fisiológica y de rendimiento a la fertilización de cultivares de ciclo corto de *Solanum tuberosum* en tres ambientes altoandinos

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Keywords:
Andigena
Growth index
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Plant nutrition
Productivity

ABSTRACT

The variability of potato cultivars and environments in the production area of Nariño-Colombia, demands the adjustment of agronomic recommendations for the improvement of crop management. Physiological behavior and yield of four short-cycle potato cultivars were evaluated: *Solanum tuberosum* Phureja groups (Mambera, Ratona Morada and Criolla Colombia) and Andigena (Morasurco), under three environments, characterized edaphoclimatically (AH1, AH2, and AH4) and three fertilization levels. The yield components did not present differences between these levels, except for AH2, where level 3 surpassed the others in yield, and AH3 for harvest index with differences between levels. Regarding the cultivars, there were statistical differences in all environments; the highest yield was obtained by Ratona Morada and Mambera in AH4, Criolla Colombia in AH1, and a homogeneous behavior for Morasurco in all environments. In AH1 and AH2 the physiological indexes were similar in proportion and occurrence, while in AH4 the behavior was variable, with higher values in Morasurco and Mambera. ANOVA and discriminant analysis of principal components (DAPC) differentiated Morasurco from Phureja cultivars and Criolla Colombia from Ratona and Mambera. The grouping of environments in the DAPC ratifies the classification of the environments. The nutritional requirements of the genotypes can be limited by elements different from those evaluated; the productivity was mainly influenced by the environments; Mambera and Ratona Morada are established as alternatives in the AH4 environment, and Criolla Colombia and Morasurco in the other environments.

Palabras clave:
Andigena
Índice de crecimiento
Genotipos nativos
Phureja
Nutrición vegetal
Productividad

RESUMEN

La variabilidad de cultivares de papa y ambientes en la zona productora de Nariño-Colombia, demanda el ajuste de recomendaciones agronómicas para mejorar el manejo del cultivo. Se evaluó el comportamiento fisiológico y el rendimiento de cuatro cultivares de papa de ciclo corto: *Solanum tuberosum* grupos Phureja (Mambera, Ratona Morada y Criolla Colombia) y Andigena (Morasurco), bajo tres ambientes caracterizados edafoclimáticamente (AH1, AH2 y AH4) y tres niveles de fertilización. Los componentes de rendimiento no presentaron diferencias entre estos niveles, a excepción de AH2, donde el nivel 3 superó a los demás en rendimiento y AH3 para índice de cosecha con diferencias entre niveles. Con respecto a los cultivares, hubo diferencias estadísticas en todos los ambientes; el mayor rendimiento lo obtuvo Ratona Morada y Mambera en AH4, Criolla Colombia en AH1 y un comportamiento homogéneo para Morasurco en todos los ambientes. En AH1 y AH2 los índices fisiológicos fueron similares en proporción y ocurrencia, mientras en AH4 el comportamiento fue variable, con mayores valores en Morasurco y Mambera. El ANOVA y el análisis discriminante de componentes principales (DAPC) diferenciaron a los cultivares Morasurco de Phureja y a Criolla Colombia de Ratona y Mambera. La agrupación de ambientes en el DAPC ratifica la clasificación de los ambientes. Los requerimientos nutricionales de los genotipos pueden estar limitados por elementos diferentes a los evaluados; la productividad fue influenciada principalmente por los ambientes; se establece a Mambera y Ratona Morada como alternativa en el ambiente AH4, y Criolla Colombia y Morasurco en los otros ambientes.

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Potato, along with wheat, rice, and maize are of great global importance as a food source, due to their nutritional and medicinal value (Arcos and Zúñiga, 2016). In *Solanum tuberosum*, the Tuberosum and Andigena groups are the most important, by area planted and production volume, and the Phureja group, to which the so-called creole potatoes belong, is of special relevance in Colombia due to its acceptance among consumers, for its good taste, nutritional quality, agro-industrial suitability, lower costs and production cycles (Molina-Cita *et al*., 2015).

In Colombia, potato is grown in high areas of the three mountain ranges, 90% corresponds to the Andigena group and the remaining 10% to the Phureja group (Fedepapa, 2017). Of the Andigena group, few cultivars have productive cycles of less than 5 months; Morasurco is one of them, which the farmer relates to the Phureja group because of the length of the cycle, the growing conditions and its management in the field, and post-harvest (Moreno, 2000). It is also a cultivar little described in the literature. Colombia is the main producer worldwide of Morasurco. In addition, it is the food base of peasant communities, especially in the South of the country, where it is considered its origin (Ñústez, 2018).

The development of the crop has a wide variability of production conditions, which depend on environmental heterogeneity. Thus, the agronomic management activities obey the specific behavior of the cultivars and their interaction with the environments and systems. One of the main parameters that defines the type of activities in the cultivars corresponds to the tuber production cycles; these can be defined as early, medium, or late tuberization (Moreno, 2000).

For potato production in Colombia, regardless of the group or cultivar used, fertilization is one of the items with the highest share in production costs (approximately 20%). This practice is generally carried out based on empirical knowledge and in the absence of soil analysis. However, studies conducted by Bautista *et al*. (2012), on nutrient absorption in the Criolla Colombia variety, indicate that the elements N, P, K, Ca, Mg, Zn, B, and S are the most important and with the highest absorption up to 113 days after planting (DAP). Likewise, Torres and Suarez (2014) did not find differences in absorption levels after 77 DAP, when evaluating commercial doses in short cycle genotypes. Muñoz and Lucero (2008) found higher yields for commercial varieties when they added between 800 and 1200 kg ha^{-1} of organic matter and 300 kg ha^{-1} of 13-26-6 (NPK -nitrogen-phosphorus-potassium).

In addition to the nutritional component, factors such as environment and cultivar and their interaction determine yield capacity. Yield studies of the Phureja group vary significantly when contrasting traditional cultivars with commercial varieties. For example, yields from 6.2 to 27 t ha^{-1} have been reported for the former (Gómez-García, 2017) and for the latter up to 41.8 t ha^{-1} (Santos, 2010; Bautista *et al*., 2012). Silva *et al*. (2017) evidenced effects on physiological parameters in creole potato with variation in doses of potassium fertilizers and different sowing densities, works that suggest variability in the responses associated with the interaction of environmental, nutritional, and genetic factors. Therefore, it is convenient to study fertilization on cultivars, in addition to nutrient doses, management systems, and interaction with particular soil and climatic conditions.

Marcillo *et al*. (2021) and Benavides *et al*. (2021) compiled relevant information and research results regarding potato cultivation in the department of Nariño, Colombia. In this sense, it is highlighted the definition of four zones or homogeneous environments with particular edaphoclimatic characteristics. These have among them differences in the mineral composition of the soils, management practices and socio-cultural aspects, which imply diversity of responses of the genotypes identified as predominant in the potato zone of the department.

Based on the above considerations, this study aimed to evaluate the response of four short-cycle potato cultivars (three genotypes of *S. tuberosum* Phureja group and one of *S. tuberosum* Andigena group) to differential fertilization levels and soil and climatic conditions.

MATERIALS AND METHODS

Location

The study was carried out in the South of the department of Nariño - Colombia, with evaluations in three of the four homogeneous environments characterized by Marcillo *et al*. (2021), for the potato-producing area (AH1, AH2 and AH4, respectively), which have particular edaphoclimatic

characteristics of altitude, precipitation, temperature, slope, texture and soil pH (Table 1). The experimental plots were located in farmers' properties in the municipalities of Guaitarilla, Providencia and Guachucal, respectively. The climatic conditions in each environment evaluated were: in Guaitarilla (AH1) an accumulated rainfall of 543.9 mm, distributed throughout the crop cycle and an average daily temperature (ADT) of 13.7 °C (Figure 1a); in Providencia (AH2) an accumulated rainfall of 534.8 mm, with little rainfall at the beginning and end of the crop and an ADT of 12.1 °C (Figure 1b), lower than the historical range and, in Guachucal (AH3), accumulated precipitation of 479.4 mm, with low rainfall regimes at the end of the crop and an ADT of 9.9 °C (Figure 1c).

Table 1. Edaphoclimatic characteristics of three homogeneous production environments of *S. tuberosum* Phureja and Andigena groups in Nariño-Colombia and location of experimental plots.

Characteristics	Homogeneous Environment *		
	1 (AH1)	2 (AH2)	4 (AH4)
Location of the evaluation plot	1° 8` 8.87" N 77° 32`46.53" W	1° 11`26.55" N 77° 35`13.67" W	1° 1`50.83" N 77° 45`42.24" W
Locality	Guaitarilla	Providencia	Guachucal
Altitude (**masl**)	2200-2600	2600-2800	3000-3400
Precipitation (mm $year^{-1}$)	800-3000	800-3000	800-2800
Temperature (°C)	14.2-17.2	13.0-14.8	9.4-12.4
Pending (%)	5-12	25-50	50-75
pH	4.7-5.4	5.1-5.8	5.5-6.1
Organic matter (%)	6.0-8.0	5.0-10	20-35
Phosphorus (mg kg^{-1})	60-115	45-120	80-190
Potassium ($cmol^{+}$ kg^{-1})	0.6-0.9	0.4-0.8	0.6-10
Calcium ($cmol^{+}$ kg^{-1})	2.0-4.1	2.5-4.5	3.0-6.4
Magnesium ($cmol^{+}$ kg^{-1})	0.7-1.6	0.5-0.9	0.3-0.8

* Adapted from: Benavides *et al.* (2021)

Plant Material

The traditional cultivars Mambera and Ratona Morada and the varieties Criolla Colombia and Morasurco were evaluated. These were classified as the most frequently cultivated, in the short-cycle group, based on the characterization of the production system in Nariño (Benavides *et al.*, 2021). Mambera has a semi-extended growth habit, green stems, round tubers with deep eyes and red skin with cream spots. On the other hand, the growth habit of Ratona Morada is semi-erect, pigmented stem, purple-skinned tuber and white pulp (Tinjacá and Rodriguez, 2015). Criolla Colombia shows semi-erect plants, with thin stems and round tubers with yellow pulp and skin (Moreno, 2000). Morasurco has erect plants, thick dark green stems, flattened round tubers, purple skin with cream spots and cream-colored pulp, and has a similar crop cycle to Criolla Colombia, Mambera and Ratona Morada, which is why it was included in this study.

Experimental design

An experiment was established in each environment under a randomized complete block design with a split-plot arrangement and three replications. The main plot included three fertilization levels based on input from average soil analyses for the area and requirements of commercial varieties (Table 2) and the subplot included the four cultivars mentioned above. The experiments were established considering a planting density of 33.333 plants ha^{-1}. Each experimental plot occupied an area of 42 m^2, of which 25 m^2 were used for the evaluations.

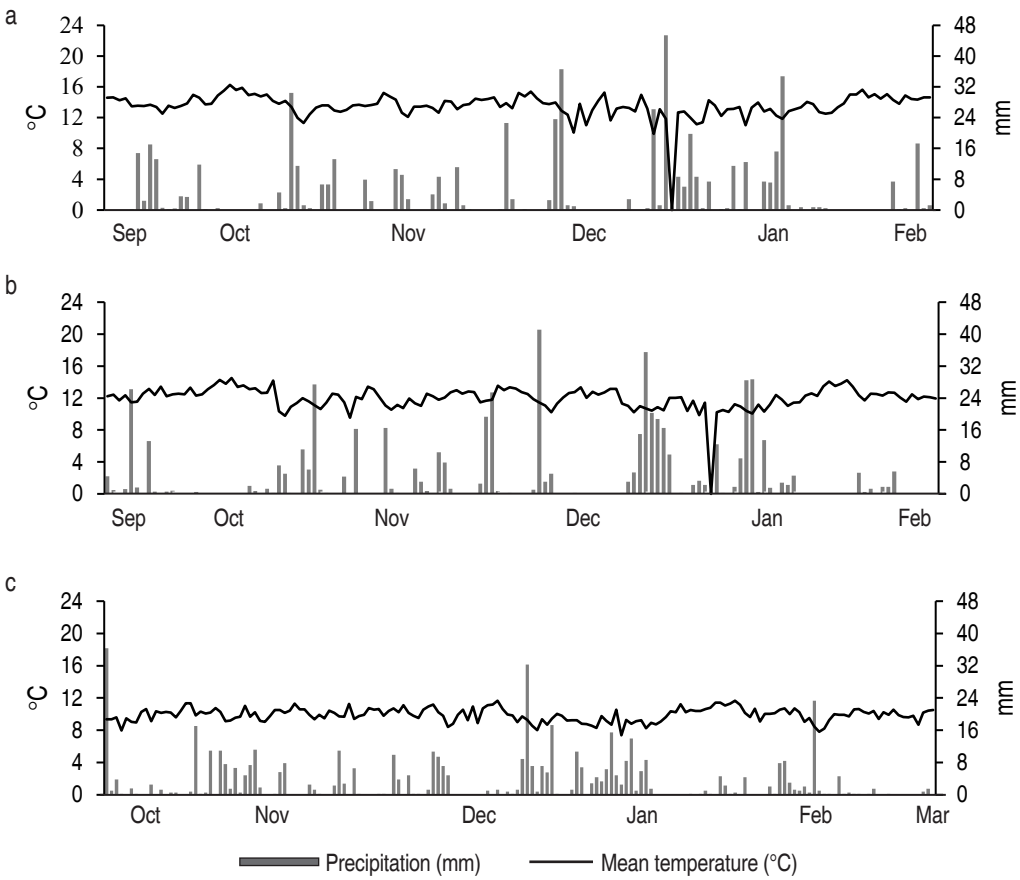

Figure 1. Climogram of experimental plots corresponding to classifications AH1, AH2 and AH4 in three municipalities of Nariño-Colombia: (a) Guaitarilla, (b) Providencia and (c) Guachucal, during the evaluation period (2017-2018).

Agronomic and physiological variables

Variables evaluated were selected according to the yield parameters. Destructive sampling was done every two weeks, from emergence to harvest, for a total of 24 plants per treatment; the samples were taken to an oven, Memmert brand, and kept at 70 °C until constant dry weight. With the information obtained, the accumulation of dry matter of the plant until the harvest was calculated. In addition, the leaf area was determined and in the field the soil area covered by the foliage, to estimate along with the above information: leaf area index (LAI), net assimilation rate (NAR), crop growth rate (CGR), and relative growth rate (RGR), according to methodologies adapted from Pandey *et al*. (2017). Harvest index (HI) was calculated as the ratio of tuber dry matter to that of the total plant, according to Mohamed *et al*. (2017).

Table 2. Fertilization levels for four short-cycle potato cultivars of the Phureja and Andigena groups in three environments.

Level	Contribution kg ha^{-1}		
	N	P_2O_5	K_2O
N1	25	75	50
N2	40	100	75
N3	50	125	100

In each experimental unit, in addition to the total yield, the number, size, and weight of tubers were recorded. A tuber size scale was generated for the cultivars of the Phureja group, according to the commercial classification in the area (Table 3). For Morasurco, the Colombian Technical Standard NTC 341 (ICONTEC, 1996) was used. The results of tubers of size zero and first in a single group were considered because they represent a higher commercial value.

Table 3. Classification of tubers by diameter for Criolla Colombia, Mambera and Ratona Morada.

	Diameter (cm)		
Size	**Criolla Colombia**	**Mambera**	**Ratona Morada**
Zero	>5.0	>8.0	>7.0
First	3.5 to 5.0	6.0 to 7.9	5.5 to 6.9
Second	2.0 to 3.4	2.5 to 5.9	4.0 to 5.4
Third	<2.0	<2.5	<4.0

Statistical analysis

Analysis of variance (ANOVA) was performed for each yield component and comparison of means with Tukey's test (P=0.05). Models were generated to explain dry matter accumulation by cultivar, in each evaluated environment. Discriminant analysis of principal components (DAPC) was performed with yield variables and physiological indices. This is a multivariate method to identify and describe groups of related individuals, which allows visual assessment of the differentiation between groups. The packages ExpDes (Ferreira *et al*., 2013), Adegenet (Jombart, 2008) were used in the statistical program R, version 4.0.3. (R Core Team, 2020).

RESULTS AND DISCUSSION

Performance Components

In environment AH1 the highest yield was reached by Morasurco (38.5 t ha^{-1}) and the lowest by Ratona Morada (19.4 t ha^{-1}). The yield of tubers of greater commercial value (size zero and first) of Criolla Colombia was 18.7 t ha^{-1}, which represents 80% of its total yield, contrasting with 24.0 t ha^{-1} in Morasurco, corresponding to 62%. In the number of tubers per plant, Criolla Colombia presented the highest value (24.5), while at the other extreme, Mambera and Ratona Morada presented 13.2 and 12.8, respectively. The HI for this environment ranged between 0.40 and 0.71, with statistical differences between Criolla Colombia (0.40) and the other cultivars.

In the AH2 environment, the averages were below those obtained in AH1. However, the cultivars benefited with the highest fertilization level (N3), increasing the yield by about 7 t ha^{-1} with respect to the averages reached with the other two levels (Table 4). There was no interaction effect between levels and cultivars, but there were differences between cultivars for the variables yield, number of tubers per plant and HI. For these variables, Morasurco presented the highest values, while Criolla Colombia, Mambera and Ratona Morada presented similar values between them, but lower than those observed in AH1.

Although AH4 produced 5 t ha^{-1} more at the N3 level than N1 and N2, there were no statistical differences (Table 4). Fertilization levels also did not affect the number of tubers per plant, but the HI was higher than those observed in the other environments. In this environment there were differences for cultivars in yield and number of tubers per plant; the genotypes with the highest yield were Morasurco (33.0 t ha^{-1}) and Mambera (30.8 t ha^{-1}). In the case of the number of tubers per plant and number of tubers of higher commercial value per plant stood out was Criolla Colombia.

In the evaluation of fertilization levels in *S. tuberosum*, Sifuentes *et al*. (2013) did not find differences in the extraction rates, but in the absorption levels. They inferred that this result is due to genetic and environmental factors, which determine the differential extraction between cultivars. This agrees with the results of the present work since differences were observed between genotypes within the environments, but it was not conclusive between the fertilization levels evaluated.

In Colombia, the importance of minor elements in potato fertilization is underestimated, since their application is not considered, although in the soil there are low levels of nutrients (Gómez *et al*., 2006). According to Marcillo *et al*. (2021), magnesium is one of the elements that presents lower concentrations in soils of the producing zone of the department of Nariño, being this element a possible influential factor on the productivity of the crop.

Table 4. Average yield components for four short-cycle potato cultivars in three environments in the department of Nariño.

Environment/ Factor	Yield (t ha^{-1})	Yield of tubers of highest commercial value (t ha^{-1})	Number of tubers per plant	Number of tubers with the highest commercial value per plant	Harvest Index
		Homogeneous Environment 1 (AH1)			
Level	0.724 ns	0.935 ns	0.531 ns	0.554 ns	0.332 ns
N1	28.8 a	20.9 a	18.0 a	10.1 a	0.62 a
N2	27.1 a	19.7 a	16.0 a	8.4 a	0.56 a
N3	27.0 a	20.6 a	17.6 a	8.7 a	0.54 a
Cultivar	<0.001 ***	0.005 **	<0.001 ***	0.002 **	<0.001 ***
Criolla Colombia	23.4 bc	18.7 ab	24.5 a	12.5 a	0.40 c
Ratona Morada	19.4 c	15.6 b	12.8 c	8.3 b	0.55 b
Mambera	29.3 b	23.3 a	13.2 c	8.6 b	0.64 ab
Morasurco	38.5 a	24.0 a	18.3 b	6.9 b	0.71 a
Level x Cultivar	0.981 ns	0.620 ns	0.615 ns	0.451 ns	0.175 ns
CV (%)	20.4	24.1	20.2	29.1	5.5
		Homogeneous Environment 2 (AH2)			
Level	0.044 *	0.252 ns	0.165 ns	0.436 ns	0.351 ns
N1	16.3 b	11.1 a	12.6 a	5.7 a	0.60 a
N2	17.1 b	10.7 a	15.2 a	5.9 a	0.59 a
N3	23.7 a	15.0 a	15.8 a	7.2 a	0.56 a
Cultivar	<0.001 ***	0.171 ns	0.003 **	0.103 ns	<0.001 ***
Criolla Colombia	12.5 b	8.9 a	18.6 a	8.4 a	0.55 b
Ratona Morada	14.6 b	10.0 a	11.1 b	4.5 a	0.55 b
Mambera	17.2 b	14.0 a	11.1 b	6.4 a	0.54 b
Morasurco	31.2 a	16.3 a	17.4 a	5.8 a	0.69 a
Level x Cultivar	0.891 ns	0.675 ns	0.982 ns	0.835 ns	0.825 ns
CV (%)	41.9	61.9	31.8	49.8	13.9
		Homogeneous Environment 4 (AH4)			
Level	0.503 ns	0.284 ns	0.734 ns	0.192 ns	0.002**
N1	25.1 a	13.9 a	20.3 a	5.8 a	0.67 a
N2	25.9 a	15.6 a	21.2 a	7.4 a	0.74 b
N3	30.7 a	20.3 a	21.3 a	9.8 a	0.70 c
Cultivar	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Criolla Colombia	22.5 b	14.7 b	29.9 a	11.9 a	0.62 a
Ratona Morada	22.7 b	15.7 b	16.6 b	6.9 b	0.73 b
Mambera	30.8 a	24.5 a	16.5 b	8.6 b	0.72 b
Morasurco	33.0 a	11.4 b	20.7 b	3.3 c	0.74 b
Level x Cultivar	0.331 ns	0.518 ns	0.800 ns	0.455 ns	0.009**
CV (%)	15.5	30.9	24.5	36.6	5.0

* Significance level $P<0.05$. ** Significance level $P<0.01$. *** Significance level $P<0.001$. ns: not significant. In each variable, within each variation factor, the averages with equal letters are not statistically different according to Tukey α <0.05). CV: coefficient of variation.

Considering the above, the fact that no differences were found between NPK fertilization levels in the evaluated cultivars, could indicate that the yield improvement for these cultivars may be conditioned to other elements of low availability.

Besides yield, it is important to analyze quantitative variables and yield components, as a base for the estimation of parameters to intervene in the agronomic management of the crop (Hay and Walker, 1989). The behavior of the yield components in the cultivars was

different in each environment, especially in the Criolla Colombia variety, which constitutes an advantage that can be used in the response and specific yield. It would be valid to propose particular practices between environments within cultivars and to identify productive alternatives by region (Huerta-Fernández *et al*., 2021).

Regarding HI results, the interaction level by cultivar was only significant in AH4 (Table 4; Figure 2); level N2 obtained higher HI for Criolla Colombia and Mambera, while N3 for Morasurco. Under harvest stages similar to those of the present study (senescent plant), Santos (2010) obtained values in Phureja genotypes from 67 to 73% at 2859 masl and from 51 to 66% at 2572 masl, ranges similar to those found in the present work.

For varieties of *S. tuberosum* of the Andigena group, Ñústez *et al*. (2009) found HI of 71 to 85%, coinciding with the behavior of the Morasurco variety in this study, which obtained in all environments the highest value (between 69 and 74%). The same authors suggest that parameters such as HI are essential in yield since it can be improved by agronomic practices that lead to a greater allocation of dry matter to the tubers.

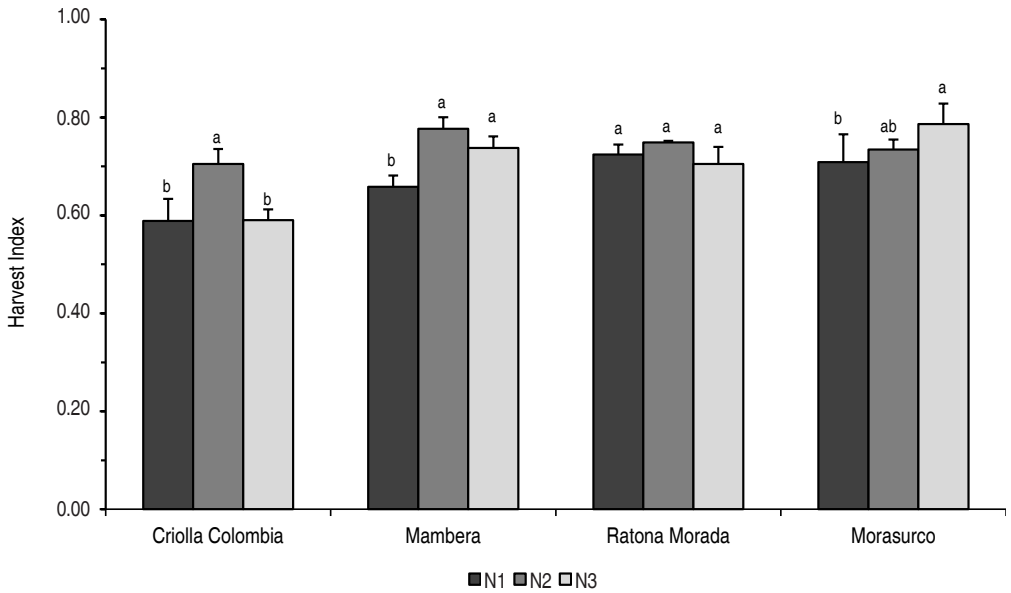

Figure 2. Harvest index in response to three levels of fertilization for four potato cultivars, in homogeneous environment 4 (AH4: Guachucal-Nariño locality). Within each cultivar, means with equal letters are not statistically different (P<0.05).

Dry Matter Accumulation and Physiological Indices

In the three environments, dry matter accumulation of all cultivars was adjusted to a quadratic model (Figure 3 a-c), with highly significant adjustments (R^2=0.97; P<0.01). In AH1 and AH2 the length of the growing season was similar, but dry matter accumulation was lower in AH2. At the end of the cycle, the cultivar with the lowest dry matter accumulation in AH1 was Criolla Colombia (176.9 g plant^{-1}), followed by Mambera (250.5 g plant^{-1}) and Ratona Morada (288.5 g plant^{-1}) and the highest accumulation was in Morasurco with 350.2 g plant^{-1} (Figure 3a), and a cycle 15 days longer than the other three. For environments AH2 and AH4, dry matter accumulation was in the same order, with lower values for Criolla Colombia, followed by Mambera, Ratona Morada, and Morasurco, with 133.3 g plant^{-1}, 161.8 g plant^{-1}, 186.6 g plant^{-1} and 278.7 g plant^{-1} in AH2 (Figure 3b) and 218.9 g plant^{-1}, 213.9 g plant^{-1}, 272.7 g plant^{-1} and 302.8 g plant^{-1} in AH4 (Figure 3c), respectively.

The dry matter content of a plant is related to the efficiency of the foliage to intercept and use solar radiation during its productive cycle. Besides being a genetically controlled trait, it is affected by genotype-by-environment interaction and management practices (Hay and Walker, 1989). This aspect was especially observed in AH4, where

the accumulation was slower, consistent with what was expected in the environment of higher altitude and lower average temperature.

Saldaña-Villota and Cotes-Torres (2020) evaluated this variable in three potato cultivars of the *Phureja* group at 2575 masl and found that regardless of the fertilization level used, the highest accumulation occurred in the same phenological stage, with a similar behavior among genotypes and values around 220 g plant^{-1}. These values were higher than those found in the present work, even for Morasurco (Andigena), which, despite having an early tuberization cycle (Moreno, 2000), has a different architecture and leaf mass than the Phureja group. This result is possibly related to the climatic conditions during the evaluation, which was a relatively dry period (below requirements) in all environments.

Regarding the Leaf Area Index (LAI) in the AH1 environment, the highest values found were 4.1, 3.4, and 2.9 m^2 m^{-2} at 78 DAP for Mambera, Morasurco and Ratona Morada, respectively (Figure 3d), while for Criolla Colombia, it occurred at 92 DAP with 2.7 m^2 m^{-2}. In AH2, the LAI presented peaks at 56 and 84 DAP, for the four cultivars (Figure 3e), the latter being more pronounced. Ratona Morada showed the highest index (4.2 m^2 m^{-2}), while the lowest value was for Morasurco (3.5 m^2 m^{-2}). In AH4 there were also two defined peaks for LAI values, in the four cultivars: the first one close to 48 DAP and the second one at 76 DAP. The highest index value occurred in Ratona Morada (7.7 m^2 m^{-2}), although the other three cultivars presented similar values (Figure 3f).

The LAI obtained its highest value in the tuberization phase and decreased from that moment until harvest.

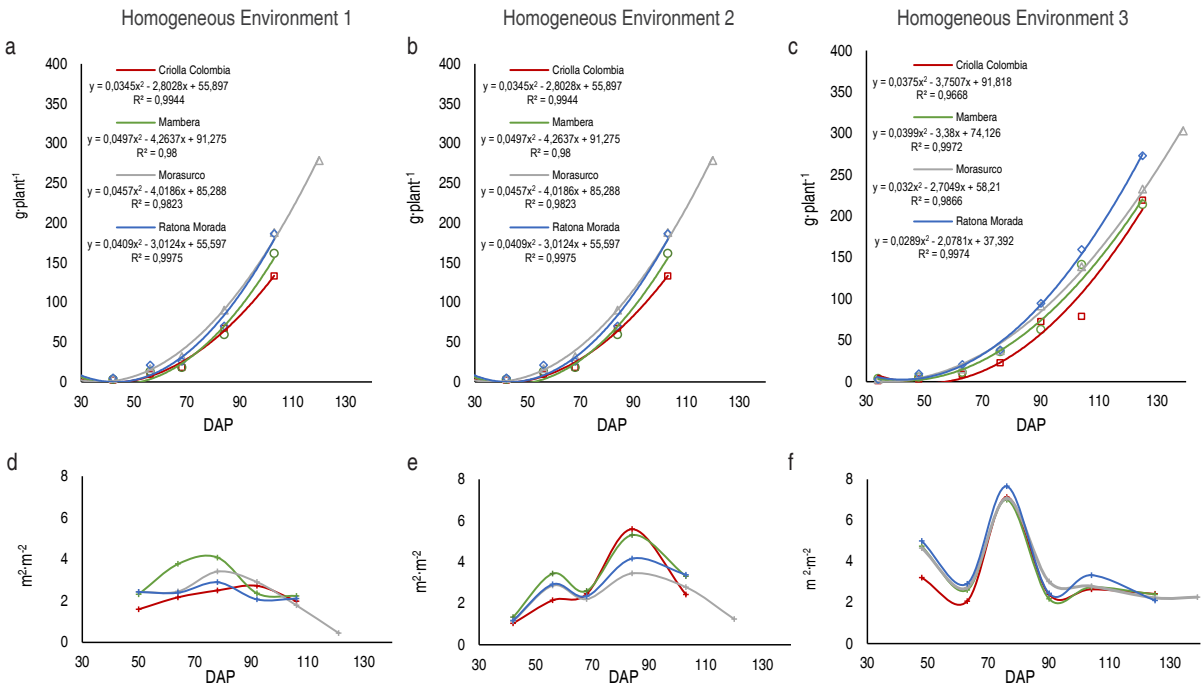

Figure 3. Average accumulated dry matter (a, b, c) and leaf area index (d, e, f) of four potato genotypes in three environments. DAP: days after planting.

This behavior was also observed by Saldaña-Villota and Cotes-Torres (2020), in the evaluation of Phureja cultivars with different fertilization levels, which also agrees with the observations made by Santos-Castellanos *et al*. (2010). A decrease in the LAI value prior to the maximum peak was also observed, especially in AH2 and AH4, behavior that is the result of agronomic practices or by translocation of assimilates that cause leaf abscission at the end of the cycle, that affect the components of the index (Montoya *et al*., 2016). On the other hand, the differences in altitude between the evaluated environments, which would suppose a

higher thermal utilization index in lower altitudes or with less accumulated thermal time (Lizarazo, 2020), for the present study the time to tuberization was similar between environments, both for the cultivars of the Phureja group and for the Andigena group, due to lower precipitation in AH4.

The values obtained for the Net Assimilation Rate (NAR) were below 0.1 g dm^{-2} day^{-1}. The maximum values were reached near 60 DAP for all cultivars in AH1 and AH2, while in AH4 they had a differential behavior among cultivars (Figure 4). This index is an indicator of photosynthetic efficiency, which relates the dry matter produced to the leaf area present during a given period of plant growth.

In AH1 all cultivars presented the highest NAR value at 64 DAP (0.095 g dm^{-2} day^{-1}), then it decreased gradually, with a slight increase in the last period evaluated, except in Criolla Colombia, whose values decreased until the end of the period. The cultivars Criolla Colombia and Ratona Morada decreased in smaller proportion with respect to Mambera and Morasurco, which decreased to 0.019 and 0.029 g dm^{-2} day^{-1}, respectively. In AH2, the maximum values was presented at 56 DAP, followed by the lowest rates at 68 DAP for all cultivars. In this environment, Criolla Colombia and Morasurco were the cultivars with less extreme variations, unlike Mambera and Ratona Morada, in which the NAR dropped to 0.020 and 0.007 g dm^{-2} day^{-1}, respectively (Figures 4a and 4b).

The NAR for AH4 presented a diverse behavior in each cultivar. Morasurco and Ratona Morada presented the highest value at 90 DAP (0.069 and 0.071 g dm^{-2} day^{-1} respectively). In Mambera the maximum NAR was presented at 104 DAP (0.086 g dm^{-2} day^{-1}) and its lowest value at 125 DAP, inverse behavior to that of Criolla Colombia, which presented at 104 DAP with the lowest rate (0.009 g dm^{-2} day^{-1}) and at 125 DAP the highest value (0.082 g dm^{-2} day^{-1}) (Figure 4c).

The CGR was particular in each environment, in AH1 the maximum value was found in one growth stage, in AH2 two stages, and in AH4 there was not a predominant behavior (Figure 4). In AH1 the highest values obtained were 0.10-0.13 g g^{-1} day^{-1}, at 64 DAP for all cultivars (Figure 4d). From this point, Criolla Colombia was the most efficient since it maintained higher values during the rest of the crop cycle. On the other hand, in AH2, Morasurco had only one maximum stage, in contrast to the other cultivars, which presented the first stage at 56 DAP and the second at 84 DAP (Figure 4e). In the case of AH4, the relative growth rates were lower than 0.09 g g^{-1} day^{-1}. In the latter, the values for Mambera, Morasurco and Ratona Morada were not lower than 0.02 g g^{-1} day^{-1} in the whole cycle (Figure 4f), unlike what was presented in the first two environments, where the CGRs went down to 0.01 g g^{-1} day^{-1}. These results coincide with those reported by Santos (2010), who observed the highest values in the early stages of the crop cycle. In the case of Criolla Colombia, they agree that the minimum values of NAR were concordant with the minimum values of RGR under the influence of the low level of fertilization.

Similarly, in AH1, the highest value of CGR expressed as weight gain per unit area and time, occurred at different times in each cultivar and environment: for Mambera at 64 DAP (0.18 g dm^{-2} day^{-1}), Morasurco at 78 DAP (0.14 g dm^{-2} day^{-1}), Criolla Colombia 92 DAP (0.10 g dm^{-2} day^{-1}) and Ratona Morada at 106 DAP (0.12 g dm^{-2} day^{-1}). The last two presented the most stable growth throughout the cycle (Figure 4g). The CGR in AH2, in agreement with the RGR, presented two periods of increase in the rate, the first at 56 DAP and the second later at 84 DAP (Figure 4h). The four cultivars presented the highest growth rates in AH4, at 76 DAP, where 0.301 g dm^{-2} day^{-1} was registered in Criolla Colombia, 0.273 g dm^{-2} day^{-1} in Mambera, 0.287 g dm^{-2} day^{-1} in Morasurco and 0.213 g dm^{-2} day^{-1} in Ratona Morada. A second stage occurred in Ratona Morada and Mambera at 104 DAP (Figure 4i).

In contrast to this work, Saldaña-Villota and Cotes-Torres (2020) evidenced differences in growth through physiological parameters, as a response to various levels of fertilization. These authors observed higher growth in the Criolla Latina variety with the highest dose, while in the Guaneña variety with a lower dose, indicating different levels of earliness. However, similar to this study, there was a decrease in growth rates at the end of the cycle. Santos (2010) evaluated genotypes of Phureja in differential environmental conditions, who also observed the increase of growth rates until around the middle of the productive cycle, and then decrease until the end.

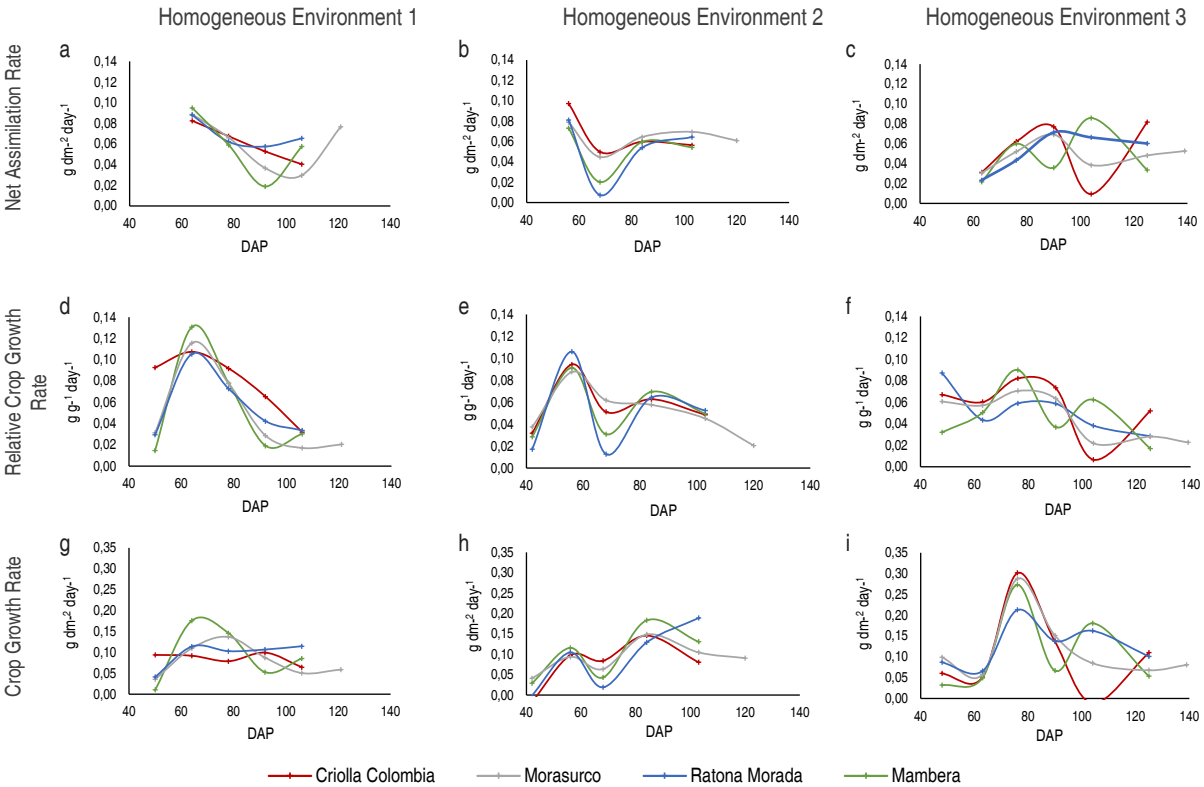

Figure 4. Growth rates in four short cycle potato genotypes in three environments: Net Assimilation Rate (a, b, c); Relative Crop Growth Rate (d, e, f); Crop Growth Rate (g, h, i). DAP: days after planting.

The behavior for the parameters NAR, RGR, and CGR, was predictable in terms of the altitudinal differential of the locations evaluated, showing the highest values in the highest environments. Therefore, these parameters should be analyzed at the cultivar level in each environment.

Discriminant analysis of principal components.

In the analysis of the physiological and yield variables, including information from the three environments, were summarized in the first two principal components of DAPC (Figure 5). The cultivars showed a response pattern where the Morasurco cultivar differs from the genotypes of the Phureja group and among these Criolla Colombia separates from Ratona Morada and Mambera that, according to the evaluated variables, have a similar behavior. The variables that had the greatest influence were HI, NAR, RGR, and CGR.

This result agrees with the differences captured in the analysis of variance between cultivars, within each environment (Table 4), which can be attributed to their genetic variability. This aspect was also considered by Huerta-Fernández *et al.* (2021), who attributed these differences to the genetic factor when they evaluated the yield of genotypes of the Phureja group in differential environments over time.

Two main groups can be found in the biodiversity of cultivated potatoes. The first group includes the so-called traditional or native varieties, which are the result of a process of domestication, selection, and ancestral conservation, and are generally associated with marginal production zones in high altitude or moorland areas. In the second are located the varieties that result from a process of genetic improvement; these possess attributes in terms of yield potential, resistance to phytopathogens, and organoleptic characteristics, among others (Monteros and Reinoso, 2010). The results evidenced in the present study agree with this classification. The genetic variability is represented initially by the Andigena group, with the variety Morasurco, coming from genetic improvement, while in the Phureja group the variety Criolla Colombia is a variety improved by clonal selection, which is separated from Ratona Morada and Mambera, which are traditional or native cultivars (Figure 5).

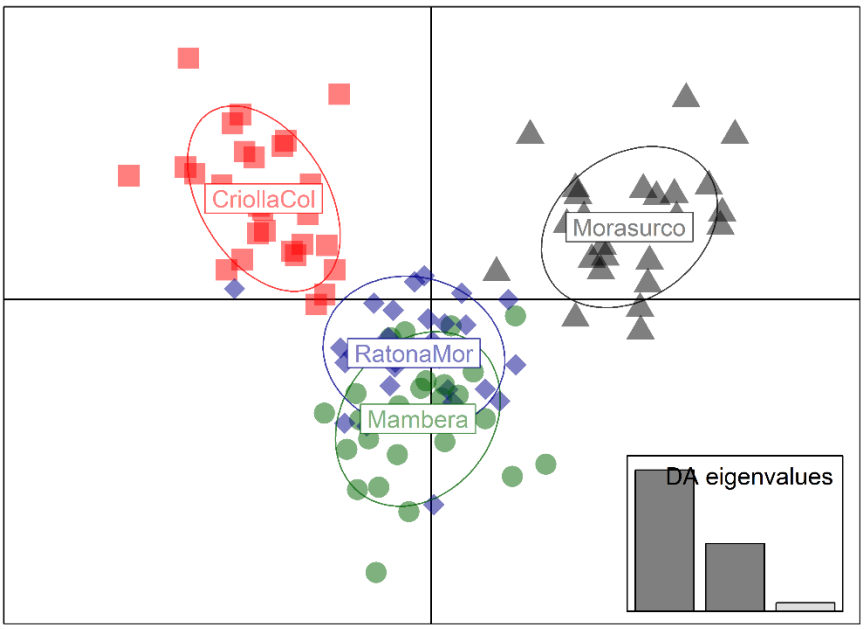

Figure 5. Biplot of DAPC of four short-cycle potato cultivars. The dispersion in the first two principal components is plotted, using the cultivars as a priori groups. The ellipses of inertia are subscribed according to the experimental units of the cultivars, in the three homogeneous environments evaluated, represented with squares for Criolla Colombia, diamonds for Ratona Morada, circles for Mambera and triangles for Morasurco.

On the other hand, the conformation of homogeneous environments based on altitude, climatic information, relief, and soils (Marcillo *et al*., 2021), generated differential responses in the cultivars. The physiological variables NAR, RGR, and LAI had the greatest influence on the discrimination of environments in the DAPC (Figure 6).

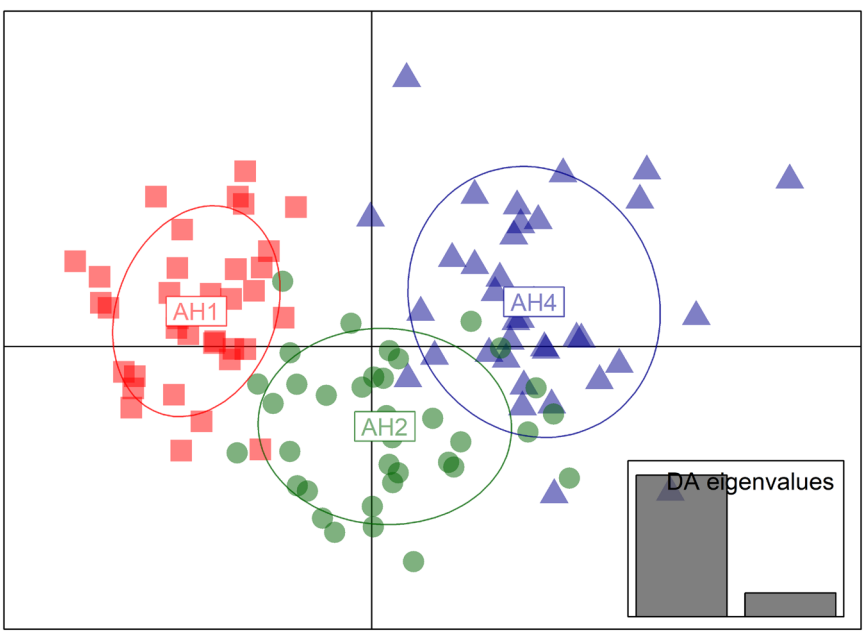

Figure 6. Biplot of DAPC of short-cycle potato cultivars in three homogeneous environments (AH). The dispersion of four cultivars in the first two principal components of the DAPC is plotted, using the homogeneous environments as a priori groups. The inertia ellipses underwrite the experimental units of the cultivars, which for AH1 are represented by squares, for AH2 by circles and for AH4 by triangles.

The research of potato cultivars in different environments has been mainly focused on the genetic factor, caused in part by the processes of domestication, cultural exchange, and native or improved varieties that, according to the area where it is produced, acquire management plans associated with edaphoclimatic requirements, without considering fundamental aspects that can differentiate them. The choice of experimental plots in the present study was due to an adequate selection of environmental diversity for the producing area, ratifying the importance of the evaluation of practices or management systems of cultivars, which sometimes could be more efficient in specific areas.

CONCLUSIONS

Considering yield and DAPC, differences in behavior were observed for the Morasurco cultivar with respect to the Phureja cultivars and within this group Criolla Colombia differed from the other two. The productivity of the evaluated cultivars was mainly influenced by the environments. Ratona Morada and Mambera are alternatives for high zones because they presented higher yield in AH4, an aspect possibly associated with its traditional character, which suggests better adaptation in high zones and, on the contrary, Criolla Colombia when evidencing better yield in AH1 and AH2 indicates its adaptation with respect to zones of higher altitude. Morasurco presented a similar behavior in the three environments, indicating wide adaptability of the cultivar in different productive conditions.

Except for the AH2 environment, for the variable yield per hectare, and AH3 for HI, the fertilization levels evaluated did not present statistical differences. This behavior was observed in the yield components as well as in the physiological parameters. Possibly the nutritional requirements of the evaluated genotypes are limited by elements different from those evaluated and it is required to deepen the evaluation of sources or levels related to secondary and minor elements that allow improving the productivity.

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EDITORIAL

SESENTA AÑOS DEL PROGRAMA DE ZOOTECNIA EN LA UNIVERSIDAD Y EN COLOMBIA

Tendríamos que remontarnos a los hallazgos arqueológicos pre-neolíticos para encontrar las primeras formas de interacción del hombre con los animales, de donde se confirmaría una dependencia milenaria que, a raíz de la domesticación, más bien pudiera llamarse de "interdependencia", como quiera que desde la Revolución Neolítica hasta nuestros días se trata de una coevolución biológica de muchas especies animales y del mismo hombre.

De la caza furtiva y la pesca, se pasó pronto a la cría y el levante en condiciones de domesticación. Tener rebaños y animales de corral para llevar consigo en las largas jornadas migratorias del hombre primitivo, a la par con las semillas y frutos cosechados en sementeras, fue el impuso necesario para que el hombre diera el salto al pensamiento, a la cultura, al arte y a la ciencia. Tal vez no haya otra relación más virtuosa en la larga historia de la evolución del hombre en la Tierra que la mantenida con los animales.

Carne, huevos, leche, pieles, grasas, compañía, fuerza de tracción, son entre muchos otros los bienes y servicios derivados de los animales. Y el acervo de conocimientos se ha venido desarrollando desde antaño en numerosas culturas y lugares, dando lugar a lo que hoy conocemos como la profesión de la Zootecnia, del griego "zoon" y "tekhne", la ciencia que se dedica al estudio del aprovechamiento de los animales domésticos, considerando su reproducción, desarrollo y utilización, en condiciones de garantizar su bienestar integral en las explotaciones pecuarias.

A principios del siglo XX, cuando la población colombiana era de apenas unos cinco millones de habitantes, los productos para la alimentación provenían exclusivamente de la agricultura campesina (*lato sensu*), realizada en pequeñas granjas con técnicas simples derivadas de la tradición indígena y española; eran entonces muy evidentes los problemas ligados a la salud animal que de varias maneras repercutían en la salud humana. Fue este uno de los intereses que llevaron a la creación por parte de la Gobernación de Antioquia de la Escuela de Agricultura Tropical y Veterinaria, ideada en el año 1911, pero formalizada por la Asamblea Departamental de Antioquia en 1914. Esta necesidad, conjuntamente con el interés por desarrollar la caficultura y el buen manejo de las granjas con sus hatos, avícolas, piaras y cultivos, permitieron conformar por primera vez para el país el programa profesional de Ingeniería Agronómica.

Pero lo que resulta realmente interesante de verificar en el plan de estudios de esa novel carrera, es la inclusión de seis asignaturas relacionadas con lo que hoy llamamos Zootecnia: Zoología, Animales de Corral (dos cursos), Zootecnia (dos cursos) y Tecnología (obtención y transformación de productos de origen animal), lo que equivaldría a un 15% de todo el pensum de la carrera. Es así como puede considerarse que el surgimiento de la Zootecnia en la actual Facultad de Ciencias Agrarias data de 1914, no obstante la creación formal de la carrera de Zootecnia y del Departamento de Producción Animal se haya dado el 2 de julio de 1962.

Habiendo llegado entonces a la efeméride notable de los 60 Años de creación de esta carrera en la Facultad, a la sazón la primera ofertada en el País y muy seguramente la primera en Latinoamérica, reconocemos en este proyecto de permanente evolución el talento y esfuerzo dedicado por los profesores de varias generaciones que han transitado por el Departamento de Producción Animal, teniendo como punto de partida a los fundadores: Lucio Rodríguez Galindo, considerado "Padre de la Zootecnia en Colombia", a Samuel Posada Saldarriaga (exDecano)

y a Francisco Villegas Bernal. Los cientos de zootecnistas egresados, dispersos por toda Colombia y varios países del mundo, los actuales estudiantes y todos los importantes desarrollos en investigación contextualizada a las condiciones intertropicales de Colombia, son la materialización de un proyecto académico visionario que hoy sigue rindiendo frutos notables para el agro colombiano y para la ciencia.

El Primer Congreso de Producción Animal de Colombia UNAL2022, COPACO, "60 Años de la Zootecnia en Colombia", reunido en el campus de la Facultad, en la ciudad de Medellín, Colombia, entre el 21 y 23 de julio de 2022, con la participación de expertos nacionales e internacionales y decenas de asistentes, ha sido la pausa apropiada para dar a conocer a los actores personales, empresariales e institucionales de la Zootecnia, los desarrollos y avances en las ciencias y técnicas que le son propias, y también la ventana propicia para mirar el futuro retador y prometedor que se abre cada día para las ciencias de la producción animal. Enhorabuena, el COPACO exalta la profesión que celebramos por sus 60 Años, con su origen virtuoso en la muy noble y centenaria Facultad de Ciencias Agrarias.

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A methodological proposal for quality control of the soil moisture variable, measured in Colombian automatic agrometeorological stations

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Propuesta metodológica para el control de calidad de la variable humedad del suelo, medida en las estaciones agrometeorológicas automáticas colombianas

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ABSTRACT

Methodological criteria for data quality control with geophysical range and spectrum consistency were evaluated, establishing flags and quality indicators for soil moisture data records, in a range of depths between 10, 30, and 50 cm, from automatic agro-meteorological stations located in the most important agricultural regions of Colombia. Data for analysis were collected from 105 stations of the IDEAM network, in an observation window from 2001-2020. The results showed that 40.3% of the soil moisture data were of good quality, 12.9% were questionable due to spectrum flags, 14.3% were questionable due to geophysical range and 32% were erroneous because the values were not possible and/or missing. The depth closest to the surface had the highest number of quality flags, suggesting that the soil layer has the highest error detection rate associated with soil moisture condition recording; the most common quality flag was C02: "Soil moisture >60% & ≤100%", detected in 93% of the sensors, and the second most frequent flag was C01: "Soil moisture ≥0% & <3%". It was concluded that the proposed methodology provides highly satisfactory results in the detection of anomalous soil moisture records, in order to make adjustments to the environmental conditions of Colombia.

Keywords:
Agrometeorology
Physical limits
Soil moisture
Spectrum consistency

RESUMEN

Se evaluaron criterios metodológicos para el control de calidad de datos con rango geofísico y consistencia del espectro, estableciendo banderas e indicadores de calidad para los registros de datos de humedad del suelo, en un rango de profundidades comprendido entre los 10, 30 y 50 cm, de las estaciones agrometeorológicas automáticas ubicadas en las regiones agrícolas más importantes de Colombia. Los datos para su análisis fueron recopilados en 105 estaciones de la red del IDEAM, en una ventana de observación comprendida entre los años 2001-2020. Los resultados evidenciaron que el 40,3% de los datos de humedad del suelo eran buena calidad, el 12,9% eran dudosos por banderas de espectro, el 14,3% eran dudosos por rango geofísico y el 32% eran erróneos porque los valores no eran posibles y/o faltaban. La profundidad más cercana a la superficie tuvo el mayor número de banderas de calidad, lo que sugiere que la capa de suelo presenta la mayor tasa de detección de errores asociada con el registro de la condición de humedad del suelo; la bandera de calidad más común fue C02: "Humedad del suelo > 60% & < = 100%", detectada en el 93% de los sensores, y la segunda bandera más frecuente fue C01: "Humedad del suelo > = 0% & <3%". Se concluyó que la metodología propuesta proporciona resultados altamente satisfactorios en la detección de registros anómalos de la humedad del suelo, para efectuar ajustes a las condiciones ambientales de Colombia.

Palabras clave:
Agrometeorología
Límites físicos
Humedad del suelo
Consistencia del espectro

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Soil moisture is defined as the surrounding water content in the root zone, which can reach a depth of up to 200 cm, used for the growth and development of plants (Houser, 2010; Sonkar *et al*., 2019). The soil moisture content describes temporal water availability for plants and provides an integrated assessment of the relative water supply status versus demand (Deng *et al*., 2016; Wyatt *et al*., 2021). *In situ* soil moisture measurements are fundamental inputs for evaluating and adjusting moisture estimates derived from numerical and satellite models (Dorigo *et al*., 2013; Liu *et al*., 2021).

Automated Quality Control (QC) methods are widely used in various geophysical disciplines, especially for variables such as air temperature and precipitation (Hubbard *et al*., 2005). There are two QC methodologies, the first one using data from multiple sites that are compared to neighboring stations (Hubbard, 2001; Sanhudo *et al*., 2021) and the second one using data from the same site (Meek and Hatfield, 1994; Yu *et al*., 2018).

The quality of *in situ* soil moisture information is essential to improving the reliability of subsequent validations or adjustment studies with numerical models and satellite products (Wang *et al*., 2021; Dorigo *et al*., 2015; Albergel *et al*., 2012).

QC algorithms consist of procedures or rules that evaluate data to detect errors; each procedure will accept data as true or reject the data and label the data as an outlier or doubtful. If data are valid and accepted or data are invalid and rejected, the QC procedure works correctly. When valid data are rejected by QC, a type I error is confirmed; if the data are invalid but accepted by QC, a type II error is committed (Hubbard *et al*., 2005).

Soil moisture is influenced by atmospheric variables (precipitation, temperature, wind speed, and evapotranspiration, among others), geomorphological and physical conditions, and land cover surrounding the sensor, such as soil texture, terrain slope, and vegetation cover (Hubbard *et al*., 2005; Lu *et al*., 2021).

The most common methods for the detection of outliers are based on thresholds that can be static or variable (Journée and Bertrand, 2011; Dorigo *et al*., 2013; Sanhudo *et al*., 2021). Spectrum-based approaches study the structure of a measured time series to identify outliers with reliable, long-term climates (>30 years), which are not available for the soil moisture variable in Colombia.

In the sites of each station, the physical conditions of soil and vegetation cover are different, so the quality control of the soil moisture variable must be carried out with data from the site where the sensor is found, moreover, the sensors within the network are spaced apart, making the use of neighboring stations for the QC process unfeasible.

In this sense, this study aimed to evaluate the quality of soil moisture measurements by Instituto de Hidrología, Meteorología y Estudios Ambientales (IDEAM) network automatic stations with measurement marking methodologies developed by Dorigo *et al.* (2013) and expanded by Heer (2017). The evaluated soil moisture values were classified into quality flags that determined geophysical range limits and unusual behaviors (Hernández *et a*l., 2021).

MATERIALS AND METHODS

Soil moisture data

The QC methodologies for the soil moisture data were adjusted for the stations' hourly data with the quality controls of geophysical range and spectrum consistency for the IDEAM agrometeorological station network, examining the soil moisture time series structure with first and second derivatives from the Savitzky-Golay filter (Xaver, 2015; Torres *et al*., 2020), the International Soil Moisture Network (ISMN) methodology review (Dorigo *et al*., 2013), and adjustment of the algorithms to the particular conditions of Colombia (Fischer *et al.,* 2022).

The data were compiled by the IDEAM network of automatic agrometeorological stations, which recorded soil moisture contents at three (3) different depths, along with other atmospheric variables with data quality processes (precipitation, temperature, wind velocity, and solar radiation), with TRIME-EZ soil moisture sensors (IMKO GmbH, German) based on Time Domino Reflectometry (TDR).

Quality Control Methodology

The best quality control for moisture data is visual inspection, with variable inputs such as precipitation, soil temperature, and air temperature (Xaver, 2015). However, for hourly data with large volumes of data and operational applications, this is impractical, inefficient, and time-consuming. Therefore, the ISMN Quality Control process is used to identify and mark spurious observations automatically (Dorigo *et al*., 2013), as adapted by Heer (2017) who included other spectrum consistency flags. Quality control does not manipulate or alter data but denotes data with quality flags. The different quality flags are encoded with letters according to the Code of Practice (CEOP). Quality control is classified into two flag groups:

- Quality control for geophysical range inspection
- Quality control with spectrum-based methods

Spectrum-based errors are sudden and unnatural changes in a soil moisture time series. These suspect measurements can be caused by a sensor malfunction, a lack of response from the sensor, a problem with the power supply, or a connection problem when registering the data. These errors can be detected by examining the time series structure (hourly) of the soil moisture and analyzing the first and second derivatives. These faults can generally be categorized into three types: jumps, peaks, and constant values. (Dorigo *et al*., 2013; Xaver, 2015; Heer, 2017).

The ISMN quality indicators are divided into three categories: 1) out-of-limit values, 2) geophysical consistency checks, and 3) spectrum-focused (Table 1), errors based on out-of-limit values were not evaluated, IDEAM performs this type of control before delivering the data.

Table 1. ISMN quality categories and flags, adjusted for this study.

Category	Flag	Description
Outside of limit	C01*	Soil moisture >=0% & <3% m^3 m^{-3}
Outside of limit	C02*	Soil moisture > 60% & <=100% m^3 m^{-3}
Outside of limit	C03	Soil moisture> saturation point (derived from HWSD parameter values) Harmonized World Soil Database
Geophysical consistency	D01	*In situ* soil temperature <0 °C in the corresponding depth layer.
Geophysical consistency	D02	*In situ* air temperature <0 °C
Geophysical consistency	D03	GLDAS soil temperature <0 °C in the corresponding depth layer.
Geophysical consistency	D04	Soil moisture shows peaks with no in situ precipitation event prior to 24 h.
Geophysical consistency	D05	Soil moisture shows peaks without GLDAS precipitation even 24 h earlier.
Spectrum	D06*	Peak in the soil moisture spectrum.
Spectrum	D07*	Negative jump in the soil moisture spectrum.
Spectrum	D08	Positive jump in the soil moisture spectrum.
Spectrum	D09	Low constant values (minimum 12 h) in the soil moisture spectrum
Spectrum	D10	Saturated plateau (minimum 12 h) in the soil moisture spectrum.
Spectrum	D11*	Suspect value before NaN (Not a Number).
Spectrum	D12*	Suspect value after NaN (Not a Number).
Spectrum	D13*	Severe drop in soil moisture.
Spectrum	D14	Alternate values.
Spectrum	D15	Constant Values.
Spectrum	D16	Highly marked spectrum.

Source: Adapted from Heer (2017).

Negative values and values greater than 100% input were marked as erroneous (M) since the sensor cannot register these values. Additionally, these records are associated with a malfunction in the sensor and/or data recording system (Table 2).

Some ranges were modified, such as quality controls for out-of-range values, and flags that are activated by soil moisture values that are theoretically possible but unlikely under Colombian conditions (C01) because of the peculiarities of this variable. Also, some threshold modifications were made for the spectrum consistency flags. For this reason, the flags were grouped into a few categories, which excluded measurements such as negative soil moisture values and values higher than 100%, which physically are not possible, and which were initially marked as wrong values.

Table 2. Soil Moisture Quality Control Indicators.

Indicator	Description	Flag
G	Good (Standard for all flags)	None
D	Doubtful Spectrum Consistency	D04, D05, D06, D07, D08, D09, D10, D11, D12, D13, D14, D15 and D16
R	Doubtful geophysical range	C01, C02, C03, D01, D02 and D03
M	Wrong or lost	Lost data and/or out of range of sensor readings

C03, D03, and D05 flags are used when information is only available from a soil moisture sensor; therefore, estimates from numerical or satellite models are needed for synthetic data on precipitation, air, and soil temperatures.

Quality control of out-of-range values

C01 and C03 flags were used if a value exceeds the established minimum or maximum limits. These limits in the ISMN are defined for moisture variables between 0% and 60% of the volumetric water content in the soil as a maximum value although, under Colombian climatic and edaphic conditions, soil moisture values of 0% are extremely unlikely. Therefore, a soil moisture value of 0% is due to a sensor failure resulting from a loss of the power supply.

The dry season or periods with less frequent rainfall (rain supply less than the potential evapotranspiration demand) do not exceed eight (8) months, an insufficient period for soils to naturally reach moisture values of 0% In addition, at depths close to 10 cm, the relative humidity in the equatorial region is high, and the Intertropical Convergence Zone (ITCZ) in Colombia contributes to an increase in cloudiness that affects the evaporation process because of the low incidence of solar radiation and the prevalence of calm winds or low wind speeds. These meteorological aspects do not actively contribute to soil moisture loss, so the minimum limit was set at 3%, which is the lower limit of the permanent wilting point for a coarse soil texture (a texture that has the least possibility of storing water) (Figure 1 A,B).

Quality control of geophysical consistency. D01 and D05 flags were used for soil moisture values when inconsistencies or incongruities are recorded with other geophysical variables, such as soil temperature, air temperature, and precipitation. An inconsistency with temperature has to do with the process of freezing and thawing of the soil, where the sensor erroneously registers lower moisture in frozen soils because the dielectric conductivity of solid water (ice) is significantly lower than liquid water. Frozen water in the soil leads to a significantly lower soil moisture content (Dorigo *et al.*, 2013).

Soil takes longer to freeze when it has vegetation cover and/or snow because the cover acts as thermal insulation. Freezing only begins when the average air temperature is below –10 °C for at least 2-3 consecutive days. A soil layer at approximately 20 cm takes 10-12 days to freeze under these air temperature conditions (Endla *et al.,* 2017; Cao *et al.*, 2021).

The purpose of the IDEAM agrometeorological network is to monitor agrometeorological conditions in the vicinity of agricultural areas (below 300) with actively growing

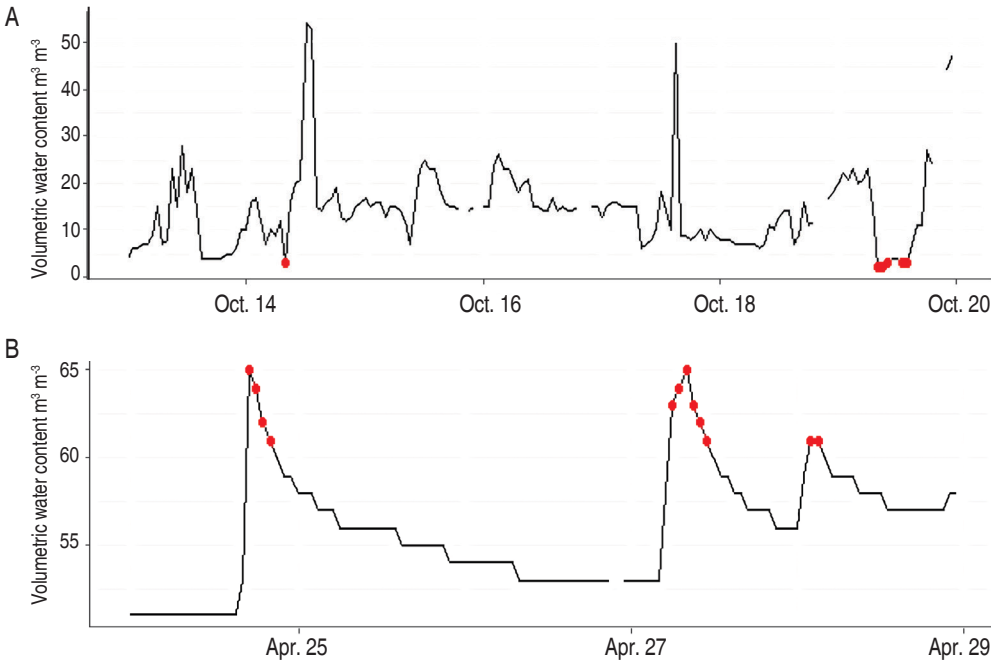

Figure 1. Limits (minimum and maximum) of soil moisture. A. Moisture values <3 %., B. Moisture values >60 %.
Source: author's elaboration based on IDEAM data.

vegetation cover. In Colombian agricultural areas, mean temperatures or daytime averages <0 °C are not possible. It is probable, that some specific points in Colombia have records <0 °C but this temperature is temporary (a few hours or days), and insufficient time for the soil to freeze and affect measurements by moisture sensors, so these flags were not used for the development of this study.

The first approach for the relationship between precipitation and responses in soil moisture to denote spurious moisture observations was proposed by You *et al.* (2010). However, Dorigo *et at.* (2013) started denoting measurements as suspicious when an increase in moisture is recorded without the occurrence of significant rainfall in the previous 24 h (equations 1 and 2.

$$x_t > x_{t-1} \quad (1)$$

$$x_t - x_{t-24} > 2\sigma_{x[t-24,t]} \quad (2)$$

Where x_t is the value of the soil moisture at time t in h, and $\sigma_{x[t-24,t]}$ is the standard deviation of x in the previous 24 h. The first equation ensures that only soil moisture rises, and the second one identifies increases that exceed daily variation. For each identified measurement, the occurrence and amount of precipitation in the 24 h before the measurement were verified.

The D04 quality flag, based on precipitation, only applies to measurement depths <10 cm, showing a direct response to precipitation (Dorigo *et al.*, 2013).

Spectrum consistency quality control. Spectrum-based errors are sudden and unnatural changes in a soil moisture time series. These suspicious measurements can be caused by a sensor malfunction, a lack of sensor response, or a power supply or connection problem when registering the data. These errors can be detected by examining the time series, and analyzing the first and second derivatives; these faults can generally be categorized into jumps, peaks, and constant values.

A peak is an event that lasts for a single time step (measurement) and differs markedly from the prior and subsequent values. This value can be above or below the measured soil moisture values. Taking into account the meteorological and edaphic conditions of Colombia

(a Tropical region with a strong influence of the ITCZ, which causes intense rainfall and soils of medium textures with a porosity that favors the passage of water through the soil profile), the algorithm was adjusted by modifying the range from 5 to 10% for a moisture change in a time step to be considered a peak, after visual inspection where natural increases in soil moisture of less than 10% were evidenced in a single time step.

1. Single measurement that differs significantly from observations x_{t-1} and x_{t+1} of x_t, x_t must vary less than 10% (equations 3 and 4).

$$\frac{x_t}{x_{t-1}} < 0.9 \text{ or } \frac{x_t}{x_{t-1}} > 1.1 \text{ with } x_{t-1} \neq 0 \text{ and } \left|x_{t-1} - x_t\right| \quad (3)$$

$$\frac{x_t}{x_{t+1}} < 0.9 \text{ or } \frac{x_t}{x_{t+1}} > 1.1 \text{ with } x_{t+1} \neq 0 \text{ and } \left|x_{t+1} - x_t\right| \quad (4)$$

2. In the first derivative, the values before and after the peak are ideally the same. This does not occur in actual measurements and varies because of constant increases or decreases in the spectrum. Therefore, equations 5 and 6 in the second derivative should satisfy the same condition although they seem redundant; both avoid different cases of overmarking.

$$\frac{x'_{t-1}}{x'_{t+1}} \approx -1 \quad \text{with} \quad x'_{t+1} \neq 0 \quad (5)$$

$$\frac{x''_{t-1}}{x''_{t+1}} \approx 1 \quad \text{with} \quad x''_{t+1} \neq 0 \quad (6)$$

3. The third condition represents the peaks in the second derivative that are twice as large as the smallest peak in the marked measurement. Since moisture measurements vary, large peaks only have to be larger than small ones (equations 7 and 8).

$$\frac{x''_t}{x''_{t-1}} < -1 \quad \text{with} \quad x''_{t-1} \neq 0 \quad (7)$$

$$\frac{x''_t}{x''_{t+1}} < -1 \quad \text{with} \quad x''_{t+1} \neq 0 \quad (8)$$

4. To take into account the noisy data of the series, the mean calculation in the first derivative applied to the time series was done at an interval of 6 h before and after (+/- 6 h) the potential peak, without the marked value (equation 9).

$$\left|\mu\left(\left[x'_{t-6,}, x'_{t+6}\right] \setminus \left\{x'_{t-1}, x'_t, x'_{t+1}\right\}\right)\right| < 0.5 \quad (9)$$

Jumps are characterized by a sudden change in soil moisture measurements from one time step to another. Unlike a peak, after the jump, the moisture does not return to the initial value but remains modified until a certain time. A Negative Jump is a measurement in time *t* that meets the following conditions:

1. The ratio between values x_t and x_{t-1} must be at least 10%, and the previous soil moisture must be > 0, (equation 10).

$$\frac{x_t}{x_{t-1}} < 0.9 \quad \text{with} \quad x_{t-1} \neq 0 \quad \text{and} \quad x_t - x_{t-1} < -0.5 \quad (10)$$

2. In the first derivative, the value before and after the jump are ideally the same but have different directions. In the second derivative, the condition is the same, but x'_{t-1} must be negative. Both conditions complement each other because the first derivative takes into account its neighbors, and the second one does not (equations 11 and 12).

$$\frac{x'_t}{x'_{t-1}} \approx 1 \text{ with } x'_{t-1} \neq 0 \quad (11)$$

$$\frac{x''_t}{x''_{t-1}} \approx -1 \text{ with } x''_{t-1} \neq 0 \quad (12)$$

3. A negative jump is seen with a negative second derivative, with a positive value for *t*+1, which must be maintained after the marked change (equation 13), equation 14 was added where the division was reversed since x''_{t-1} and x''_t should not be zero; in any event, x''_{t-2} and x''_{t+1} is often zero.

$$\left|\frac{x''_{t-2}}{x''_{t-1}}\right| < 0.15 \text{ with } x''_{t-1} \neq 0 \quad (13)$$

$$\left|\frac{x''_{t+1}}{x''_t}\right| < 0.15 \text{ with } x''_t \neq 0 \quad (14)$$

4. The spectrum before and after the jump must be fairly smooth to avoid excessive signaling, where the mean of the first derivative is used in 6 h before and after the potential jump, without the marked value or the value above (equation 15).

$$\left|\mu\left(\left[x'_{t-6,}, x'_{t+6}\right] \setminus \left\{x'_{t-1}, x'_{t}\right\}\right)\right| < 0.5 \quad (15)$$

5. The equation (16) differs from the previous one by adding the derived value before x_t, with the first derivative rounded.

$$x'_t + x'_{t-1} < -10 \cdot \left|\mu\left(\left[x'_{t-6,}, x'_{t+6}\right] \setminus \left\{x'_{t}, x'_{t-1}\right\}\right)\right| \quad (16)$$

A Positive Jump does not differ much from the negative one although there are difficulties in its detection because of the similarity in behavior with natural increases caused by precipitation.

1. For a positive jump, the x_t and x_{t-1} ratio must be greater than 10%, similar to the range for a negative jump (equation 17), and the moisture in x_{t-1} must be >0.

$$\frac{x_t}{x_{t-1}} > 1.1 \text{ with } x_{t-1} \neq 0 \text{ and } x_t - x_{t-1} > 0.5 \quad (17)$$

2. The second condition is exactly the same as in a negative jump (equations 18 and 19).

$$\frac{x'_t}{x'_{t-1}} \approx 1 \text{ with } x'_{t-1} \neq 0 \quad (18)$$

$$\frac{x''_t}{x''_{t-1}} \approx -1 \text{ with } x''_{t-1} \neq 0 \quad (19)$$

3. The third condition is also equal to the one for a negative jump (equations 20 and 21).

$$\left|\frac{x''_{t-2}}{x''_{t-1}}\right| < 0.15 \text{ with } x''_{t-1} \neq 0 \quad (20)$$

$$\left|\frac{x''_{t+1}}{x''_{t}}\right| < 0.15 \text{ with } x''_{t} \neq 0 \quad (21)$$

4. The spectrum around the jump must be very smooth, so the first derivative is evaluated in 6 h before and 6 h after x_t (equation 22), similar to a negative jump.

$$\left|\mu\left(\left[x'_{t-6,}, x'_{t+6}\right] \setminus \left\{x'_{t-1}, x'_{t}\right\}\right)\right| < 0.5 \quad (22)$$

5. For a positive jump, the sum of x'_t y x'_{t-1} must be 10 times greater than in the spectrum of the first derivative (equation 23).

$$x'_t + x'_{t-1} > 10 \cdot \left|\mu\left(\left[x'_{t-6,}, x'_{t+6}\right] \setminus \left\{x'_{t}, x'_{t-1}\right\}\right)\right| \quad (23)$$

6. Finally, an additional condition is added to avoid excessive signaling from natural precipitation events. After the jump, the soil moisture maintains a constant level because of new rainfall and may even increase or decrease because of the natural drying process; this situation is excluded with equations 24 and 25.

$$\frac{x_t}{x_{t+1}} > 0.99 \text{ and } \frac{x_t}{x_{t+1}} < 1.01 \text{ with } x_{t+1} \neq 0 \quad (24)$$

$$\frac{x_{t+1}}{x_{t+2}} > 0.99 \text{ and } \frac{x_{t+1}}{x_{t+2}} < 1.01 \text{ with } x_{t+2} \neq 0 \quad (25)$$

Plateaus are defined as relatively constant values. They can be low or high, with defined characteristics; therefore, detection algorithms are different. The ISMN defines a plateau as lasting for at least 12 h.

Low-level plateau. Low constant values are mainly the result of a poor sensor power supply, following a negative jump with inadequate readings for low soil moisture.

1. The first condition for a low-level plateau is the presence of a negative jump because a low-level plateau starts at x_t with $t = t_{pl\_start}$

2. They are usually at values close to 0; within the plateau, the variation must be minimal and last for at least 12 h (equation 26).

$$\sigma^2\left(\left[x_{t,x_{t+n}}\right]\right) < 0.01 \text{ with } n \geq 12 \text{ and } t = t_{pl\_start} \quad (26)$$

3.To ensure an increase in moisture once the plateau is finished and avoid excessive signaling from the natural drying process in soil, which can be quite slow, equation 27 is tested.

$$x'_t > 0 \text{ with } t = t_{pl\_end} \quad (27)$$

Saturated plateau. This event is easily confused with the natural drying process. On many occasions, it occurs with one or more precipitation events, which triggers a high moisture measurement at the beginning and a moisture drop at the end. For a saturated plateau to be recorded, the following conditions must be met:

1. An increase in soil moisture must occur to achieve a saturated plateau; this increase must happen, at a maximum, in the previous 3 h and must be greater than for the flags described above (equation 28).

$$\sum_{i=0}^{3}(x_{t-i-1}-x_{t-i})>0.5 \text{ and } x_t-x_{t-1}>0.1 \text{ with } t=t_{pl\_start} \quad (28)$$

2. The variance of the entire plateau and each time step of the 12 h is calculated separately; the variation of the additional limit avoids marking the drying process, but, if the plateau lasts for a long period, the added value would not have much influence. Therefore, both limits are required (equations 29 and 30).

$$\frac{\sigma^2([x_t,x_{t+n}])}{\mu([xt,x_{t+n}])}<0.0075 \text{ with } n\geq 12 \text{ and } t=t_{pl\_start} \quad (29)$$

$$\frac{\sigma^2([x_{t-12},x_t])}{\mu([x_{t-12},x_t])}<0.005 \quad \forall\left\{t \mid t_{pl\_start}+12\leq t\leq n\right\} \quad (30)$$

3. To detect large moisture differences between two neighboring values, which should not occur within a plateau, equation 31 is applied.

$$\frac{x_i-x_{i-1}}{\mu([x_t,x_{t+n}])}<0.01 \quad \forall x_i\in[x_{t+1},x_{t+n}] \text{ with } n\geq 12 \text{ and } t=t_{pl\_start} \quad (31)$$

4.The last condition indicates that it will only be marked as a saturated plateau if it is in the top 5% of the soil moisture time series readings (equation 32).

$$\mu([x_{pl\_start},x_{pl\_end}])>\max([x_{t_0},x_{t_{end}}]\setminus\{x_t>100\})\cdot 0.95 \quad (32)$$

Suspicious values around missing values. Values before or after a sensor failure are often atypical, especially after long periods of sensor record drops.

Suspicious values before missing values. To analyze this questionable data before missing values, the first and second derivatives are quite useful.

1. A sensor failure in a time step does not normally lead to suspect values before or after failure. To avoid over-marking, a minimum of 3 h of sensor downtime is required (equation 33).

$$\nexists[x_{t+1},x_{t+n}] \text{ with } n\geq 3 \quad (33)$$

2. The difference with the value before the suspect value must be greater than a certain limit; Heer (2017) defined it as 5%. For the meteorological and edaphic conditions of Colombia, a visual inspection to avoid over-marking of this flag, this limit was determined as at least 10% (equation 34).

$$\frac{x_t}{x_{t-1}}<0.9\frac{x_t}{x_{t-1}}>1.1 \text{ with}\left|x_t-x_{t-1}\right|>0.5 \text{ and } x_{t-1}\neq 0 \quad (34)$$

3. The spectrum before the suspect value has to be smooth to differentiate between natural moisture variations and potentially doubtful values (equation 35).

$$x'_{t-1}<-10\cdot\left|\mu([x'_{t-2},x'_{t-6}])\right| \text{ with } \left|\mu([x'_{t-2},x_{t-6}])\right|<1 \quad (35)$$

4. For a breakout, the first derivative in the suspect value must be much higher than in the last spectrum (equation 36).

$$x'_{t-1}+x'_t>10\cdot\left|\mu([x'_{t-2},x'_{t-6}])\right| \text{ with } \left|\mu([x'_{t-2},x_{t-6}])\right|<1 \quad (36)$$

Suspicious values after missing values. The algorithms are analogous to those used for suspect values before NA (Not a Number). As above, the relative change after the suspect value must be at least 10%.

Severe drop in soil moisture. Because the soil drying process is progressive and can be relatively slow, it makes sense to introduce a flag that marks a negative change in soil moisture that occurs within 1 h beyond a certain threshold. Unlike the negative jump, the fall must be much greater. Heer (2017) defined the fall as at least 25%.

Taking into account the physical conditions of the soil and variables such as texture, porosity, apparent density, and hydraulic conductivity, among others, determine the speed with which it can dry or favor the passage of water through it.

Therefore, this threshold was modified since an excessive moisture drop for a fine texture differs in terms of infiltration speed when compared to soils with a medium and coarse texture. Taking into account aspects such as porosity and soil texture, the following thresholds were established for Colombian conditions.

- Fine texture: a drop of at least 8.1% (Clay, Sandy clay, and Silty clay), equation 37.

$$\frac{x_t}{x_{t-1}} < 0.918 \text{ with } x_{t-1} \neq 0 \text{ and } x_t - x_{t-1} < -0.5 \quad (37)$$

- Average texture: a drop of at least 12.2% (sandy loam, loam, silty loam, silty clay loam, and silt), equation 38.

$$\frac{x_t}{x_{t-1}} < 0.878 \text{ with } x_{t-1} \neq 0 \text{ and } x_t - x_{t-1} < -0.5 \quad (38)$$

- Coarse texture: a drop of at least 27% (sand, clay sand, loamy sand, coarse sand), equation 39.

$$\frac{x_t}{x_{t-1}} < 0.73 \text{ with } x_{t-1} \neq 0 \text{ and } x_t - x_{t-1} < -0.5 \quad (39)$$

Alternate values: In some soil moisture time series, sensor malfunctions result in alternating values. The readings constantly change between fairly high and low soil moisture readings. Five conditions must be met to mark these values as alternates. The moisture readings are classified in two sets, which must include at least three (3) measurements; their union is the period of erroneous data with at least 13 values (equation 40).

$$\exists\{X\} : |X| \geq 3 \text{ and } \exists\{Y\} : |Y| \geq 3 \text{ and } X \cup Y = [x_t, x_{t+n}] \text{ with } n \geq 12 \quad (40)$$

5. Each group must include a value that has a predecessor and a successor in time from the other group (equations 41 and 42).

$$\exists x \in X : t_{y1} < t_x < t_{y2} \quad y1, y2 \in Y \quad (41)$$

$$\exists y \in Y : t_{x1} < t_y < t_{x2} \quad x1, x2 \in X \quad (42)$$

6.The variance of each group must be less than 0.5% (equation 43).

$$\sigma^2(X) < 0.5 \quad \wedge \quad \sigma^2(Y) < 0.5 \quad (43)$$

7.The relative difference between the sets must be at least 25% (equation 44).

$$\frac{\mu(X)}{\mu(Y)} < 0.75 \quad \vee \quad \frac{\mu(X)}{\mu(Y)} < 1.25 \quad (44)$$

8. Each value in each set should differ from the mean of the set by a maximum of 1% to avoid outliers that should not occur in that set and therefore avoid over-marking (equations 45 and 46).

$$|\mu(X) - x| < 1 \quad \forall_x \in X \quad (45)$$

$$|\mu(Y) - y| < 1 \quad \forall_y \in Y \quad (46)$$

Constant values: Normally, moisture values are variable, even in the absence of precipitation events, because they are influenced by the seasonal cycle. Daily variation is also recorded because of the effect of solar radiation and temperature. For this reason, it is unlikely that soil moisture will remain constant for a period longer than 24 h.

The condition that is set to mark a value as constant is a measured value that does not change for at least three days (72 h), where three daily cycles should be visible.

Highly marked spectrum: Many random erroneous observations that cannot be detected by specific algorithms, and data values that are within a spectrum where most of the data sets are flagged. Therefore, there is a flag that indicates when more than half of the data sets are flagged within 24 h, before and after a suspect value detection.

RESULTS AND DISCUSSION

The results show that 40.3% of the soil moisture records were good (G), 12.9% were doubtful for the spectrum (D), 14.3% were doubtful for the geophysical range (R) and the remaining 32% of the data were erroneous (M), either because they were outside the geophysical range and/or data were missing (Figure 2).

When performing the flag quality analysis by depth (Table 3), the one closest to the surface (10 cm) had the highest number of reported quality flags, with 134 observations marked per 1,000 records, followed by 120 records marked for the 30 cm depth and 95 records marked for the 50 cm depth.

Of the out-of-limits and geophysical consistency flags, the quality flag with the highest number was C02 “Soil moisture >60% & <=100%”, with 215 detections per 1,000 records. This flag was detected in 93% of the stations with sensors

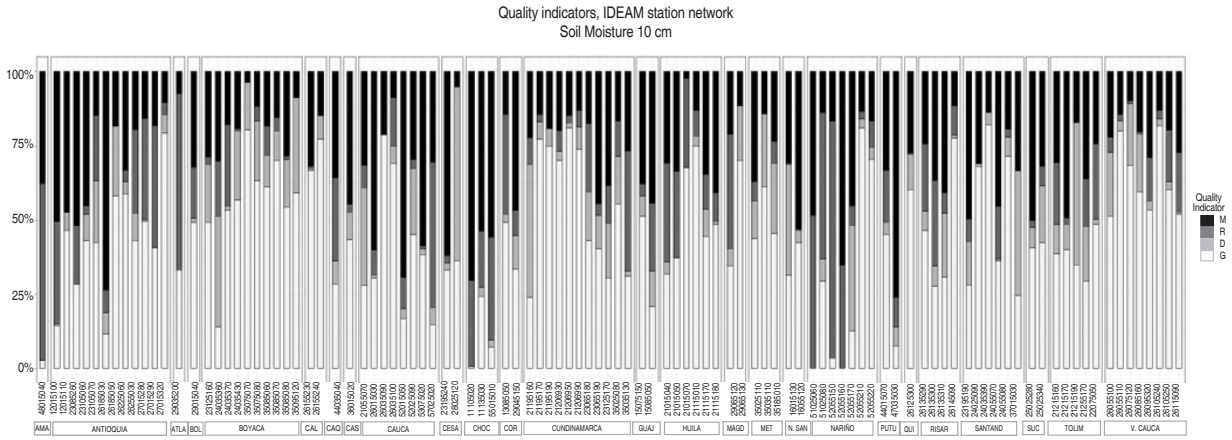

Figure 2: Quality indicators for a depth less than 10 cm, measured in the IDEAM Automatic Agrometeorological Stations Network**.** Source: author's elaboration, based on IDEAM data (2021).

at 10 cm, and 91% and 82% of the stations with sensors at 30 cm and 50 cm deep, respectively. The second most frequent quality flag was C01 "Soil moisture >=0% & <3%", with 78 detections per 1,000 records.

When analyzing the spectrum consistency flags, the most frequent quality flag was D13 "Severe Drop in Soil Moisture", with 4.9 observations marked per 1,000 records. This flag was detected in 98% of the stations with sensors at 10 cm and 30 cm deep, and 90% of the stations with sensors at 50 cm. The second most frequent spectrum consistency flag was D09 "Low constant values (minimum 12 h) in the soil moisture spectrum", with 2.9 observations marked per 1,000 records.

The soil layer closest to the surface is the most influenced layer by the atmosphere and physical and vegetation cover conditions for the soil moisture content. The most common flag in the geophysical range check was C02 (Figure 3).

Table 3. Quality flags detected (* in thousands) by depth. Data series from the IDEAM agrometeorological network**.**

Quality Flag	30 cm depth (SMD30)			50 cm depth (SMD50)					
	Detected stations	Total observations*	Marked observations*	Detected stations	Total observations*	Marked observations*	Detected stations	Total observations*	Marked observations*
C01	97	7.699	558	85	6.702	500	50	3.814	357
C02	97	7.492	971	86	6.566	884	50	3.600	728
D04	75	6.281	1.1	67	5.280	0.8	31	2.568	0.3
D06	95	7.521	3.4	86	6.614	2.8	48	3.715	1.1
D07	83	6.542	2.2	65	5.199	1	39	2.944	0.8
D08	96	7.627	3.3	86	6.811	2.8	48	3.670	1.8
D09	61	5.010	16.1	47	3.811	9.9	32	2.396	7.6
D10	40	3.312	6.6	49	3.859	3.6	29	2.284	6.8
D11	79	6.437	0.3	60	4.714	0.3	33	2.738	0.1
D12	76	5.873	0.3	59	4.799	0.3	37	2.991	0.1
D13	103	7.831	45.6	92	7.035	32.4	55	3.937	15.4
D14	1	78.9	0.0	-	-	-	2	97.3	17.6

SMD: Soil Moisture Depth.

The high soil moisture values were in areas with rain between 8-10 months per year, which favors moisture saturation and soil supersaturation, with values higher than 60% of the volumetric moisture content. In addition, the moisture fell to values of 0% and stayed at this value for a long time. Additionally, during the day, the values appeared to be normal, and, at night, the values rapidly dropped to 0%, indicating that, when a decrease or loss of energy was registered by a sensor, the registered soil moisture value was 0%.

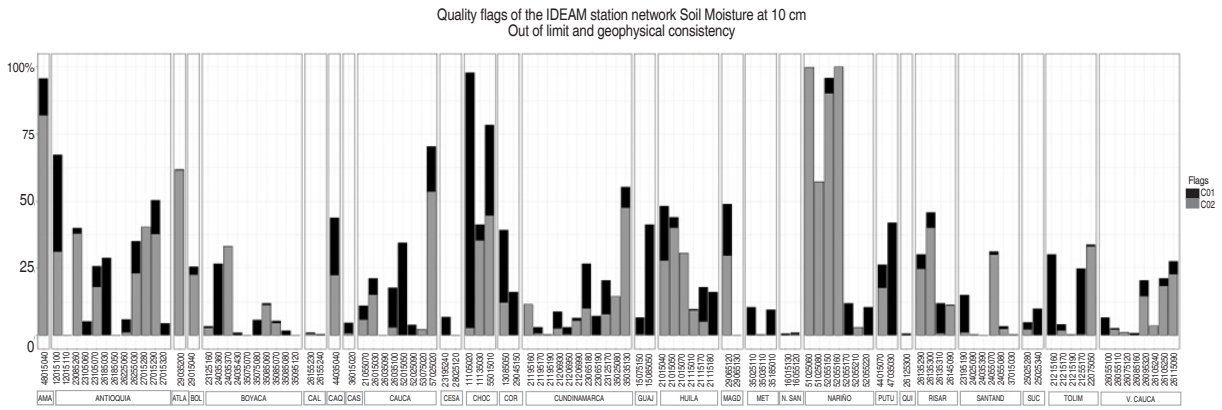

Figure 3. Percentage of out-of-limit quality flags and geophysical consistency at a depth of 10 cm in the data series from the IDEAM agrometeorological network.

This flag is highly related to the C01 Geophysical flag and the D13 spectrum flag since the former shows values lower than 3%, and the latter indicates a severe moisture drop. Therefore, when a station suffers a power loss, the moisture drops to values close to 0%; the C01 flag is activated. When this occurs in a single time step, a severe moisture drop is registered (D13). When this record is maintained for a period greater than 12 h until the power supply is restored, the third flag, D09, is triggered, corresponding to a low-level plateau.

There are a couple of complementary flags that were calculated within the spectrum analysis, the D15 "Constant Values" flag and the D16 "Highly marked spectrum" flag. These flags show complementary information at the spectrum level, providing information on particular behaviors in a time series, such as constant values, which show the number of observations where the sensor between one-time step and another does not have any response to a variation in moisture for 72 h (low-level plateaus and saturated plateaus).

The flag for a highly marked spectrum takes into account neighboring measurements that are marked by a flag, which indicates the quality of the record concerning the quality of the 48 neighboring measurements 24 h before the measurement and 24 h after the measurement. Table 4 shows quality indicators of soil moisture records for the stations of the IDEAM network and the number of total records by sensor depth, where G (Good Data), D (Doubtful spectrum), R (Doubtful Geophysical Range) and M (Erroneous or missing data).

Colombia has a variable supply of rainfall, both in volume and distribution, but is among the rainiest places on the planet. When checking the dynamics of soil moisture with values> 60%, it was observed that this condition resulted from strong, isolated rainfall events or consecutive events, as seen in most of Colombia in the rainy months, which in some areas can be up to 8-10 months of the year. This suggests that the range defined for the C02 flag may be below the registered values for soil moisture, resulting in over-marking for this quality flag and suggesting the need for specific studies to determine the soil moisture saturation limit for Colombian conditions**.**

Previous studies on quality controls applied to the ISMN soil moisture series (Dorigo *et al*., 2013; Xaver, 2015 and Heer, 2017) indicate that the C01 flag "Soil moisture <0%"

works well for arid and seasonal regions, where there is a long time with limited rainfall and low relative humidity that favor the gradual drying process in soil (Brutsaert, 2014; Tugwell-Wootton *et al.*, 2020).

It does not apply to equatorial regions such as Colombia where there is climatic seasonality, there are different rain regimes that range from the absence of a dry season to the presence of two dry seasons in the year and relative humidity that does not drop below 40%, conditions that do not favor the loss of moisture in the soil at values close to 0%; therefore, this flag was adjusted for the Colombian territory and was defined as "soil moisture> = 0% & <3%".

Table 4. Network of IDEAM agrometeorological stations. Quality indicators, soil moisture records by sensor depth

Station Code	Number of records	(10 cm depth) SMD10				(30 cm depth) SMD30				(50 cm depth) SMD50			
		G	D	R	M	G	D	R	M	G	D	R	M
		%											
11105020	121,915	0.5	0.1	29.0	70.4	2.8	0.3	26.2	70.8	0.2	0.0	29.1	70.6
11135030	117,767	24.3	2.9	19.0	53.8	21.0	3.4	21.8	53.8	13.5	1.9	30.8	53.8
12015100	134,550	14.4	0.5	34.6	50.5	32.3	13.9	6.4	47.4	30.6	3.8	15.5	50.1
12015110	135,197	46.5	6.0	0.0	47.4	43.4	3.6	2.8	50.2	3.2	0.4	49.0	47.4
13085050	46,532	49.0	3.1	33.5	14.4	53.5	12.2	16.5	17.8	61.7	23.8	0.0	14.4
15075150	131,601	51.2	6.8	4.1	37.8	-	-	-	-	-	-	-	-
15085050	132,245	20.7	12.0	22.9	44.4	10.1	18.7	25.8	45.3	9.2	45.6	2.5	42.7
16015130	120,020	31.3	37.5	0.4	30.8	20.3	13.7	35.2	30.8	-	-	-	-
16055120	120,020	42.2	4.3	0.4	53.0	32.6	13.9	0.4	53.0	28.7	4.7	12.9	53.8
21015040	129,680	31.9	3.9	33.3	30.9	21.9	3.2	44.0	30.9	-	-	-	-
21015050	130,375	37.2	0.0	29.1	33.7	49.2	1.5	13.7	35.6	44.1	8.8	10.9	36.2
21015070	12,014	67.6	0.0	30.0	2.4	0.0	0.0	97.6	2.4	3.3	1.7	92.6	2.4
21055070	126,377	28.0	32.9	7.5	31.6	37.6	26.6	3.6	32.1	-	-	-	-

SMD: Soil Moisture Depth.

The series analysis for some stations marked with quality flag C01 saw a drop in moisture values to lower than 3% in less than 2-3 time steps in the late afternoon and evening, suggesting that these sudden drops in soil moisture have to do with a power supply power for the sensor.

One of the main problems of spectrum consistency quality control is the presence of missing values, causing difficulties in the detection of suspicious measurements. Spectrum consistency flags are based on the smoothing of the series by applying the Savitzky-Golay filter, which implements the first and second derivatives that require a complete series of values on an hourly scale.

Dorigo *et al.* (2013) and Xaver (2015) set the change in soil moisture in a time step for the detection of peaks and plateaus at 5% for quality control in the ISMN soil moisture series, which generated an excessive markup for Colombian conditions because of the frequency and intensity of rainfall. For this study, this threshold was modified to 10%, avoiding over-marking as the result of rainfall events.

Heer (2017) developed a new quality flag to detect severe drops in moisture, which set a 25% decrease in moisture in a time step (hour) and does not adjust for the equatorial conditions of Colombia. It was decided to define the drop in moisture based on the soil texture

conditions, resulting in improvements in the detection of this type of quality flag, avoiding under-marking.

CONCLUSIONS

IDEAM's network of agrometeorological stations has good data in 40.3% of its records, a volume of data that can be used for subsequent validations with estimated data. In addition, when analyzing the trend of a quality flag marking throughout the operational years of the IDEAM series, there were no increasing trends for any flag, neither for geophysical range nor for spectrum consistency. This suggests that the sensors are still adequately recording the soil moisture condition, indicating that the quality of the records has not undergone significant changes that would show deterioration after about 20 years of network operation. Some peculiarities were detected in the quality control:

- Problems with missing values in the application of the Savitzky-Golay filter, based on derivatives of the first and second degree, which requires a time spectrum with measurements and is interrupted when a lost value is recorded; this causes undermarking of quality flags based on spectrum consistency. The flags most affected by this condition are plateaus and jumps, which have interrupted detection with the appearance of missing values.

- Abnormally low values, soil moisture <3%, where the moisture drops to values of 0% in a few hours; for some stations, this occurred in the evening hours, suggesting they are due to failures in the electrical supply.

- A high percentage of high moisture records> 60% were marked because of the established geophysical limit, but, when analyzing some series, it was evidenced that they are the result of intense and/or continuous rainfall and wetting and drying processes in the soil. This suggests that the geophysical range marking was good, along with the soil moisture measurements, which, for some stations and areas of Colombia, may become normal.

- The typical cloud formation of the intertropical convergence zone (ITCZ), which intercepts a high percentage of incident light and reduces the supply of solar energy for the panels on the automatic stations, means the stations must have a power system with solar panels and batteries. When a station sensor perceives a decrease in power supply, low values are recorded.

- The quality control methodology, with adjustments for local Colombian conditions, provided satisfactory results in the detection of anomalous records of soil moisture, providing soil moisture data with quality flags, which can be used in subsequent validation analyses of and studies on the dynamics of this variable in Colombia. However, more in-depth studies should be carried out for the geophysical limits of Colombian regions where rainfall is usually frequent and intense.

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Automatic delimitation and morphometrics analysis of watersheds and sub-watersheds using a digital elevation data set in the Cornare Antioquia, Colombia jurisdiction

Delimitación automática y análisis morfométrico de cuencas y subcuencas usando un conjunto digital de datos de elevación en la jurisdicción de Cornare, Antioquia, Colombia

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ABSTRACT

Basin hydrological and morphometric parameters are elements that must be made on a map with sufficient information. This information collected on-site has been limited by public order problems. Basin circularity index (Ci) is among the morphometric indices, which has not been calculated yet for the basins under study. Basins automatic delimitation carried out from Geographic Information Systems (GIS), has allowed its research and management due to efficiency in data processing, ease, and low cost; in addition, increase images and databases availability. The work aim was to evaluate the Digital Elevation Model (DEM) SRTM proposed methodology in the Corporación Autónoma Regional de las Cuencas de los Ríos Negro y Nare (Cornare) jurisdiction, extracting from it the numerical drainage, in order to delimit basins and sub-basins, as well as calculate indices, such as drainage density (Dd) and Ci. The declivity map showed terrain with very soft to very strong surface relief. Basin´s automatic delimitation based on the drainage order ≥ 6 was the one that came closest to the basin actual characteristics; regarding the sub-basins delimitation, it was the one based on the ≥ 3 order. Dd and Ci indices indicated that the sub-basins have moderate drainage, runoff level, and infiltration capacity and tend to be more elongated than circular.

Keywords:
Circularity index
Digital elevation model
Drainage density
Morphometric characterization
Watersheds

RESUMEN

Los parámetros hidrológicos y morfométricos de una cuenca constituyen estimaciones que deben realizarse sobre un mapa con suficiente información. La recopilación de esta *in situ* se ha visto limitada por problemas de orden público. Entre los índices morfométricos de una cuenca se encuentra el índice de circularidad (Ic), que hasta el momento no ha sido calculado para las cuencas en estudio. La delimitación automática de cuencas realizada a partir de Sistemas de Información Geográfica (SIG), ha permitido la investigación y manejo de las mismas debido a la eficiencia en el procesamiento de datos, facilidad y bajo costo, además de la creciente disponibilidad de imágenes y bases de datos. El objetivo de este trabajo fue evaluar la metodología propuesta para utilizar el Modelo Digital de Elevación (MDE) SRTM de la jurisdicción de la Corporación Autónoma Regional de las Cuencas de los Ríos Negro y Nare (Cornare) y extraer de este el drenaje numérico, para delimitar cuencas y subcuencas, así como calcular índices, tales como densidad de drenaje (Dd) e Ic. El mapa de declividad mostró terrenos con relieve muy suave a muy fuerte. La delimitación automática de cuencas con base en el orden de drenaje ≥ 6 fue la que más se acercó a la realidad; en cuanto a la delimitación de subcuencas, fue aquella con base en el orden ≥ 3. Los índices Dd e Ic, indicaron que las subcuencas tienen drenaje, nivel de escorrentía y capacidad de infiltración moderados y tienden a ser más alargadas que circulares.

Palabras clave:
Índice de circularidad
Modelo digital de elevación
Densidad de drenaje
Caracterización morfométrica
Cuencas

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Basin hydrological and morphometric parameters constitute a set of elements made, for planning, exploration, and management (Lux, 2012). These elements and parameters must be made on a map with sufficient hydrographic and topographic information, which has been facilitated by satellite images and Geographic Information Systems (GIS), therefore, it is no longer necessary to compute the indices in spreadsheets. Information collection *in situ* has been limited by public order problems in the region. Among basin morphometric indices, is the circularity index, which so far has not been calculated for the basins in the region under study.

In Brazil, a hydrographic basin is considered a territorial unit of reference and intervention, since it has a good part of the cause and effect relationships that involve the local reality, and there must be an integration of the local basic sanitation policies, uses, occupation, and soil and environment conservation with water resources policies (Lei, No. 9.433, 1997). Colombia has had extensive regulations on the management of hydrographic basins in recent decades, in which technicality predominates, but the social and conservation dimension has not been consolidated for different reasons, mainly due to violence (Bermúdez, 2014).

The hydrographic or hydrological basin is the area that collects or captures naturally water from rainfall, in which there is a convergence of a set of interrelated flow channels to a single outlet, called water intake. The water amount that reaches the river courses depends on the area size occupied by the basin, the total rainfall, and its regime, and on the losses due to evapotranspiration and infiltration (Silveira, 2001).

The automatic delimitation of hydrographic basins has become a constant study. This delimitation, made from GIS, has enabled several results that can be used in the basins study and management due to the effectiveness in data processing, ease, and low cost, since it can be performed, even, in free GIS; also, images and databases increasing availability such as SRTM (Shuttle Radar Topography Mission) images, has provided support in the basins physiographic characterization process (Cordeiro *et al.*, 2014).

In Colombia, there are some works using SRTM images. Correa (2012) developed a method to delimit, characterize and represent cartographically terrain shapes in mountain zones in the department of Cauca, using SRTM and ASTER images. Grande (2015) used SRTM and Landsat TM 5, 7, and 8 images to develop multitemporal analysis and morphometric modeling (contour lines generation, drainage network, accumulation flows, drainage network direction), from the Molino River sub-basin in the municipality of Popayán, Cauca. Castañeda (2016), with SRTM and ASTER images, developed a semi-automated methodology, using object-based image interpretation for the terrain shapes classification and surface relief types in the municipalities of Tenjo and Tabio in the department of Cundinamarca. With Landsat images, WorldView2, and an SRTM topographic map, Cifuentes (2016) identified changes in the coastline and the climate change effects of increasing sea levels in the Buenaventura District. López *et al.* (2005), compared the information obtained on the ground through the geological study of outcrops in Valle del Cauca with that obtained through SRTM images, aerial photographs, and Landsat images, concluding that the tilting and bulging of the land, counterscarps, flexure scarps, and terraces, reveal the activity of partially or totally covered folds generated by the growth of reverse faults that cut increasingly older sediments towards the orogen and increasingly younger sediments towards the alluvial Cauca river valley.

A basin morphometric characterization has numerous applications, such as hydrological processes modeling, pollutants transport and deposition, and floods prediction (Moura, 2013). Studying physical aspects through morphometric analysis is possible to determine which points of the hydrographic basin are more likely to receive greater and lesser water loads (Freire and Monteiro, 2015). To evaluate the basin morphometric characteristics, the drainage density (Dd) and the circularity index (Ci) can be calculated from the basin's areas, perimeters, and drainage network.

Drainage density indicates the effectiveness of the drainage from the basin. It can vary from 0.50 km km^{-2} in basins with poor drainage to 3.50 km km^{-2} or more in well-drained basins. The higher the index, the lower the capacity to infiltrate water; low values indicate that the region is

more favorable to surface infiltration and percolation that contributes to the water table (Villela and Mattos, 1975). This variable is directly related to the climatic processes operating in the studied area, which influence the dendritic material supply and transport or indicate the anthropic manipulation degree.

For the same climate type, the drainage density depends on the rock's hydrological behavior. Thus, in the most impermeable rocks, the conditions for surface runoff are better, enabling channel formation, consequently, increasing the drainage density. The opposite happens with coarse-grained rocks. The drainage density can be classified as low when it is less than 1.50; moderate between 1.50 and 2.25 and high when it is greater than 2.25 (Horton, 1945).

The circularity index tends to unity when the basin approaches the circular shape and decreases when the shape becomes elongated (Tonello, 2005). This index represents the relationship between the basin total area and a circle area of the equal perimeter to the basin total area, which, in areal expansion, is better related to river runoff. Thus, Circularity Index between 0.45 and 0.55 represents a runoff moderate level, not contributing to the water concentration that allows rapid flooding. Values greater than 0.55 indicate that the basin tends to be more circular, favoring flooding processes (fast floods). Values smaller than 0.45 suggest that the basin tends to be more elongated, favoring the runoff process (Müller, 1953; Schumm, 1956).

This work aimed to establish a methodology for the use of the SRTM digital elevation model in the automatic delimitation and morphometric analysis of basins and sub-basins in the jurisdiction of Cornare, Antioquia, Colombia.

MATERIALS AND METHODS

The digital elevation model SRTM was used, which after being processed gave rise to the hydrologically consistent digital elevation model, from which were derived the declivity map, the drainage network, and the hydrographic basins and sub-basins of the jurisdiction of Cornare, Antioquia, Colombia. Also were calculated the morphometric indices drainage density and circularity index.

The Corporación Autónoma Regional de las Cuencas de los Ríos Negro y Nare (Cornare) is an autonomous and independent public entity, integrated by municipalities that, by their characteristics, geographically constitute the same ecosystem or form a geopolitical, biogeographic, or hydrogeographic unit (Cornare, 2005).

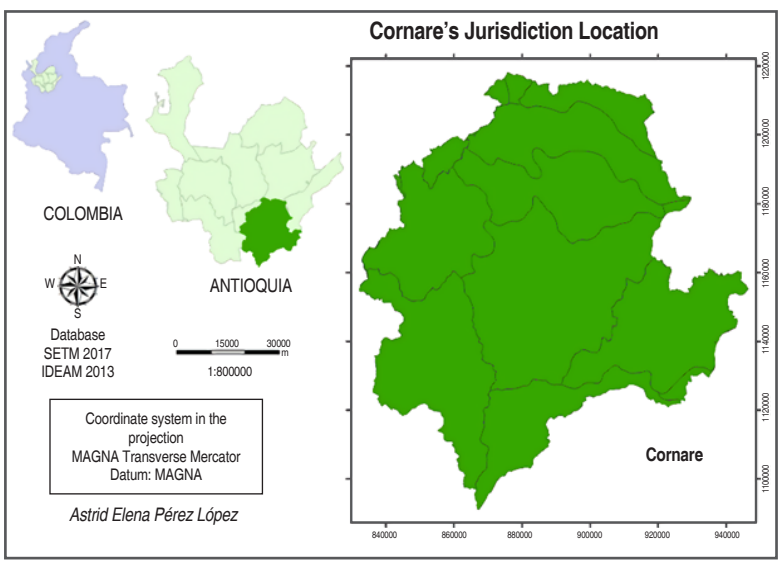

Figure 1. Location Jurisdiction of Cornare.

Cornare, by law, has the function of managing, within its area of jurisdiction, the environment, and renewable natural resources, besides working for their sustainable development, following the policies of the Ministerio de Ambiente y Desarrollo Sostenible (Cornare, 2005).

Cornare's jurisdiction is located in the East of the Department of Antioquia, extreme Northwestern of Colombia, between the geographic coordinates 5° 24' 39.77" N, 75° 34' 58.92" W and 6° 35' 11.78" N, 75° 13.58" W, with an approximate area of 8,318 km^2 (Figure 1). It has high rainfall levels, with an annual average of 2,500 mm; great climatic variety, with a minimum altitude of 200 m in the Magdalena Medio plain and a maximum of 3,340 m in the hill of Las Palomas in the moor of Sonsón, which allows for areas between cold and hot (Cornare, 2005).

The jurisdiction is divided into eight basins, namely: Porce, Nus, Nare, Rio Negro, Arma, Samaná Norte, Samaná Sur, and Cocorná Sur (Cornare, 2005), (Figure 2).

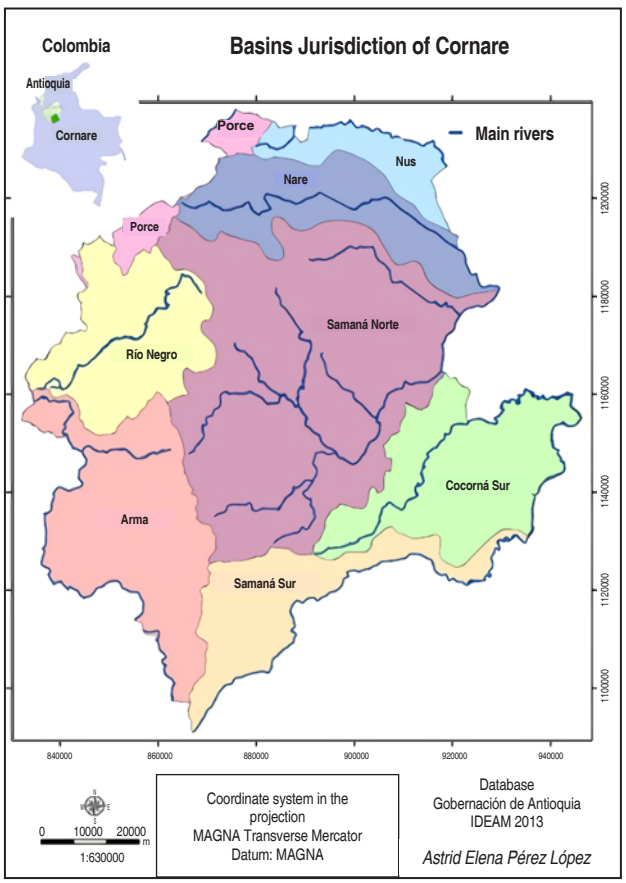

Figure 2. Basins of Cornare's jurisdiction.

The databases used were: Colombian Basins mapped by the Instituto de Hidrología, Meteorología y Estudios Ambientales (IDEAM) and the hydrography mapped by the Dirección de Sistemas de Información y Catastro del Departamento Administrativo de Planificación de la Gobernación de Antioquia. The procedures in ArcGis 10.1 according to Fernandes *et al.* (2015) were as follows:

1. SRTM digital elevation model acquisition, 90 m resolution, on the website of Consortium for Spatial Information (CGIAR-CSI, 2017).

2. Mosaic elaboration. The study area covers two grids, so it was necessary to mosaic the digital elevation model, obtaining a single file. Then, a cutout of the digital elevation model was made using the study area as a limit.

3. Coordinate system conversion. The SRTM digital elevation model was obtained with coordinates in decimal degrees and Datum WGS84. However, to extract numerical drainage and to obtain river length measurements, and basin areas, as well as to derive the declivity map, the elevation model must be in metric coordinates. Thus, the SRTM digital elevation model was redesigned for the projection MAGNA Transverse Mercator and Datum MAGNA.

4. Elimination of systematic errors from SRTM digital elevation models. The digital elevation model presents some negative altitude values, which are characterized as noise and occur due to failures in the model's acquisition system and/or processing. The noises are usually located in regions close to water bodies and/or mountainous relief areas. In order to correct such errors is necessary to generate a hydrologically consistent digital terrain model. For this, the *Raster Calculator* tool was used, using equation 1. This equation makes all cells with a negative value become null (NO DATA).

SetNull("mosaic"<=0," mosaic") (1)

Cells with NO DATA value were filled with the mean value of the three closest cells values, considering a circle with a center in the cell to be filled. For this, the R aster Calculator tool was used, using equation 2:

filled01 = Con(IsNull("rastercalc"), FocalStatistics(Raster(r"rastercalc"), NbrCircle(3,"CELL"), "MEAN",""),"rastercalc") (2)

5. Hydrologically consistent digital elevation model.
When the spurious (erroneous) values are eliminated and the hydrograph is mapped, the watercourses must follow the correct runoff direction, going from the cell with the highest to the lowest altitude value. For this, the *Fill* tool was used. The model obtained is the hydrologically consistent digital elevation model. To present the digital elevation model (DEM), the *Hillshade* tool or shading map was used, thus emphasizing the texture, roughness, and surface relief shape characteristics. Also, from the DEM and with the *Slope* tool, the declivity map in degrees was generated to define the surface relief categories according to IDEAM (2013).

6. Hydrographic basins delimitation.
The basins are delimited based on the runoff direction from the drainage network. The *Flow Direction* tool defines for each grid cell the runoff direction based on the neighborhood altitude of a given model cell. The direction is determined considering that water can follow one of the eight directions given by neighboring cells in a 3×3 matrix (Figure 3). Thus, the map with the correctly defined direction must assume one of the eight matrix directions. This approach is commonly referred to as the eight directions flow model (D8) presented in Jenson and Domingue (1988).

Basins delimitation was obtained from the runoff direction, using the *Basin* tool. The basins were delimited considering the water flow direction in each drainage network segment. They were converted from matrix to vector format, obtaining a shapefile, with the *Raster to Polygon* tool. To extract Cornare's jurisdiction map, the *Extract by Mask* tool was used.

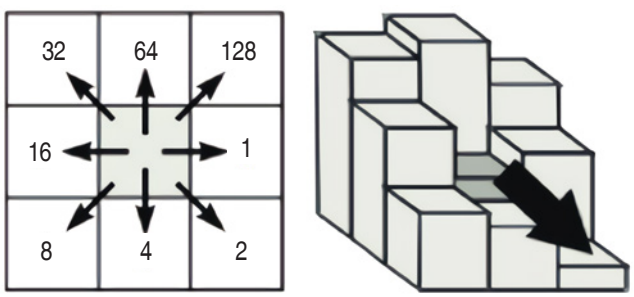

Figure 3. D8 encoding of flow direction.

7. Accumulated runoff flow map.
Then, the accumulated runoff flow was obtained with the *Flow Accumulation* tool, to map the drainage network defined by cells with the highest water flow.

8. Numerical drainage.
The drainage visualization can be done from the classification of the accumulated runoff flow map into two classes, where the values vary from the minimum to a value chosen by the operator as the threshold for the drainage network occurrence. Then, the real drainage can be compared with the numerical based on the superposition of the two classes defined above and presented in contrasting colors such as blue and red.

The numerical drainage network must have as few ramifications as possible, which are false drainage channels. The definition of the drainage line was made by several attempts with different values of accumulated runoff flow: 10, 20, 30, 40, 50, 60, 70, and 80. The map used for comparison was provided by the Information Systems and Registration of the Dirección de Sistemas de Información y Catastro del Departamento Administrativo de Planificación de la Gobernación de Antioquia, on a scale of 1: 25,000.

The *Raster Calculator* tool was used, with equation 3:

Dren10 = Con("flowacc_Cornare">=10.1) (3)

Where: *Dren10* was the name of the generated file, whose drainage was composed of cells that receive the contribution of more than 10 cells. *Con* was the condition for selecting cells that accumulated the flux coming from more than 10 cells. The same equation was used, only changing the number of cells by 20, 30, 40, 50, 60, 70, and 80.

9. Order of drainage of the basins.
This order is obtained from the channels hierarchy, using the *Stream order* tool. The order of drainage always starts with the value one (1), assigned to the first channel that forms the watercourse upstream of the basin. The highest order is attributed to the channel that receives the greatest flow of water, located further downstream from the basin.

The drainage order map was generated with all drainage channels grouped into the same class without it being possible to select an individual segment. However, to delimit the basins based on a certain order, it is necessary to individualize the channels. The drainage regionalization was done using the *Region Group* command.

10. Sub-basins delimitation.
To delimit the sub-basins, orders six (6), five (5), four (4), three (3), and two (2) were chosen, so a selection was made by channels atributes of higher-order or equal than six (≥ 6), five (≥ 5), four (≥ 4), three (≥ 3) and two (≥ 2). Finally, the sub-basins were delimited using the *Watershed* tool. These maps were compared with the maps made by the Instituto de Hidrología, Meteorología y Estudios Ambientales – IDEAM (basins) and the Dirección de Sistemas de Información y Catastro del Departamento Administrativo de Planificación de la Gobernación de Antioquia (sub-basins).

11. Morphometric indices determination.
The drainage network in raster format was converted to vector format, using the *Stream to feature* tool. Also, the sub-basin files generated from drainage orders greater than or equal to three (≥ 3), five (≥ 5), and six (≥ 6) were converted from raster format to vector format, using the *Raster to Polygon* tool. To calculate the drainage density, the sum of the total rivers length in each sub-basin was obtained in the same table. To do so, procedures for the spatial union of attributes of drainage tables and sub-basins with the *Spatial Join* tool were performed. The drainage file obtained after the spatial union has, in the attributes table, the ID field, which is the identifier of each sub-basin.

To calculate the drainage length, the *Calculate Geometry* tool was used. To sum up the drainage by sub-basin, the *Summarize* tool was used in the ID field. The table resulting from this procedure was joined to the sub-basins attribute table, through the *Join* tool, having as reference the common field ID.

The sub-basin attributes table contains the drainage length values for each sub-basin. To obtain the drainage density and the circularity index, four numerical fields were inserted with the names: area, perimeter, drainage density, and circularity index. Area and perimeter values were calculated with the *Calculate Geometry* tool. Drainage density and circularity index values were calculated with the *Field Calculator* tool using equations 4 and 5:

$$Dd = Lt / A \quad (4)$$

Where: Dd is the drainage density (km km^{-2}), Lt is the total rivers length in each sub-basin (km) and A is the drainage area (km^2).

$$Ci = (12.57 \times A) / P^2 \quad (5)$$

Where: Ci is the circularity index (km^2 km^{-2}), A is the drainage area (km^2) and P is the perimeter (km).

RESULTS AND DISCUSSION

The hydrologically consistent digital elevation model of the Cornare's jurisdiction is shown in Figure 4. The DEM shows land with elevations from 134 m to 3,378 m, which agrees with what was described by Cornare (2005), who stated that the jurisdiction counts with a minimum altitude of 200 m on the plain of the Magdalena Medio and a maximum of 3,340 m on the hill of Las Palomas in the Paramo of Sonsón.

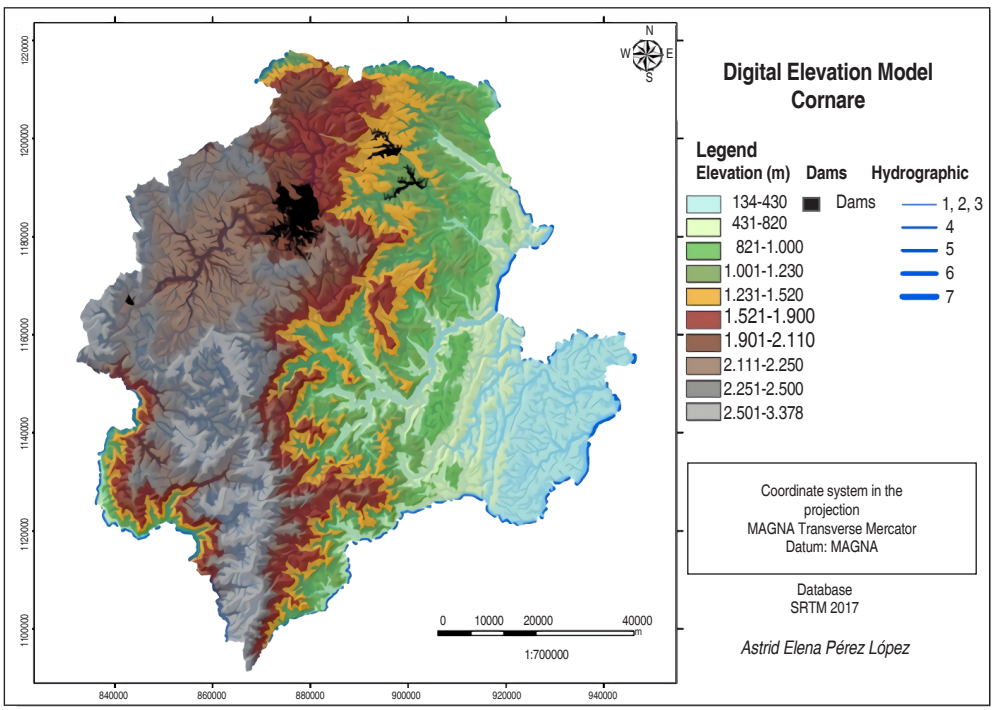

Figure 4. Cornare's Digital Elevation Model.

The declivity map is shown in Figure 5. The surface relief categories according to IDEAM (2013) are shown in Table 1. The jurisdiction of Cornare has terrains with very low, low and slightly moderate surface relief (0°-7°) mainly in the Cocorná Sur and Rionegro basins; moderate and moderately strong (7°-30°) in most of the territory and strong and very strong (30°->60°) mainly in the Samaná Norte, Samaná Sur and Arma basins; thus having geoforms from flood plains to rocky escarps passing through Vegas and alluvial piedmonts, torrential cones, thick and thin colluvial slopes, and rocky slopes.

Table 1. Surface relief categories according to IDEAM (2013).

Range (°)	Name	Common Geoforms
0.00-0.50	Very low	Flood plains
0.50-2.00	Low	Vegas and alluvial piedmonts
2.00-7.00	Slightly moderate	Torrential cones
7.00-14.00	Moderate	Thin colluvial slopes
14.00-30.00	Moderately strong	Thick colluvial slopes
30.00-60.00	Strong	Rocky slopes
>60.00	Very strong	Rocky escarpments

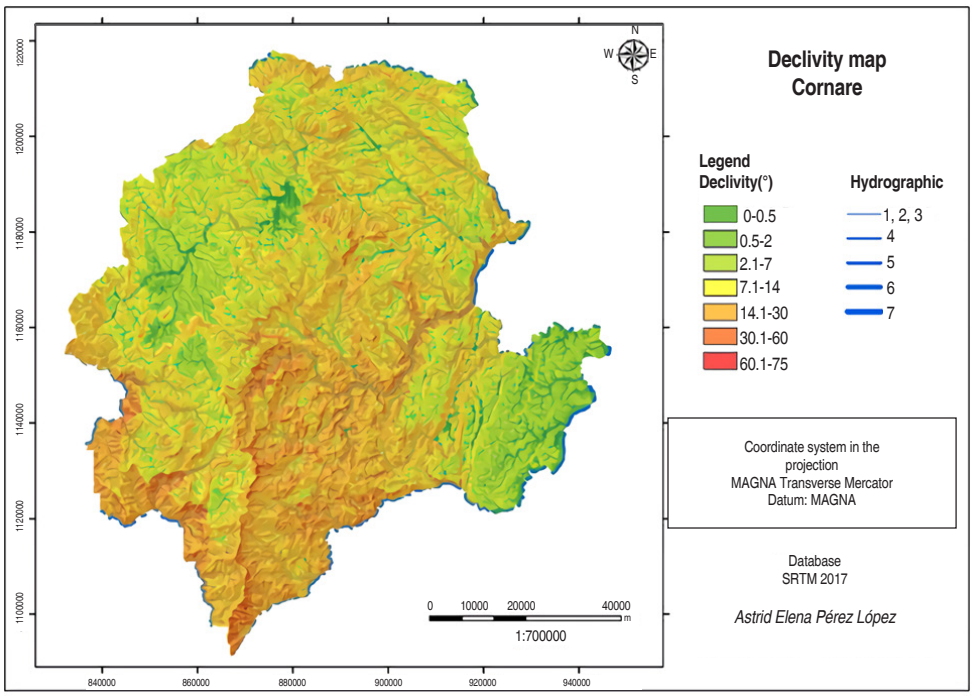

Figure 5. Cornare's Declivity Map.

Figure 6 shows the comparisons between the real drainage mapped by the Dirección de Sistemas de Información y Catastro del Departamento Administrativo de Planificación de la Gobernación de Antioquia in blue color and the numerical drainage with 10, 20, 30, 40, 50, 60, 70 and 80 contribution cells in red color, in the jurisdiction of Cornare. To obtain the numerical drainage, it has a lower restriction, that is the lower number of accumulated flow cells, it causes a larger drainage network, since having a lower threshold, the network becomes denser.

With 10, 20, 30, and 40 cells, several false drainage channels are presented. With 50 and 60 cells, the drainage channels were more representative. With 70 and 80 cells, the numerical drainage did not reach the length of several channels, although they were the ones with the fewest false drainage channels. By visual comparison of a larger area, it was highlighted that the numerical drainage with 60 contribution cells was the one that best represented the hydrography of the Cornare's jurisdiction.

Figure 7 shows a cutout of the numerical drainage network with 60 contribution cells in Cornare's jurisdiction based on the order. The automatic delimitation calculated eight (8) drainage orders, one (1) being the drainage line with the least water and eight (8) the drainage line receiving the most water.

Figure 8 shows the hydrographic map of the Cornare's jurisdiction based on the channels of 4th, 5th, 6th, and 7th order when the numerical drainage was obtained with 60 contribution cells.

Figure 9 shows the comparisons between the automatic delimitation of basins in the jurisdiction of Cornare based on drainage orders greater than or equal to six (≥ 6), five (≥ 5), and four (≥ 4) and the delimitation of basins made by the Instituto de Hidrología, Meteorología y Estudios Ambientales - IDEAM.

It was observed that the automatic delimitation based on the drainage order greater than or equal to six (≥ 6) (Figure 9A) was the one that came closest to the delimitation of hydrographic basins made by IDEAM, although there is a basin that grouped four (violet color). It is noteworthy that the basins limits correspond exactly to those of the basins delimited in the region, which

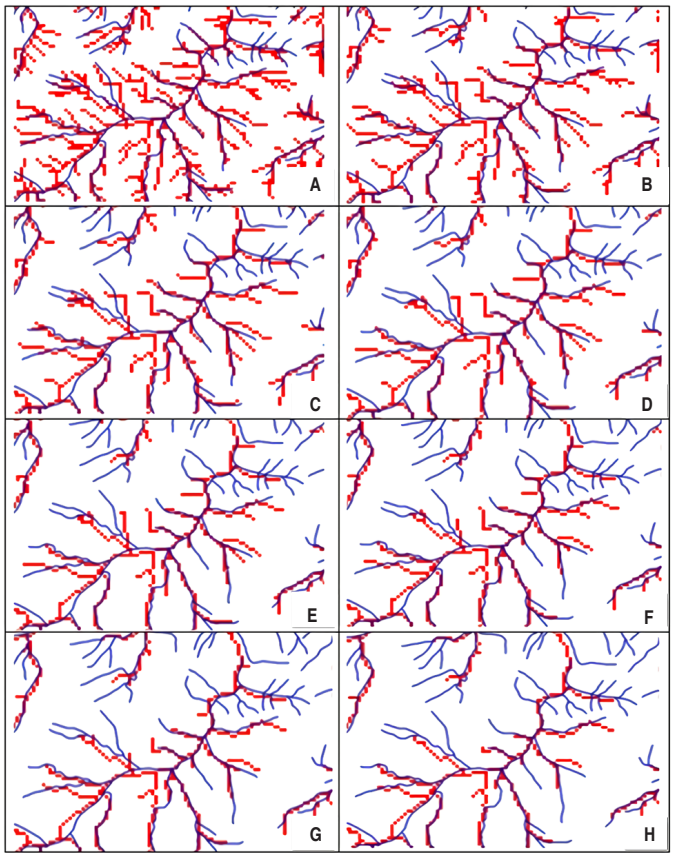

Figure 6. Comparison between the real drainage in blue and the numerical drainage in red, with a different contribution cells number: A) 10, B) 20, C) 30, D) 40, E) 50, F) 60, G) 70, H) 80.

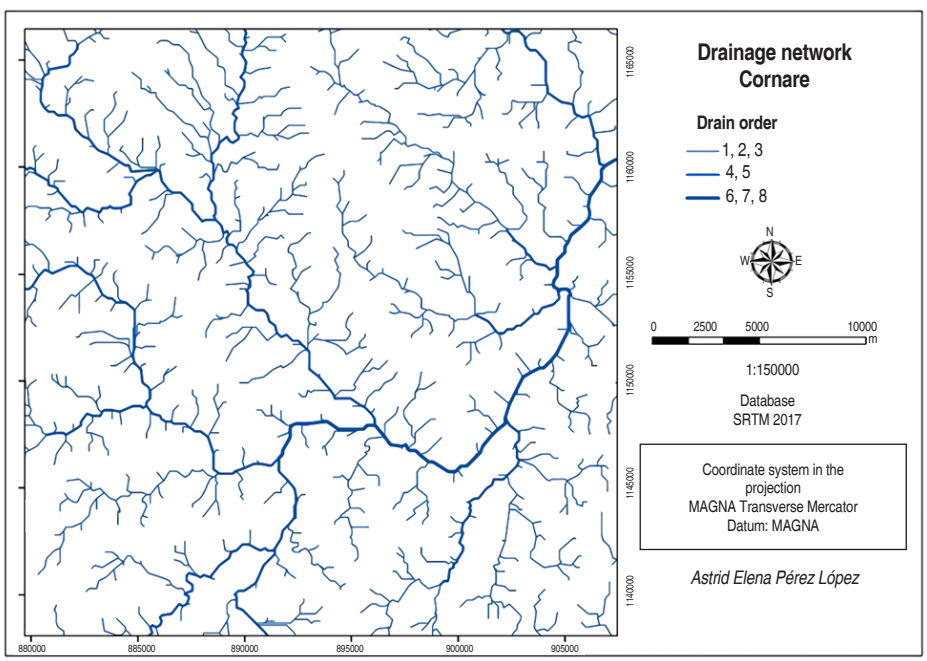

Figure 7. Cutout of the drainage network with 60 cells, from Cornare's jurisdiction based on the order.

confirms that the numerical drainage obtained with 60 contribution cells was the most indicated. Delimitations based on drainage orders greater than or equal to five (≥ 5) and four (≥ 4) (Figure 9B and 9C) present a greater basins number, 14 and >50, respectively, against eight (8) basins delimited in the region. The delimitation based on the drainage order greater than or equal to seven (≥ 7) divides the jurisdiction of Cornare into only two basins.

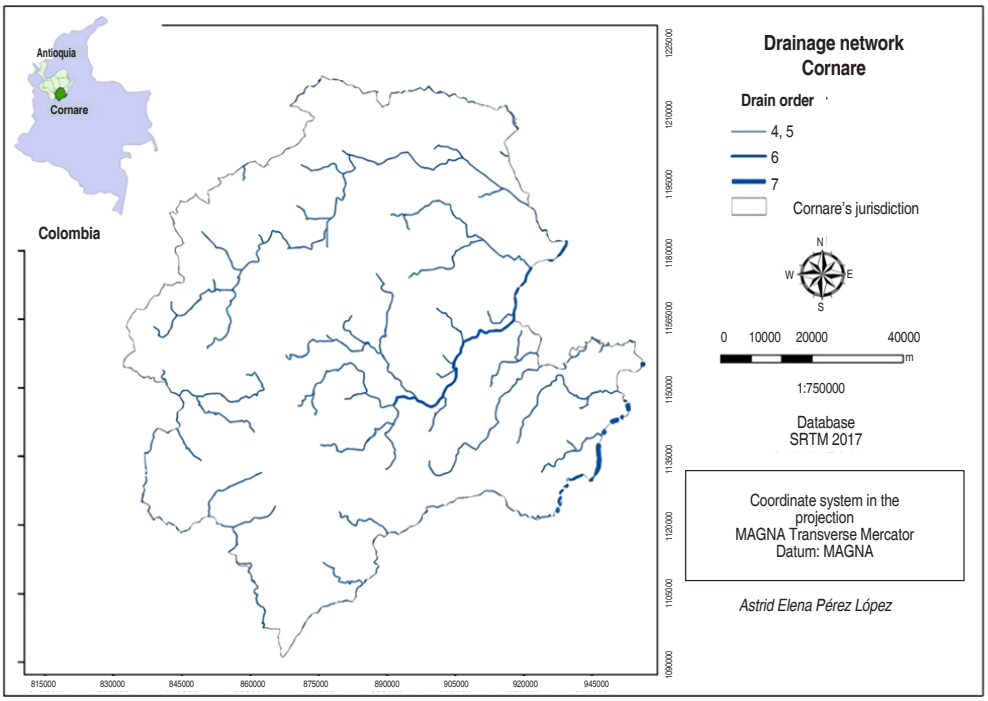

Figure 8. Hydrographic map of Cornare's jurisdiction based on channels of order greater than or equal to four (≥ 4).

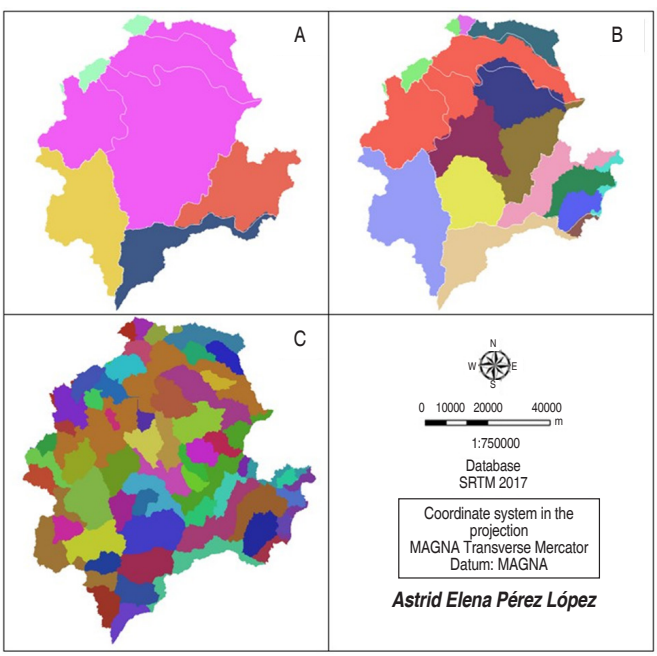

Figure 9. Comparisons between the automatic delimitation of basins in the jurisdiction of Cornare based on drainage orders greater than or equal to six (A), five (B) and four (C) and the basins delimitation made by IDEAM (white limit).

Figure 10 shows the comparisons between Cornare´s jurisdiction sub-basins automatic delimitation based on drainage orders greater than or equal to five (≥5), four (≥4), three (≥3) and two (≥2) and the sub-basins delimitation made by the Dirección de Sistemas de Información y Catastro del Departamento Administrativo de Planificación de la Gobernación de Antioquia.

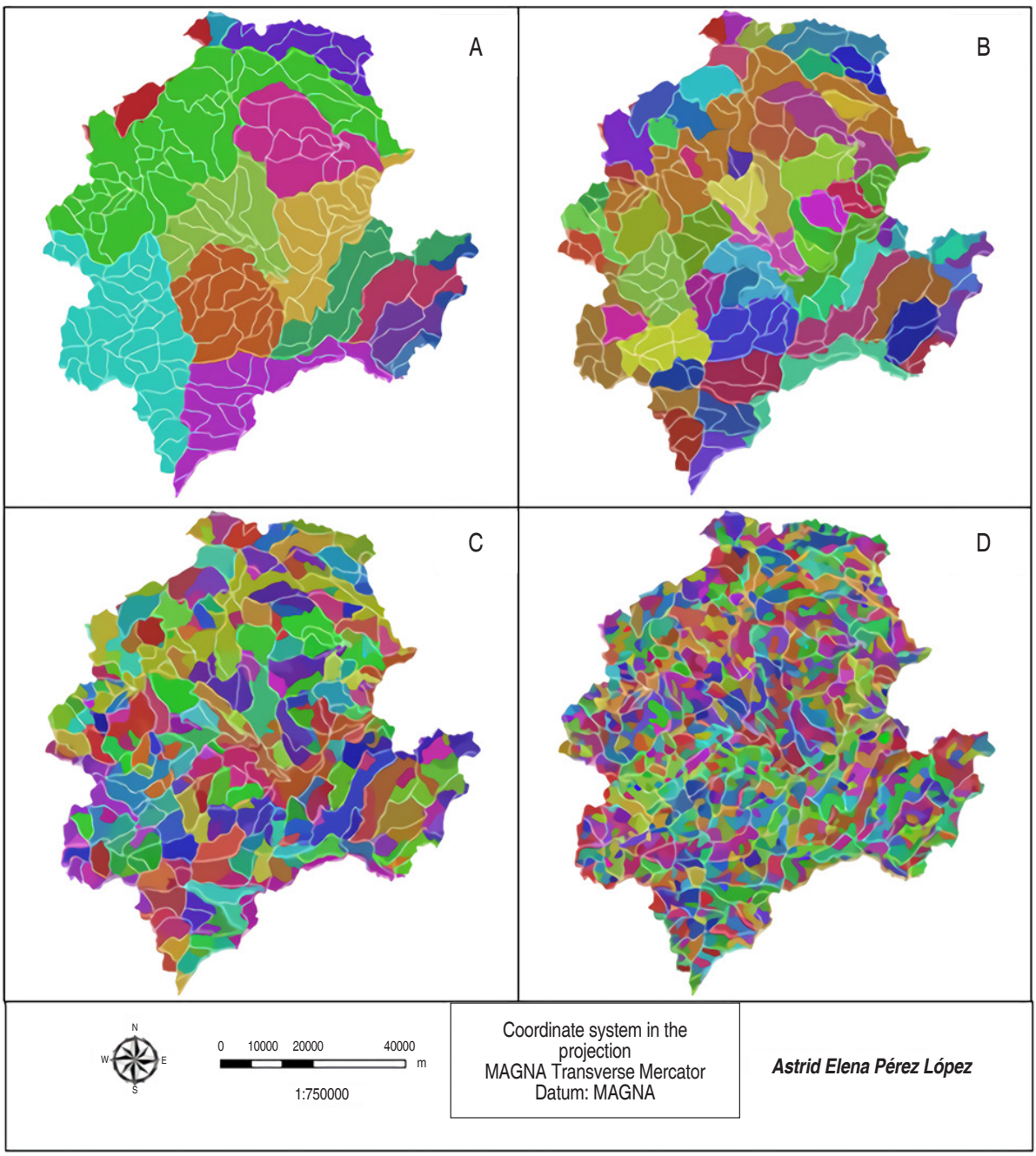

Figure 10. Comparisons between the automatic sub-basins delimitation in the Cornare's jurisdiction based on drainage orders greater than or equal to five (A), four (B), three (C), and two (D) and the sub-basins delimitation made by the Gobernación de Antioquia (white limit).

The automatic delimitation based on the drainage order greater than or equal to three (≥ 3) (Figure 10C) with 226 sub-basins was the closest to the hydrographic sub-basins delimitation by the Gobernación de Antioquia, to which it counts 165 sub-basins. The difference of 61 sub-bases shows that this automatic delimitation is not reliable or that the delimitation made by the Gobernación de Antioquia is obsolete. In comparison, the automatic delimitation based on the drainage order greater than or equal to five (≥ 5) (Figure 10A) inferred 15 sub-basins.

The automatic delimitation based on drainage order greater than or equal to four (≥ 4) (Figure 10B) calculated 63 sub-basins. The automatic delimitation based on drainage order greater than or equal to two (≥ 2) (Figure 10D) shows a number much greater than 226 sub-basins.

Determining morphometrics drainage indices orders greater than or equal to three (≥ 3), five (≥ 5), and six (≥ 6) different drainage density (Dd) and circularity index (Ci) values were found (Table 2).

Table 2. Averages morphometrics sub-basins indices in the jurisdiction of Cornare, according to the chosen drainage order.

Order	Sub-basins (#)	Average area (km^2)	Average perimeter (km)	Average drainage density ($km\ km^{-2}$)	Average circularity index ($km^2\ km^{-2}$)
≥ 3	561	33.51	30.92	0.25	0.48
≥ 5	23	741.17	166.55	0.09	0.34
≥ 6	7	2,304.32	244.62	0.10	0.39

Differences in values are because Dd is inversely proportional to the area, so with a lower drainage order number, there is a greater sub-basins number and a smaller area for each one, so there is a Dd bigger. Also, by having a smaller area, there is a smaller total rivers length, which is directly proportional to Dd. According to Horton's classification (Horton, 1945), the Dd is between low and moderate, that is, the sub-basins in the jurisdiction of Cornare have moderate drainage, medium runoff effectiveness, and moderate infiltration capacity.

As for the Ci, this is inversely proportional to the perimeter, so with a smaller drainage order number, there is a smaller perimeter and a higher Ci. According to Müller (1953) and Schumm (1956) classification, the Cornare jurisdiction sub-basins have a moderate runoff level, tending to be more elongated than circulars, disfavoring the flooding processes and benefiting the runoff process. This agrees with the slope map generated by the DEM.

As the Ci tends to unity, the basin torrentiality increases, since the relative distances of the partition points, about a central one, do not present great differences, and the concentration-time becomes smaller; therefore, increases the possibility that the flood waves are continuous.

In elongated basins, the concentration times are quite different for almost all points in the basin, so the waters will gradually flow towards the main channel. In this way, the main channel will have a greater opportunity to drain part of its volume, before receiving rainwater in the most distant points and, therefore, the flow variations will be less wide and fast, reducing the risk of overflow and flooding in the watershed areas deposition. (Londoño, 2001).

According to Cornare (2012), for 186 sub-basins studied in the municipality of La Ceja, Dd values between 0.98 and 4.21 $km\ km^{-2}$ were obtained. In the La Guayabal sub-basin in the municipality of Cocorná, the Dd was 3.61 $km\ km^{-2}$ and its Ci was 0.39. The sub-basin order is four with 63 tributaries of order one, 16 of order two, and four of order three (Cornare, 2009b).

Montoya and Montoya (2009) found that the Los Andes sub-basin stream in the municipality of El Carmen de Viboral has a high Dd (7.17 $km\ km^{-2}$), it has an area of only 2.87 km^2, 94 tributaries with a total length of 20.58 km. Ramírez *et al.* (2007) found that the sub-basin of the El Salto stream, in the municipality of El Santuario has a Dd of 5.65 $km\ km^{-2}$. Cornare (2009a) describes the La Corozal sub-basin of the municipality of Puerto Triunfo, with a main channel length of 4.06 km, a total tributary sum of 20.59 km, and a Dd of 3.18 $km\ km^{-2}$. The sub-basin order is 4, with 27 tributaries of order one, five of order two, and two of order three.

The main rivers information is recent. According to the POMCAS (2017), the hydrographic basins of the Nare,

Rionegro, Cocorná, Samaná Sur, Porce, and Arma rivers are well drained and elongated. Nevertheless, the Samaná Norte river basin is well-drained, but it has an oval round to oval oblong shape, that is, it can present torrential risks. Figure 11 shows an order greater than or equal to five (≥5) sub-basins Dd map in the Cornare´s jurisdiction, by obtaining the numerical drainage with 60 contribution cells. The Dd values ranged between 0.04 and 0.22 km km^{-2}.

Figure 12 shows order greater than or equal to five (≥5) sub-basins Ci map in Cornare´s jurisdiction, with 60 contribution cells to achieve the numerical drainage. The Ci estimates oscillate between 0.08 and 0.45 km^2 km^{-2}.

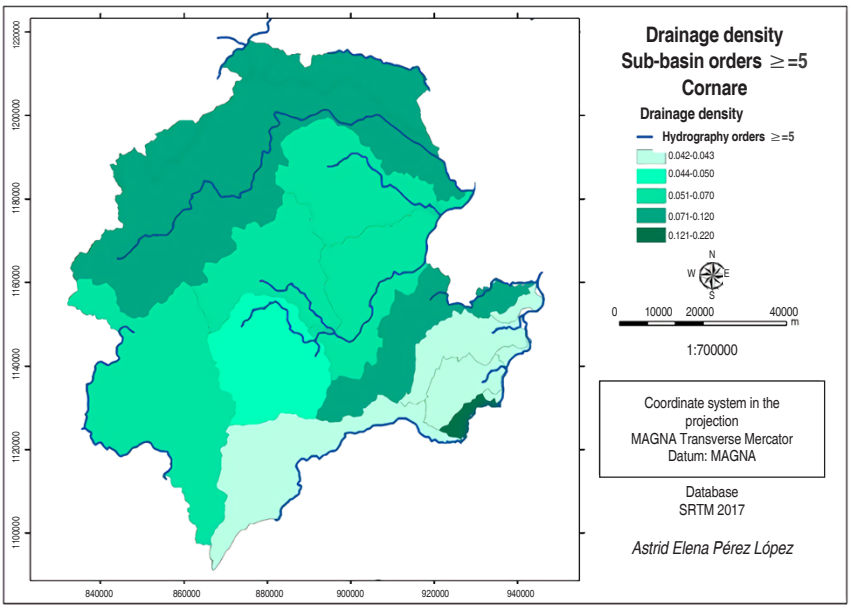

Figure 11. Map drainage density sub-basins order greater or equal to than five in the jurisdiction of Cornare.

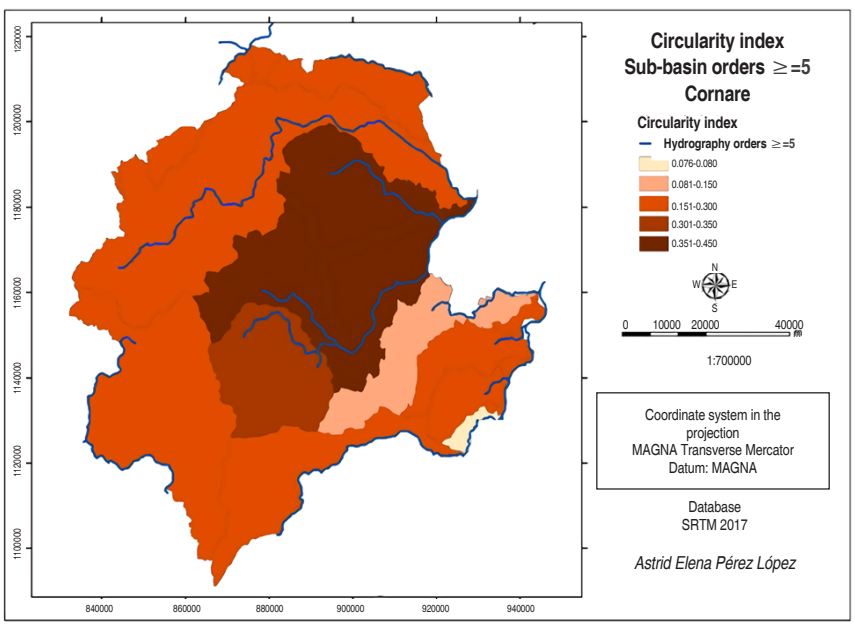

Figure 12. Order greater than or equal to five sub-basins circularity index map in Cornare's jurisdiction.

CONCLUSIONS

Digital elevation models SRTM availability and their information quality saves time and resources for conducting research and diagnostics aimed at the hydrologically consistent digital elevation models generation, which can also be used in areas that don´t have cartographic data available, as they allow to obtain information in a fast, efficient and standardized way.

In hydrographic basins and sub-basins automatic delimitation with the proposed methodology should pay special attention to determining the contribution cell number used to obtain the numerical network.

Basins and sub-basins automatic delimitation based on their drainage order did not come close to reality according to the databases used. This may be because the proposed methodology is unreliable or the databases used obsolete.

The morphometric indices drainage density and circularity index indicated that the sub-basins in the jurisdiction of Cornare have moderate drainage, moderate runoff level, and moderate infiltration capacity; they tend to be more elongated than circulars, disfavoring the flooding processes and benefiting the flow process.

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Short article

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Effect of removal of the upper stem of the ear (topping) and nitrogen application on maize for dual-purpose production of fodder and seed

UNIVERSIDAD NACIONAL DE COLOMBIA

Efecto de la eliminación del tallo superior de la mazorca (poda) y aplicación de nitrógeno en el maíz para la producción de forraje y semilla de doble propósito

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ABSTRACT

Maize is a plant cultivated as fodder or seed. The seeds of this plant are used for poultry and thefresh fodder or silage is required for animal husbandry. In the research, the dual-purpose cropping possibility of maize for fodder and seed harvesting under nitrogen application was assessed. A field experiment and a laboratory experiment were conducted in 2014. The field experiment was performed as a split-plot design. Studied factors were the topping factor (topping (stem removal at the top of the ear) or no topping) and nitrogen fertilizer factor (nitrogen application of 0, 75, and 150 kg ha^{-1}). In the laboratory experiment, germination traits of produced seeds from the maternal plant under topping and nitrogen were studied. Results showed that no topping had the highest ear weight. Topping and nitrogen did not affect seed number per maize ear row. Topping had a lower 100-seed weight and seed yield than no topping. Nitrogen did not affect seed yield and 100-seed weight. Topping and nitrogen application did not affect germination percentage, radical length, caulicle length, and vigor of produced seed at the seed milking stage. Nitrogen application at this stage could not compensate for the damage caused by the topping. Maize topping at the seed dough stage may be evaluated in future studies.

Keywords:
Dual-purpose cropping
Fertilizer
Seed germination
Seed weight

RESUMEN

El maíz es una planta cultivada como forraje o semilla. Las semillas de esta planta se utilizan para la avicultura y su forraje fresco o ensilaje se requiere para la cría de animales. En la investigación se evaluó la posibilidad de cultivo de doble propósito del maíz para forraje y cosecha de semillas bajo aplicación de nitrógeno. Para lograr el propósito, en 2014 se llevaron a cabo un experimento de campo y uno de laboratorio. El experimento de campo se llevó a cabo como un diseño de parcela dividida. Los factores estudiados fueron el factor poda (con poda (eliminación del tallo en la parte superior de la mazorca) o sin poda) y el factor fertilizante nitrogenado (aplicación de nitrógeno de 0, 75 y 150 kg ha^{-1}). En el experimento de laboratorio, se estudiaron los rasgos de germinación de semillas producidas de la planta materna bajo poda y nitrógeno. Los resultados mostraron que ninguna poda tuvo el mayor peso de mazorca. La poda y el nitrógeno no afectaron el número de semillas por hilera de mazorca de maíz. La poda tuvo un peso de 100 semillas y un rendimiento de semillas más bajos que el maíz sin poda. El nitrógeno no afectó el rendimiento de semillas ni el peso de 100 semillas. La poda y la aplicación de nitrógeno tampoco no afectaron el porcentaje de germinación, la longitud de las raíces, la longitud de la caulícula y el vigor de la semilla producida en la etapa de ordeño de la semilla. La aplicación de nitrógeno en esta etapa no pudo compensar el daño causado por la poda. Se sugiere que la eliminación del tallo superior de la mazorca del maíz en la etapa de masa de semilla se evalúe en estudios futuros.

Palabras clave:
Cultivo de doble propósito
Fertilizantes
Germinación de semillas
Peso de semillas

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Maize is a plant cultivated as fodder or seed. The seeds of this plant are used for poultry and fresh fodder or silage is required for animal husbandry. Maize residues after seed production become woody and hardly rot in the soil. These residues are a barrier to tillage operations. One way to use maize as a dual-purpose crop is to use the seeds and leaves of the plant (Homann *et al*., 2013).

Defoliation has been studied in many experiments by several authors (Martínez *et al*., 2014; Balducci *et al.,* 2020; Toïgo *et al.,* 2020; Wang *et al.,* 2020; Scasta *et al.,* 2021). Maize leaf removal improved photosynthesis, morphological characteristics, and seed yield in soybean under maize-soybean relay-intercropping (Raza *et al*., 2019). Weekly defoliation at low and high nitrogen levels showed that at low nitrogen levels, dry matter accumulation in shoots and roots of *Festuca rubra* decreased (Paterson and Sim, 2000). Nitrogen is an essential nutrient for plants (Albdaiwi *et al*., 2019); the availability of nitrogen improved plant growth from defoliation damage in wheat (Heidari and Nosratti, 2016). Nitrogen application of 75 kg ha^{-1} with no leaf removal (control) had the highest seed yield in chickpeas (*Cicer arietinum* L.). Nitrogen application of 75 kg ha^{-1} with complete removal of leaves had the lowest seed yield (Amini *et al.,* 2017). Defoliation affects the growth and photosynthetic capacity of plants, remobilizes carbon and nitrogen reserves, and improves source-sink relationships (Iqbal *et al*., 2012). Defoliation at the eight-leaf stage, regardless of nitrogen content, did not reduce seed yield compared to no defoliation. Nitrogen fertilizer application as a top dressing at the fifteen-leaf stage increased seed yield due to a reduction in degradation caused by defoliation. Defoliation at the tasselling stage caused a great reduction in seed yield (Sangoi *et al.,* 2014). The effect of leaf removal and nitrogen on maize showed that compared to untouched treatment, seed yield reduction under removal of upper and lower leaves was 22.2% and 4.2%, respectively. As the amount of nitrogen increased, the redistribution of stem assimilates increased and to some extent, the effect of removal of the lower leaves of maize on seed yield was compensated (Modhej *et al.,* 2014).

Optimal removal of maize leaves improved photosynthesis and sink-source ratio after silking, resulting in maximum grain yield. So, less dense cultivars should have fewer or smaller upper leaves to reduce leaf area and therefore increase the light distribution. However, for denser cultivars, the leaves below the ear should age rapidly to reduce leaf respiration (Liu *et al.,* 2020).

Among the different treatment compositions of topping and nitrogen levels, the highest plant, diameter, leaf number, leaf area, and seed yield of maize were observed under treatment composition of no topping and 150 kg N ha^{-1} (Shesh *et al.,* 2019). Topping at two weeks after anthesis had no adverse effect on seed yield. Therefore, a significant amount of fodder can be harvested without reducing grain yield. Although topping increased the 1000-seed weight compared to the control, no significant difference was observed in their grain yield (Tahmasbi *et al.,* 2001). The results of research on the response to topping and nitrogen levels showed that grain yield, cob length, and grain number per row were highest in no topping treatments. Harvest index and cob diameter increased under topping conditions. Similarly, all of these traits were superior under 150 kg N ha^{-1}. The lowest value was obtained in treatments without fertilizer application (Shesh *et al.,* 2020).

The environment of the mother plant can affect the germination of the produced seeds (Laossi *et al.,* 2010; Cendán *et al.,* 2013; Edwards *et al.,* 2016; Dewan *et al.,* 2018; Geshnizjani *et al*., 2019). Application of 100 kg N ha^{-1} in rapeseed fields produced seeds that had a higher germination percentage and shorter germination time than the control (Oskouie, 2012).

According to the literature review, most studies use the leaves of the maize plant to produce fodder; there are few studies on the effect of topping (removal of the upper stem of the ear) and nitrogen fertilization in maize to produce fodder. Therefore, this study aimed was to determine the best treatment combination of topping and nitrogen for dual-purpose production of maize grain and fresh fodder. Another objective of this project was to determine the germination traits of maize seeds produced from material plants under topping and nitrogen conditions.

MATERIALS AND METHODS

Field experiment

The field experiment was conducted as a split-plot design based on a complete randomized block design

with three replications. The studied factors were nitrogen fertilizer as a main-plot and topping factor as a subplot. Nitrogen fertilizer factor included application rates of 0, 75, and 150 kg ha^{-1}. Topping factor included topping (stem removal at the top of the ear) and no topping. This research was conducted in the arable lands of Chamchamal plain located in Kermanshah. The soil of this region is one of the most fertile soils in the west of Iran and has a wide groundwater aquifer. Most farmers in the area use wheat-maize rotation. In recent years, the area under maize cultivation in this region has grown beyond the area under wheat cultivation. At the beginning of March 2014, the soil of the field was plowed using a moldboard plow. Then, triple superphosphate fertilizer at the rate of 333 kg ha^{-1} was applied under the soil using a cultivator. Single cross 704 maize seeds were sown at a rate of 27 kg ha^{-1} using a pneumatic maize seeder with a row spacing of 75 cm. The sowing date was April 7, 2014. Urea fertilizer at the rate of 367 kg ha^{-1} was applied as a top dressing to all treatments at two stages on May 31 and June 22. Soil texture, pH, electrical conductivity, and nitrogen were silty clay loam, 7.2, 1.6 ds m^{-1}, and 0.2 %, respectively.

The most important weeds in the field were red-root amaranth (*Amaranthus retroflexus*, johnsongrass (*Sorghum halepense* (L.) Pers.), common lambsquarters (*Chenopodium album*), green foxtail (*Setaria viridis*), Chinese lantern (*Physalis alkekengi),* black nightshade (*Solanum nigrum*). Nicosulfuron and 2,4-D plus MCPA herbicides were used to control weeds.

During the growing season, the plants were irrigated eight times. Plants were sowed on the ridge and the method of irrigation was furrow. The time of application of experimental treatments was July 30 at the beginning of the seed filling period, seed milking stage. Then, topping and nitrogen were applied 114 days after sowing. The criterion for phenological stages, such as the seed filling stage, was the entry of at least 50% of plants into that stage. In the topping treatment, the maize stalk was cut just above the ear using a cutter.

At harvest time, three plants were selected from the two midlines of each plot. After drying the ears, the ear husks were weighed first. Then, the weight of the ear without husk was obtained. The seed number per ear row, the seed number per column of ear, cob length, cob weight, seed yield, and weight of 100 seeds were also calculated.

Laboratory experiment

A laboratory experiment was conducted to evaluate the effect of the mother plant environment on seed germination at the Crop Physiology Laboratory, Faculty of Agricultural Science and Engineering, Razi University in 2014. In the laboratory experiment, the germination traits of seeds produced from the maternal plant under topping and nitrogen were investigated. The laboratory experiment was conducted as a factorial experiment based on a completely randomized design with three replications. Seeds related to each field treatment were surface disinfected separately using sodium hypochlorite. Then, 10 seeds were placed on filter paper in a Petri dish. The Petri dishes were stored in a germinator at 25 °C for one week. After one week, germination traits such as germination percentage, radicle and caulicle length, and seed vigor were measured. Seed vigor was obtained by the following equation (Heidari*,* 2013).

Seed vigor (% cm) = Seed germination (%) x (Radicle length (cm) + Caulicle length (cm))

Data analysis

Data were analyzed by analysis of variance and mean data were compared using the Tukey test at the 5% probability level. Before analyzing the data, the normality test was performed. Correlation between traits was also calculated. Statistical calculations were performed using SAS, SPSS, and MINITAB software (Soltani, 2013).

RESULTS AND DISCUSSION

Field experiment

Ear husk weight and ear weight. Analysis of variance and mean comparison showed that topping and nitrogen did not affect the ear husk weight (Tables 1 and 2). This is probably because the ear cover, like the organ that covers the ear, begins to grow before other parts of the ear. This organ has probably reached its final growth at the milking stage of the seeds. The ear husk as a photosynthetic organ plays an important role in grain filling (Koocheki and Sarmadnia, 2011). Analysis of variance showed that nitrogen had no effect on ear weight, but topping had a significant effect on ear weight

(Table 1). Mean comparison showed that there was no difference between nitrogen levels in terms of ear weight but topping reduced ear weight (Table 2). These data showed that topping reduced the ear weight and nitrogen application could not reduce the damage caused by topping. The plant may have already taken the nitrogen necessary for the growth of its reproductive organs from the soil during the milking stage of the seeds, or the amount of soil nitrogen may have been sufficient before applying the nitrogen treatments. The results of soil testing showed that the amount of soil nitrogen for maize is appropriate based on the research of others (Sarafraz-hezarmasjed, 2022). In a study, nitrogen application at the stage of spike emergence reduced defoliation damage in wheat (Heidari *et al.,* 2013). Part of the difference between the present experimental results and the results of others is related to the stage of application of the treatments. In the present study, the time of nitrogen application was the milking stage of the seeds.

Table 1. Analysis of variance (mean square) of the effect of topping and nitrogen on maize traits.

Source of variation	d.f	Ear husk weight	Ear weight	Seed number per column	Seed number per row	Cob length	Cob weight	Seed yield	100-seed weight
Block	1	2.79^{ns}	95.26^{ns}	3.56^{ns}	0.027^{ns}	0.23^{ns}	0.19^{ns}	219.18^{ns}	0.26^{ns}
Nitrogen (N)	2	1.52^{ns}	1091.12^{ns}	58.80*	0.724^{ns}	13.73*	21.39^{ns}	1183.77^{ns}	1.42^{ns}
Error (a)	2	0.70	258.73	3.57	0.112	0.80	0.33	273.88	47.36
Topping (T)	1	1.66^{ns}	9733.84**	6.72^{ns}	0.497^{ns}	0.18^{ns}	28.85^{ns}	10203.54**	267.50**
N * T	2	0.63^{ns}	110.94^{ns}	19.20^{ns}	0.389^{ns}	0.92^{ns}	1.71^{ns}	172.29^{ns}	6.20^{ns}
Error (b)	9	3.17	312.77	7.64	0.516	2.44	8.22	359.6	7.62
Coefficient of variation (%)		19.31	15.44	6.80	5.28	8.81	16.3	19.2	14.73

ns, * and ** are non-significant and significant at the probability level of 5 and 1%, respectively

Table 2. Comparison of means of the effect of topping and nitrogen on maize traits.

Treatments	Ear husk weight (g plant^{-1})		Ear weight (g plant^{-1})		Seed number per column		Seed number per row	
	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
N1	9.72a	0.51	129.3a	13.13	44.2a	0.72	13.7a	0.37
N2	8.71a	0.54	111.3a	10.16	38.2a	1.37	13.9a	0.22
N3	9.23a	0.79	102.9a	13.06	39.6a	1.32	13.2a	0.14
T1	8.9a	0.44	137.7a	6.81	41.3a	1.45	13.4a	0.25
T2	9.5a	0.57	91.2b	5.88	40.0a	1.09	13.8a	0.18

Treatments	Cob length (cm)		Cob weight (g plant^{-1})		Seed yield (g plant^{-1})		100-seed weight (g)	
	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
N1	19.5a	0.67	19.6a	0.9	109.4a	12.36	19.1a	2.1
N2	16.9a	0.38	17.3b	1.04	93.8a	9.32	18.5a	1.85
N3	16.7a	0.52	15.8b	1.29	81.6a	15.66	18.6a	2.27
T1	17.8a	0.64	18.8a	0.85	118.6a	5.95	22.6a	0.94
T2	17.6a	0.57	16.3a	0.98	71.0b	7.61	14.9b	0.91

T1 and T2 correspond to the control (untouched) and topping (removal of stem at the top of the ear), respectively. N1=0 kg N ha^{-1}, N2= 75 kg N ha^{-1}, and N3= 150 kg N ha^{-1}. The means with the same letter in each trait are not significantly different according to the Tukey test at the probability level of 5%.

Seed number per column and row of the ear. Mean comparison showed that there was no difference between the nitrogen rate and the topping in terms of seed number per column and row of the ear. No significant difference between the treatments is probably because the seed number per ear of maize is determined during pollination and only the seed weight may change after pollination (Koocheki and Sarmadnia, 2011). The results of this study also confirmed that the seed weights changed. In a study on maize, it was reported that the removal of the top two leaves had no effect on the number of seeds per plant because the removal caused nitrogen remobilization from the vegetative organs to the seed and increased photosynthetic capacity to absorb nitrogen, but removing more leaves reduced the number of seeds per plant (Liu *et al*., 2017).

Length and weight of cob. Mean comparison showed that there was no difference between the nitrogen rate and the topping in terms of cob length. The topping did not affect cob weight, but nitrogen application reduced cob weight. These results indicate that high nitrogen application may stimulate root growth and prevent the remobilization of material inside the stem or root of the plant to the ear. Previous studies have reported that defoliation reduces root materials' remobilization to shoots (Paterson and Sim, 2000). However, the difference between treatments for cob is less than ear weight or grain yield, which indicates that cob growth is completed earlier than seeds. In a study on maize, treatment of removal of the lower leaves of the ear had lower cob length compared to undefoliated treatment (Umashankara, 2007).

100-seed weight and seed yield. Analysis of variance showed that nitrogen had no significant effect on 100-seed weight, but topping had a significant effect on 100-seed weight (Table 1). Mean comparison showed that topping had a lower 100-seed weight than no topping. Nitrogen did not affect the 100-seed weight of maize. Nitrogen needed to fill the seeds during the milking stage is probably supplied by nitrogen in plant organs such as leaves, stems, and roots, and the plant no longer absorbs nitrogen from the soil at this stage (Mahmoodi and Hakimian, 2019). Analysis of variance showed that nitrogen had no effect on seed yield, but topping had a significant effect on seed yield. Mean comparison showed that there was no difference between nitrogen levels in terms of seed yield but topping reduced seed yield (Table 2).

Reduction of 100-seed weight of the plant under topping conditions compared to other yield components of maize played a more important role in reducing seed yield. These results indicate that the upper stem and leaves of the plant at the time of seed filling, in addition to providing the plant photosynthesis, are probably a reservoir for the plant carbohydrate supply when the current photosynthesis stops during the remobilization process and their removal reduces maize seed yield. Nitrogen application at this time cannot compensate for the damage caused by the removal of these organs. The importance of upper maize leaves in seed filling has been proven in previous studies (Barimavandi *et al.,* 2010). The upper leaves of maize are younger and have more photosynthetic power than the lower leaves. While the lower leaves of corn are often old and sometimes act as photosynthetic consumers.

Based on the fertilizer recommendation for nitrogen (Sarafraz-hezarmasjed, 2022) and the results of soil nitrogen storage in the present study, it seems that the nitrogen requirement of the plant has been met and nitrogen application at the milking stage did not affect maize seed yield. Fanoodi *et al.* (2017) reported that in soil with 0.2% nitrogen, 200 kg N ha^{-1} is sufficient for barley. Khodshenas *et al.* (2016) reported that in soil with 0.04% nitrogen, no difference was observed between 100 to 400 kg N ha^{-1} in terms of maize forage yield. Although nitrogen is the most limiting nutrient in the growth of maize, there was probably enough nitrogen in the soil for the plant to grow, or the plant did not need to absorb nitrogen from the soil during the milking stage, and this nutrient was transferred from the plant organs to the seeds. Of course, the time of the treatment application should also be considered. If nitrogen is used at the pollination stage, its effect might have been different. In one study, four levels of defoliation (0, 33, 66, and 100% removal of leaf area) were applied on maize at stages of twelve leaves, silking, or seed milking.

Dry matter yield gradually decreased with increasing defoliation intensity and application at the twelve-leaf stage compared to silk or milking stages (Bani *et al.,* 2019). Shesh *et al.* (2019) reported that topping reduced maize seed yield, which is consistent with the results

of the present study. In the research of Tahmasbi *et al.* (2001), it was observed that there is no difference between the topping and no topping treatment of maize in terms of grain yield, which is different from the results of the present study. Maize seed yield had a significantly positive correlation with all studied traits except ear husk weight and seed number per ear row (Table 3). This result shows the importance of seed yield components such as seed weight and even organs such as cob in producing high yields.

Table 3. Pearson correlation coefficients among maize traits under topping and nitrogen.

	Ear husk weight	Ear weight	Seed number per column	Seed number per row	Cob length	Cob weight	Seed yield	100-seed weight
Ear husk weight	1							
Ear weight	0.228	1						
Seed number per column	0.37	0.538*	1					
Seed number per row	0.007	-0.268	-0.319	1				
Cob length	0.324	0.527*	0.774**	-0.202	1			
Cob weight	0.424	0.856**	0.695**	-0.221	0.747**	1		
Seed yield	0.276	0.976**	0.515*	-0.209	0.525*	0.844**	1	
100-seed weight	0.115	0.903**	0.287	-0.396	0.311	0.647**	0.893**	1

*,**: significance at the probability level of 5% and 1%, respectively.

Laboratory experiment

Means comparison showed that topping and nitrogen application on maize maternal plants at the seed milking stage did not affect germination percentage, radicle length, caulicle length, and vigor of produced seed (Table 4). However, maize topping reduced the weight of produced seeds (Table 2), and seeds with different weights had the same germination traits. A positive and significant correlation was observed between seed vigor and radicle length and caulicle length, which is because these two traits are part of seed vigor (Table 5). Some previous studies have shown a decrease in seed germination produced by the mother plant under nitrogen application conditions (Zhao *et al.*, 2021), and some other studies have indicated an increase in seed germination produced by the mother plant under nitrogen application conditions (Oskouie, 2012).

Table 4. Mean comparison of effect of topping and nitrogen on maize seed traits.

Treatments	Germination (%)		Caulicle length (cm)		Radicle length (cm)		Seed vigor (% cm)	
	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
N1	100.0 a	0	7.0 a	1.15	15.5 a	0.47	22.4 a	1.43
N2	100.0 a	0	7.5 a	1.10	17.3 a	0.79	24.8 a	1.70
N3	100.0 a	0	7.4 a	1.31	17.1 a	0.64	24.6 a	1.91
T1	100.0 a	0	7.4 a	0.66	16.9 a	0.37	24.4 a	0.92
T2	100.0 a	0	7.0 a	1.22	16.3 a	0.62	23.4 a	1.71

T1 and T2 correspond to the control (untouched) and topping (removal of stem at the top of the ear), respectively. N1=0 kg N ha^{-1}, N2= 75 kg N ha^{-1}, and N3= 150 kg N ha^{-1}. The means with the same letter in each trait are not significantly different according to the Tukey test at the probability level of 5%.

The application time of nitrogen fertilizer affects the germination traits of seeds produced from the mother plant. In the mentioned studies, nitrogen was used before the reproductive stage, but in the present study, nitrogen was used at the milking stage of the seeds. Different levels of defoliation on the mother plant did not affect the

Table 5. Pearson correlation coefficients among maize seed traits under topping and nitrogen

	Germination percent	Radicle length	Caulicle length	Seed vigor
Germination percent	1			
Radicle length	-0.080	1		
Caulicle length	-0.326	0.676**	1	
Seed vigor	-0.177	0.962**	0.852**	1

*,**: significance at the probability level of 5% and 1%, respectively.

germination percentage of produced seeds in *Triticum aestivum* (Heidari *et al.,* 2013; Rodolfo *et al*., 2017) and *Quercus ilex* (Roncé *et al*., 2020), which is consistent with the results of this study.

CONCLUSION

Topping reduced maize seed yield which was mainly due to a decrease in maize seed weight. Nitrogen application at the maize seed milking stage could not compensate for the damage caused by the topping. Nitrogen application and topping in maize did not affect the germination traits of produced seeds. It is necessary to evaluate the topping treatment at the dough stage of maize grains for dual-purpose cultivation in future studies.

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Research article

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Nitrogen and phosphorus as macronutrients of cocoa (*Theobroma cacao*) and their physiological functions in different planting patterns of cultivation in Central Java, Indonesia

Nitrógeno y fósforo como macronutrientes del cacao (*Theobroma cacao*) y sus funciones fisiológicas en diferentes patrones de plantación de cultivos en Java Central, Indonesia

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ABSTRACT

Plant physiological status during the growing season (specific leaf area (SLA), resorption of N and P) leads to knowing the best plant nutrition management (amount and time) based on the plating pattern. Furthermore, proline and glucose content in root tissues may provide a better technique to represent plant stress conditions. This study aimed to evaluate the SLA, the level of reabsorption of N and P from the leaf, and root proline and glucose content of cocoa plants in different seasons and planting patterns. This study was performed in the fields of Plana village, Somagede, Banyumas, 14 Central Java, Indonesia, and was conducted in December 2015 (rainy season) and October 2016 (dry season) on 7 years-old cocoa plants (*Theobroma cacao*). Three different planting patterns were observed; (1) only cocoa plants, (2) cocoa and coconut pattern, and (3) cocoa with shading trees. The results showed that different seasons and planting patterns affected each observed parameter differently. Cocoas' SLA was not significantly different in all areas for both 2015 and 2016. N resorption during the growing season did not change in 2015 and 2016 in all planting patterns, whereas P resorption had a significant change in 2016 in all planting patterns. The proline content was significantly different in June 2015, October 2015, and March 2016 in all planting patterns. The glucose content in roots showed insignificant differences in 2015 and 2016 in all planting patterns. These results also showed that SLA and glucose did respond to season and plating patterns. These parameters are suggested as poor indicators of physiological status. Furthermore, sowing cocoa plants with other types of plants can be used to help farmers and stakeholders in managing cocoa cultivation in efficient and sustainable ways.

Keywords:
Osmolithic
Resorption
Specific leaf area
Theobroma cacao L.
Vegetation

RESUMEN

El estado fisiológico de la planta durante la temporada de crecimiento (área foliar específica (SLA), reabsorción de N y P) conduce a conocer el mejor manejo de la nutrición de la planta (cantidad y tiempo) en función del patrón de siembra. Además, el contenido de prolina y glucosa en los tejidos de la raíz puede proporcionar una mejor técnica para representar las condiciones de estrés de la planta. Este estudio tuvo como objetivo evaluar el SLA, el nivel de reabsorción de N y P de la hoja y el contenido de prolina y glucosa de la raíz de las plantas de cacao en diferentes estaciones y patrones de siembra. Este estudio se realizó en los campos de la aldea de Plana, Somagede, Banyumas, 14 Central Java, Indonesia, y se realizó en diciembre de 2015 (temporada de lluvias) y octubre de 2016 (temporada seca) en plantas de cacao (*Theobroma cacao*) de 7 años. Se observaron tres patrones de plantación diferentes; (1) solo plantas de cacao, (2) patrón de cacao y coco, y (3) cacao con árboles de sombra. Los resultados mostraron que las diferentes estaciones y patrones de siembra afectaron cada parámetro observado de manera diferente. El SLA de cacao no fue significativamente diferente en todas las áreas para 2015 y 2016. La reabsorción de N durante la temporada de crecimiento no cambió en 2015 y 2016 en todos los patrones de siembra, mientras que la reabsorción de P tuvo un cambio significativo en 2016 en todos los patrones de siembra. El contenido de prolina fue significativamente diferente en junio de 2015, octubre de 2015 y marzo de 2016 en todos los patrones de siembra. El contenido de glucosa en raíces mostró diferencias no significativas en 2015 y 2016 en todos los patrones de siembra. Estos resultados también mostraron que el SLA y la glucosa respondieron a la estación y a los patrones de siembra. Estos parámetros se sugieren como malos indicadores del estado fisiológico. Además, la siembra de plantas de cacao con otros tipos de plantas se puede utilizar para ayudar a los agricultores y las partes interesadas a gestionar el cultivo de cacao de manera eficiente y sostenible.

Palabras clave:
Osmolítico
Reabsorción
Área foliar específica
Theobroma cacao L.
Vegetación

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Cocoa (*Theobroma cacao* L.) is a species from the Theobroma genus and the Malvaceae family. This species is the most widely cultivated plant as it has high economic value (Najihah *et al*., 2018). Cocoa beans are the raw material for chocolate and cosmetics. The average cocoa production that comes from smallholder plantations from 2011 - 2018 was 649,807 t annually or around 94.32% of total national production (Ruslan and Prasetyo, 2021). In Indonesia, cocoa cultivation is conducted using agroforestry and full-sun systems (Witjaksono, 2016). Sowing protective plants to reduce direct sunlight exposure could increase cocoa production (Wartenberg *et al*., 2019). High temperatures have negative impacts on cocoa plants, such as eco-physiological stress, environmental changes, and decreased CO_2 assimilation. Shade plants play an important role to enhance cocoa plants' physiology and the quality of soil nutrients. The benefits of cacao cultivation under coconut trees are that it can increase land use efficiency, sunlight, and control Helopeltis as the main pest of cocoa (Adam *et al*., 2017). The cocoa-coconut intercropping system does not create competition for sunlight because cocoa is a plant that can grow in 40-80% shade (Latha *et al*. 2017).

Leaves are among the vital organs of a plant, which can affect plant growth and are one of the primary organs in the assimilation process that affects plant production. Furthermore, leaves are also correlated with the physiological functions of the plants, such as specific leaf area (SLA), photosynthetic capacity, nitrogen and phosphorus content, respiration rate, and leaf age (Wright *et al*., 2004). SLA is the leaf area per dry mass that is used as a parameter to assess the plant's carbon content and water status (Ali *et al*., 2017). SLA can indicate the relative growth rate of plants. Also, changes in SLA can represent the structure of leaves and their nutrient content because SLA is mostly affected by photosynthesis activity (Gong and Gao, 2019).

Macroelements, such as nitrogen, phosphorus, potassium, and calcium, play an essential role in several physiological processes (Ashraf *et al*., 2014). Resorption is a process to use nutrients efficiently by transferring nutrients from senescence leaves to young leaves (Housman *et al*., 2012). Environmental factors, such as the dry season with low water supply, affect the resorption process. The senescence leaves use 50% of their N and P components, enzymes, lecithin, and nucleic acids to be translocated to seeds, roots, young leaves, and other parts of plants via the phloem tissue (Tang *et al*., 2013;). There were indications of NPK limitation in all cropping patterns used because the NPK concentration in cocoa was lower than the ideal level without fertilization. *C. indicum* tree planting system at a distance of 8 m×16 m and *G. sepium* at a distance of 12×12 m was preferred over *C. indicum* at a distance of 8×8 m for *T. cacao*. This was because the high-density distance can lead to the depletion of organic matter. The genotype of cocoa affects the response or interaction with the cultivation environment, and will subsequently also affect the efficiency of nutrients (Rosas-Patiño *et al*., 2019).

Plants adapt to changing water content by metabolic adaptation to deal with osmotic pressure and trigger the synthesis of the osmoprotectants, which are proline, protein, and sugar. Those osmoprotectants regulate osmotic potentials and protect the cells from drought damage and regulate drought stress (Li *et al.,* 2015). Proline is the main organic osmolyte compound during abiotic stresses. Furthermore, proline plays a critical role in the osmotic regulating compound and plant structure protection. Proline accumulation is more extensive during the day than at night to protect plants from UV radiation in Barley (Fedina *et al*., 2002). The adjustment of osmotic compounds can be evaluated by the accumulation of sugars to balance water potential during drought stress in cotton (Jamal *et al*., 2015). The more plants experience water shortages, the proline level increases as a survival effort of the plant. According to Janani *et al.* (2019), cocoa clones that were subjected to drought stress and produced the highest proline content also showed high-stress tolerance, thus potentially producing cocoa.

The influence of seasons and planting patterns on cocoa growth is needed to discover the differences in physiological responses. The present study aimed to analyze differences in SLA, N, and P resorption in leaves, and proline and glucose concentration in cocoa roots in different seasons and planting patterns.

MATERIALS AND METHODS

Experimental site and design

The present study was conducted in 2015 and 2016

in Plana village, Somagede, Banyumas, Central Java, Indonesia (Type climate A based on Kuppen climate classification). Based on information from Sugito *et al*. (2019), Plana village has an altitude of 300 m, so it is classified as medium land. The sampling areas were determined by the planting patterns of the cocoa plantation (Figure 1). This land is rainfed soil and NPK fertilizer (10 kg plant^{-1}) is only applied once a year in the early rainy season from month 1 to month 2. Leaf trimming of young shoots and twigs that had many leaves according to Angela and Efendi (2015) was carried out every 2 months depending on the vegetation's density. For maintenance pruning, removed branches had a diameter of < 2.5 cm, heavy pruning was also carried out when the plant was too dense. This pruning also aimed to stimulate the growth of flowers and fruit. Insecticides were not used in this study because of the absence of insect pests on the observed cocoa plants (cocoa hybrid clone). In areas 2 and 3, the number of cocoa plants in each area was 12 trees. The age of cocoa, coconut, and other plants are 8 years, 10 years, and about 10 years, respectively. The percentage of shade plants in area 1 was 75%. Each area was about 2000 m^2 and the planting distance between cocoa plants was 3 m (Figure 1). A completely randomized design with 12 replications was used in this study.

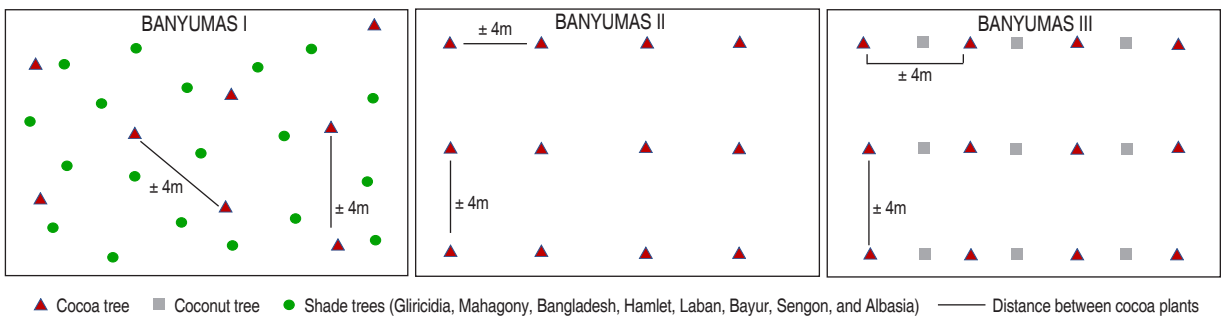

Figure 1. Three different planting patterns in sampling sites.

From 24 cocoa plants, 12 leaves per area were picked up in each area to evaluate the SLA and N and P content. The analyses on the cocoa tree roots were conducted to examine the proline and glucose content. Proline and glucose were quantified in three periods (6-7 June 2015, 31 October 2015, and 26-27 March 2016) to show a complete seasonal profile in Indonesia. According to BPS (2017), Banyumas has the highest rainfall of 113.25 mm^3 in June (rainy season) and the lowest of 0 mm^3 (dry season) in October 2015. The rainfall took place in all months observed in 2016 with 302 mm^3 of rainfall in March 2016.

Environmental and weather factors determination

The microclimate was measured from January 2015 to December 2016 every week. Air temperature, humidity, pH, temperature, and groundwater content were measured in the present study. Air temperature and humidity were measured by using a thermohygrometer. pH, temperature, and groundwater content were measured using a tensiometer from 12.00 a.m. to 02.00 p.m.

Specific leaf area (SLA) analysis

SLA is the ratio of leaf area to dry weight (cm^2 g^{-1}). SLA observations were conducted using adult cocoa leaves with a similar leaf index color according to Roderick *et al*. (1999). Leaf samples (12 leaves area^{-1}) were obtained from plant branches, wrapped in aluminum foils, and stored in a cool box to keep them fresh. The leaf area (cm^2) was measured using millimeter block paper. The leaves were dried using an oven to calculate the dry weight (cm^2 g^{-1}). SLA evaluation was conducted with three different vegetation areas in 2015 and 2016.

Analysis of nitrogen (N) and phosphorus (P)

N and P content analysis was conducted in adult and senescence leaves of cocoa plants on 26-31 January 2015 and 3-9 October 2016. The N leaf content was evaluated using the Kjeldahl method (Singh *et al*., 2015). P content of the leaf was evaluated using the Morgan–Wolf method as a sanitizer based on Jeshni *et al.* (2017). N and P resorption measurement was conducted according to Li *et al*. (2019a) by Equation 1.

$$NR = 100\% \times \frac{(N_d - N_s)}{N_d} \quad (1)$$

Where, NR: nitrogen resorption (%); N_d: adult leaf nitrogen (%); N_s: senescence leaf nitrogen (%)

Proline analysis

Fine root samples were obtained using a soil core at 20 cm depth in midday (12.00–14.00) on 6-7 June 2015 (early dry season), 31 October 2015 (late dry season), and 26-27 March 2016 (late rainy season). Proline accumulation was analyzed based on Bates *et al*., (1973) method. A total of 0.5 g of dry root was homogenized with 10 mL of 3% sulfosalicylic acid for 72 h and then filtered. About 2 mL of filtrate was reacted with 2 mL of ninhydrin acid and glacial acetic acid. The samples were heated at 100 °C for 1 h. The filtrate was then placed into a glass cup with ice. A mixture of filtrate, ninhydrin, and glacial acetic acid was added to toluene and stirred for 15–20 s. The obtained red toluene with proline was measured using a spectrophotometer at 520 nm wavelength and compared to a standard proline curve to obtain the proline level of the sample.

Glucose analysis

Phenol method with glucose solution was used to determine the root's glucose content according to Chow and Landhäusser (2004). The reagents were 5% phenol solution in water, 95.5% H_2SO_4 with a density of 1.84, and a standard glucose solution. The used equipment was a spectrophotometer and a 25 °C water bath. The standard curve was made with 2 mL of a standard glucose solution with 0, 10, 20, 30, 40, and 60 µL glucose. The fine root samples were obtained similarly to proline analysis. The obtained samples were immediately heated at 60 °C for 60 s. Then, it was added with 1 mL of 5% phenol solution and mixed. After that, it was added with 5 mL of the sulfuric acid solution for 10 min, constant shaking, and placed in a water bath for 15 min. The absorbance was measured at 490 nm for hexose and 480 nm for pentose and uronic acid.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at the significance level of 5%. The SPSS program for Windows (IBM) was used for the statistical analysis.

RESULTS AND DISCUSSION

Climatology characteristics

The climatology characteristics of the plantations' locations was shown in Table 1. The annual weekly or monthly rainfall distribution is more important than the total amount during the year because cocoa plants are more suitable in the months with not too high rainfall. Cocoa plantations generally have around 1250 to 3000-mm of rainfall every year. Based on the rainfall data of the location, the rainfall in plantations has the appropriate amount for cocoa plants' growth (Table 2). For optimal growth of cocoa plants, a maximum temperature between 30–32 °C, a minimum temperature between 18–21 °C (Sitohang and Siahaan, 2018), and relative humidity of 100% at night and between 70–80% during the day are needed conditions. Table 3 shows the organic matter, nitrogen (N), phosphate (P), and potassium (K) of the Banyumas soil (sampling site). Nitrogen, phosphorus, and potassium were 0.13-10.16%, 0.155-0.172%, and 0.03-0.1%, respectively (Table 3). Generally, the observed mineral content indicated that the soil is fertile at the sampling sites.

Table 1. Location and climatological characteristics of cocoa plantations in Plana Village, Banyumas, Central Java.

Climatological characteristics	Measurement
Altitude (masl)	105
Land slope (°)	3 – 6
Early cocoa planting	2006–2008
Air temperature (°C)	22 – 32
Relative humidity (%)	83 – 87
Groundwater content (%)	31 – 55
pH	5.8 – 6.5

Table 2. The rainfall in Plana Village, Banyumas, Central Java

Month, Year	Average rainfall (mm/month)
October – March, 2015	436.99
April – September, 2015	101.26
October – March, 2016	404.35
April – September, 2016	86.07

Source: Amini, 2019.

Table 3. Soil organic matter and soil N, P, K level.

Content	2015	2016
Organic matter (%)	2.96	2.94
N total (%)	0.15	0.15
P total (%)	0.165	0.163
K total (%)	0.065	0.048

Specific leaf area

There was no significant difference between planting patterns for specific leaf area (SLA) in January 2015. Furthermore, there was a non-significant difference in the three planting patterns in October 2016 (Table 4). At the peak of the dry season in October 2016, the SLA in plot II decreased of 8.92%, while in plots I and III increased of 24.52% and 25.76%, respectively compared to January 2015. However, the sampling was done during a relatively high rainfall period in January 2015 and a dry period in October 2016.

Table 4. Specific leaf area (SLA) of cocoa leaves.

Planting pattern	SLA (%)*	
	January 2015	October 2016
I	174.76±71.27	199.28±61.69
II	135.25±54.57	126.33±20.48
III	143.39±66.20	169.15±71.61
	***P*=0.19**	***P*=0.35**

*There is no significant difference at α=5%.
Area I: Cacao plant with several trees; Area II: Cacao without shade; Area III: Cacao plant with coconut trees.

SLA is a morphological parameter of nutrients a moisture availability that can be influenced by environmental conditions and the age of the leaf. SLA plays an important role in determining plant productivity, changes in leaf SLA indicate changes in leaf structure and nutrient content. In addition, differences in leaf SLA are influenced by soil and environmental climatic factors (Gong and Gao, 2019). Low SLA is due to the leaf's adaptation to survive, store nutrients, and avoid drought. The small size helps to regulate the temperature and water use efficiency for photosynthesis in dry and low water supply conditions (Karavin, 2013).

The increase in SLA is due to the increase in the number of cocoa leaves and may be due to a decrease in light intensity (Zang *et al*., 2016). Low SLA values do not reflect or indicate low photosynthetic activity and vice versa. However, in a water shortage condition, the temperature around the surface of the cocoa unshaded is higher than those in the shade, which causes faster groundwater evaporation, resulting in a reduced photosynthesis rate for survival (Lahive *et al*., 2019). According to Xu *et al*. (2008), decreasing soil moisture can reduce leaf size, and eventually affect the rate of photosynthesis. Photosynthetic rate is associated with SLA; by increasing the light capture area per mass, high SLA improves photosynthesis (Goorman *et al*., 2011). Photosynthetic rate is associated with SLA; by increasing the light capture area per mass, a larger leaf size, and higher photosynthesis (Huang *et al*., 2021).

Resorption of nitrogen (N) and phosphorus (P)

Table 5 shows the N and P contents of cocoa leaves. The average N content was higher in 2015 than in 2016 in areas I and II. As shown in Table 5, the N content was

increased in area III during 2015 as well. The increased N content is needed by cocoa plants that grow with coconut plants. The P content was not significantly different from N content; the P content was lower in 2015 than in 2016 in three different vegetation areas. The highest P increase was found in area II at 877.78%. Moreover, the N and P content of cocoa leaves decreased in the senescence phase (Table 6).

Table 5. Nitrogen (N) and phosphorus (P) contents of cocoa leaves.

Area	January 2015	October 2016
N (%)		
I	1.21±0.20	1.12±0.24 ab
II	1.24±0.07	0.95±0.09 b
III	1.31±1.15	1.34±0.17 a
	***P*=1.43**	***P*=0.03**
P (%)		
I	0.14±0.33	0.29±0.15
II	0.17±0.32	0.16±0.03
III	0.20±0.59	0.19±0.02.
	***P*=0.13**	***P*=0.14**

Numbers in columns followed by the same letters show no difference at α=5%.
Area I: Cacao plant with several types of trees; Area II: Cacao without shade; Area III: Cacao plant with coconut trees.

Table 6. Nitrogen (N) and phosphorus (P) contents in senescence leaves of cocoa.

Area	January 2015	October 2016
N (%)		
I	0.65±0.37	0.63±0.14 b
II	0.71±0.09	0.57±0.02 b
III	0.71±0.05	0.98±0.27 a
	***P*=0.90**	***P*=0.19**
P (%)		
I	0.10±0.03	0.17±0.08
II	0.09±0.32	0.88±0.02
III	0.09±0.44	0.12±0.05.
	***P*=0.75**	***P*=0.21**

Numbers in columns followed by the same letters show no difference at α=5%.

N and P resorption of cocoa leaves was higher in 2015 than in 2016 because of the different seasons (Table 7). The rainfall in Banyumas was low (almost 0 mm^3) in October 2015 because of still early for the rainy season. The reduction in areas I–III was 3.63, 6.44, and 38.57%, respectively. Furthermore, the N resorption in area I was not significantly decreased because of the shade plant presence. In 2016, the decrease of P resorption in areas I–III was 63.43, 13.10, and 49.26%, respectively. This shows that the most significant decrease in P resorption was in area I and the lowest P resorption was in October (*P*=0.01) (Table 8).

Table 7. Resorption of nitrogen (N) and phosphorus (P) in cocoa leaves.

Area	January 2015	October 2016
N resorption (%)		
I	44.11±31.91	42.51±16.12
II	42.41±4.34	39.68±4.07
III	45.32±9.10	27.84±14.22
	***P*=0.97**	***P*=0.27**
P resorption (%)		
I	28.36±716.67	10.37±8.78 b
II	45.66±14.17	39.68±4.07 a
III	54.87±25.81	27.84±14.22 a
	***P*=0.13**	***P*=0.01**

Numbers in columns followed by the same letters show no difference at α=5%.
Area I: Cacao plant with several types of trees; Area II: Cacao without shade; Area III: Cacao plant with coconut trees.

Nitrogen plays a vital role in the growth process, leaf production, and protein content in plants. The dry season affects the absorption and transportation of N. It may reduce the transpiration and the permeability of the membrane. Besides, nutrient transfer from senescence leaves to underground organs increases nutrient use (Yang *et al.*, 2016).

P absorption from the soil decreases in the dry season because of drought stress, reducing nutrient diffusion, and mass flow in grasses in the soil (Bista *et al.*, 2018). The resorption ability depends on the nutrients in the soil and the environmental conditions, such as temperature and light. According to some authors, plants utilize about 5–80% N and 0–95% P through leaves (Aerts and Chapin, 2000). Furthermore, N resorption for each plant species ranges from 16 to 42%. Additionally, microclimate under the canopy increases the decomposition of N content (Sari *et al.*, 2022). Area II had a good absorption of N. The root system of mature cocoa plants is stronger than the perennial or annual plant.

The most significant decrease in P resorption occurred in cocoa plants with coconut plants. Coconut trees have a root system up to 2 m wide and 0–60 cm below the soil surface making it easier to absorb nutrients than cocoa (Adam *et al.*, 2017). Generally, the ability of plants to do P resorption is at 0 until 90%. It increases when the availability of P in the soil decreases (Mayor *et al.*, 2014). The low rate of resorption is influenced by competition with other trees. In another study, N and P resorption were higher in plants affected by drought stress (Lobo-do-Vale *et al.*, 2018). On the contrary, the P resorption tends to decrease in the present study.

Table 8. The proline content of cocoa root.

Plantibng pattern	Proline content (%)		
	June 2015	October 2015	March 2016
I	1.03±0.36 b	16.70±9.16 a	0.37±0.29 b
II	2.03±1.06 ab	10.52±5.6 7 ab	1.08±0.30 a
III	4.25±2.91 a	5.30±1.46 b	0.68±0.25 b
	***P*=0.02**	***P*=0.02**	***P*=0.03**

Numbers in columns followed by the same letters show no difference at α=5%.
Area I: Cacao plant with several types of trees; Area II: Cacao without shade; Area III: Cacao plant with coconut trees.

Proline content of cocoa root

The proline content in the cocoa plant root was increased in all planting patterns from June to October 2015 (Table 8) due to the drought. Based on the planting pattern, the proline content was significantly different (*P*=0.02) in June 2015, the difference between area I and area III was significant, 1.03% and 4.25%, respectively. In Oct 2015, the proline content in area I was significant (*P*=0.02) different and lower than area III, 16.7% and 5.30%, respectively. And in March 2016, the proline content in area II (1.08%) was significantly (*P*=0.03) higher than area I (0.37%) and Area III (0.68%).

Proline accumulation was relatively higher in cocoa plants root without shade trees compared to those with cocoa plants root in the area with coconut trees (Table 8). The increase in proline is due to drought stress during the dry season (Devaranavadagi *et al.*, 2002). Proline is a substrate for respiration, a source of nitrogen, and other metabolisms during the dry season that was reported in wheat. The accumulation of proline during drought stress does not inhibit the biochemical reactions, but it acts as an osmoprotectant in corn (Molazem *et al.*, 2010). Osmotic regulation triggers cell development and plant growth in the dry season in wheat (Keyvan, 2010). Osmotic adaptation maintains the cell's turgor to enlarge, influences plant growth and allows stomata to open to assimilating CO_2 in Festuca (Man *et al.*, 2011). The highest decrease in proline was 97.78% in area I from October 2015 to March 2016. The plants in areas with diverse vegetation have a small amount of proline because of the low transpiration process. It was because plants do not need to make osmotic adjustments like when drought stress increases proline (Robakowski *et al.*, 2020).

Glucose content of cocoa root

There were no significant differences in glucose content between planting patterns during 2015 and 2016 (Table 9). The decrease in glucose levels in area I–III was 49.81%, 32.07%, and 3.8%, respectively, showing that the highest decrease in glucose content was in area I.

Table 9. The glucose content of cocoa root.

Area	Glucose content (%)		
	January 2015	October 2016	Change (%)
I	5.28±1.21	2.65±1.15	49.81
II	5.30±1.52	3.60±1.49	32.08
III	3.42±2.07	3.29±2.34	3.80
	***P*=0.11**	***P*=0.63**	***P*=0.03**

Numbers in columns followed by the same letters show no difference at α=5%.
Area I: Cacao plant with several types of trees; Area II: Cacao without shade; Area III: Cacao plant with coconut trees.

Plants have an osmotic adjustment mechanism for leaves and root turgor for growth, water absorption, cytokinin synthesis, and photosynthesis in black willow (Carpenter *et al.*, 2008). High carbohydrate storage in leaves during the dry season can be used for the respiration process of Aleppo pine (Klein *et al.*, 2011). Besides, higher amounts of sugar during the dry season are due to the translocation of carbohydrates from senescing leaves and the increased rate of photosynthesis at the beginning of the dry season. Sugars affect the growth, development, and metabolism of leaves, shoots, roots, and other plant organs (Ciereszko, 2018). According to Tezara *et al.* (2020), each clone of cocoa has a different physiological response to drought stress. Leaf N content, chlorophyll, and photochemical activity are reduced during drought, and the metabolism is disrupted. However, certain clones managed to survive in an environment with low water availability. Research by Rosas-Patiño *et al.* (2019) revealed that liming and fertilization affect nutrient efficiency and yields, in addition, the genotype of the cocoa and climate conditions also have an effect.

CONCLUSION

In conclusion, cocoa had different responses to cocoa root proline levels, leaf glucose levels, SLA, and leaf

N and P resorption to deal with seasonal changes. Further study to correlate yields with proline or glucose concentrations is needed to determine whether sowing cocoa plants with other types of plants can be used to help farmers and stakeholders in managing cocoa cultivation to increase yields in efficient and sustainable ways.

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Short article

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Does substrate influence germination of *Cinchona pubescens* Vahl. (Rubiaceae)?

¿Tiene influencia el sustrato sobre la germinación de *Cinchona pubescens* Vahl. (Rubiaceae)?

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Keywords:
Cinchona tree
Sub-irrigation chamber
Sexual propagation

ABSTRACT

Cinchona pubescens is an emblematic species of Peru, as it was used as the only effective treatment against malaria for three centuries. This species is threatened by various anthropogenic activities and its propagation depends on the dispersal of seeds whose germination power is low, therefore, it is necessary to conserve and propagate it. The objective of the study was to evaluate the effect of substrate on the germination of *C. pubescens*. A completely randomized design was applied with five treatments according to the type of substrate T1 (25% forest soil+75% sand), T2 (50% forest soil+50% sand), T3 (75% forest soil+25% sand), T4 (100% forest soil) and T5 (100% sand), the forest soil was extracted from areas where *C. pubescens* is naturally present. Three replicates and 100 seeds per replicate were used in the treatments. Germination of *C. pubescens* started 12 days after sowing until day 42. T4 had a better effect on the index (14.23±0.41), time (24.18±0.69) and germination percentage (88.3±2.88%); followed by treatments T3 and T2. While T5 was the treatment with the least effect on *C. pubescens* germination. The study indicated that the type of substrate used significantly influences the germination of *C. pubescens* seeds, so it is suggested to use substrate from natural forest without combination to achieve high germination rates and propagation of this species.

Palabras clave:
Árbol de la quina
Cámara de subirrigación
Propagación sexual

RESUMEN

Cinchona pubescens es una especie icónica de Perú ya que fue usada como único tratamiento efectivo contra la malaria por más de tres siglos. Esta especie está amenazada por diversas actividades antropogénicas y su propagación está supeditada a la dispersión de semillas cuyo poder de germinación es bajo, por ende, es necesario conservarla y propagarla. El objetivo del estudio fue evaluar el efecto del sustrato sobre la germinación de *C. pubescens.* Se aplicó un diseño completamente aleatorio con cinco tratamientos según el tipo de sustrato T1 (25% tierra de bosque+75% arena), T2 (50% tierra de bosque+50% arena), T3 (75% tierra de bosque+25% arena), T4 (100% Tierra de bosque) y T5 (100% arena), la tierra de bosque fue extraída de zonas donde *C. pubescens* está presente de forma natural. En los tratamientos se utilizaron tres réplicas y 100 semillas por cada réplica. La germinación de *C. pubescens* inició 12 días después de la siembra hasta el día 42. El T4 tuvo un mejor efecto sobre el índice (14,23±0,41), tiempo (24,18±0,69) y porcentaje de germinación (88,3±2,88%); seguido por los tratamientos T3 y T2. Mientras que el T5 fue el tratamiento con menor efecto sobre la germinación de *C. pubescens*. El estudio indicó que el tipo de sustrato empleado influye significativamente en la germinación de las semillas de *C. pubescens*, por lo que se sugiere emplear el sustrato procedente de bosque natural sin combinación para alcanzar índices altos de germinación y propagar esta especie.

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Cinchona is a genus of plants of high medicinal value, such as *Cinchona officinalis*, *C. pubescens*, *C. calisaya*, whose bark contains quinine, which was supplied as the only treatment against malaria for more than three centuries (Cóndor *et al*., 2009). The species of this genus were overexploited and their bark was traded in several countries. According to a conservative estimate, between the 17th and 18th centuries about 500,000 kg of bark was exported annually to Europe (Van Der Hoogte and Pieters, 2016).

The natural ecosystems of *C. pubescens* have suffered severe damage due to migratory agriculture, cattle ranching and logging (Arbizu *et al*., 2021; Huamán *et al*., 2019), making it difficult to find populations of this species in the forests of Peru (Buddenhagen *et al*., 2004), which has led to prioritizing the conservation and recovery of this species in Peru (Albán-Castillo *et al*., 2020). Agroforestry is an alternative for the recovery of native trees, one of the complex stages lies in the production of seedlings at the nursery level (Abanto-Rodriguez *et al*., 2016); especially in the seed germination phase, since it depends largely on quality factors such as type of medium, substrate, humidity, fertilization and botanical seed (Santos *et al*., 2010).

Cinchona pubescens is mainly propagated by seed, which is of great importance in the agroforestry management of the species (Vásquez *et al*., 2018). Under natural conditions, *C. pubescens* has a low germination and regeneration rate (Armijos-González and Pérez-Ruiz, 2016; Espinosa and Ríos, 2014), finding them only in remote sites and in small groups (Buddenhagen *et al*., 2004).

The substrate must allow good oxygenation, nutritional balance, and good water retention, in addition, it must provide a pH compatible with the species, adequate electrical conductivity, and be free of chemical elements at toxic levels (Abanto-Rodriguez *et al*., 2016). To meet the maximum of these required conditions, substrates must eventually be used in combination with each other or their natural form (Frade *et al*., 2011). Therefore, the objective of the study was to evaluate the effect of forest soil and sand on the germination of *C. pubescens*.

MATERIALS AND METHODS

Study area

The trial was conducted from November 5, 2020 to January 6, 2021 in the community of La Cascarilla (5°40'16.5"S and 78°53'11.6"W), province of Jaen in Peru, at 1810 masl. Annual precipitation is 1730 mm, minimum temperature of 13.0 °C and a maximum of 20.5 °C (Fernandez *et al*., 2021).

Collection and drying of biological material

Seeds of *C. pubescens* were collected in October 2020 from a single existing population at the locality of La Cascarilla (5° 4037.96S and 78° 5327.0W) at an altitude of 1760 m 1 kg of mature capsules (brown to brown color) were collected and packed in cloth bags for transfer to the nursery. The fruits were subjected to a drying process in a low light environment for 15 days, after dehiscence, seeds were selected in optimal phytosanitary conditions, with uniform size and purity; they were then stored in cloth bags at room temperature.

Trial set-up

A sub-irrigation chamber of 1 m long, 0.45 m wide, and 0.5 m high was divided into 15 experimental units of 0.15 m wide, 0.2 m long and 0.1 m high. In each replicate, the combined substrates were placed according to the standardized ratio (Table 1); then they were moistened to field capacity and 100 seeds of *C. pubescens* were sown per replicate, after which daily irrigation was applied (0.10 L m^{-2}) to ensure that the moisture content remained constant throughout the trial process.

Experimental design

The experiment was conducted under a completely randomized design with five treatments (Table 1) and three replicates per treatment; 100 seeds of *C. pubescens* per replicate and 1500 seeds were used throughout the trial.

Evaluation and data recording was carried out daily for 60 days, and the presence of the root apex was considered an indicator of germination.

Table 1. Classification of treatments according to the type of substrate used in the germination of *C. pubescens.*

Treatment	Description
T1	25% forest soil+75% sand
T2	50% forest soil+50% sand
T3	75% forest soil+25% sand
T4	100% forest soil
T5	100% sand

The germination rate was determined according to the following equation:

$$\%\ \text{germination} = \frac{\text{germinated seeds}}{\text{seeds sown}} \times 100$$

Additionally, parameters related to seed germination were calculated according to González and Orozco (1996):

Germination Index (GI)

$$GI = \frac{\sum(n_i t_i)}{N}$$

Average germination time (T).

$$T = \frac{\sum(n_i t_i)}{\sum n_i}$$

Germination speed (M)

$$M = \sum\left(\frac{n_i}{t}\right)$$

Where:
n_i: number of seeds germinated each day i
t_i: number of days after planting
t: time from sowing to emergence of the last seed
N: total seeds sown in the study

The assumptions of normality (Shapiro-Wilk) and homogeneity of variances (Levine test) were verified. Then, an analysis of variance (ANOVA) was performed for each response variable and mean values were compared using Tukey's HSD post hoc test (P<0.05). Data were processed in StatGraphics Centurion XVI software (StatPoint Technologies Inc, Warrenton, VA, USA).

RESULTS AND DISCUSSION

Seeds of *C. pubescens* germinated 12 days after sowing. Thereafter, germination increased daily, reaching the highest cumulative germination rate at 42 days (Figure 1). The cumulative germination curves show a quadratic polynomial trend, with a coefficient of determination close to 1 and with a certain degree of similarity in all treatments. The highest germination occurred between days 17 and 27 in all treatments and then its increase was minimal between days 28 and 37; completing the germination phase at 38 days (constant). However, the cumulative germination curve of T4 was always higher than that of the other treatments and T5 remained constant and below the mean.

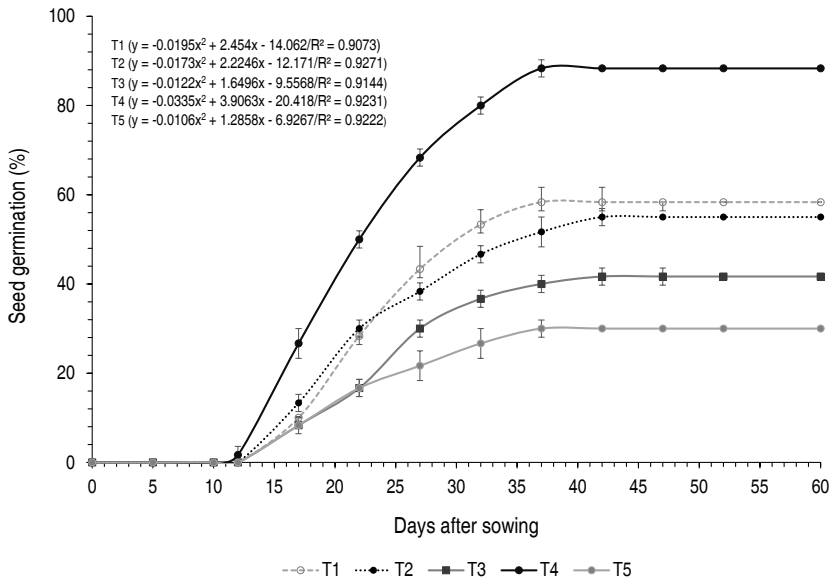

Figure 1. Cumulative germination curves of *C. pubescens* seeds sown in different substrates.

The germination process of *C. pubescens* seeds started on day 12 and concluded on day 42, when the highest cumulative germination rate was recorded; these results differ from those reported by Caraguay *et al.*(2016), who indicated that *C. officinalis* seeds began to germinate on day five and finished at 35 days; these differences may be related to genetic, physiological (phenol content) and morphological conditions of the seeds (Herrera *et al.*, 2006; Armijos-González and Pérez-Ruiz, 2016), in addition to other factors such as humidity, soil, nutrients and agricultural management (Bonfil-Sanders *et al.*, 2008; Meza *et al.*, 2004). According to the analysis of variance, the type of substrate has a significant effect (P<0.05) on the total number of germinated seeds of *C. pubescens*. Tukey's post hoc test showed that T4 had the highest germination rate of 88.3±2.88%, followed by T3 and T2 with 58.3±2.88% and 55±10%, respectively, while T5 had the lowest germination rate of 30±5%. There were significant differences (P<0.05) between T2, T3 (germination >50%) and T1 and T5 (germination <50%) (Figure 2).

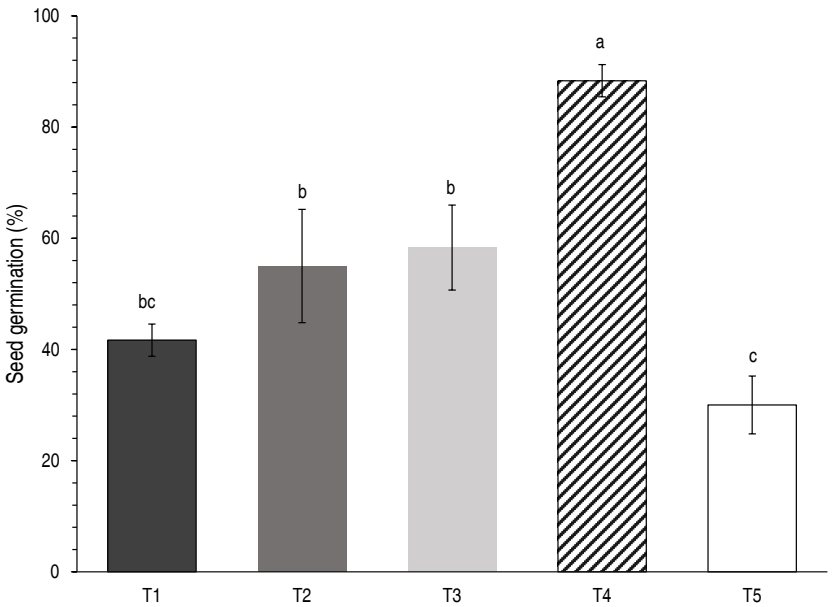

Figure 2. Effect of substrate on *C. pubescens* seed germination at 60 days. Means with the same letters per treatment indicate no significant differences by Tukey HSD test (P<0.05).

The highest germination rate (88.3%) of *C. pubescens* seeds was recorded at T4 (forest substrate), this result may be related to the high organic matter content (10.55%) and pH (4.82) of the substrate (García-Hoyos *et al.*, 2011), in addition to the texture (sandy soil) which facilitates water retention and circulation, also provide the necessary nutrients during germination (Alfonso *et al.*, 2017; Cunha *et al.*, 2006). The type of substrate influences seed imbibition, due to a series of characteristics such as water potential (Wagner *et al.*, 2006), which allows the activation of substances stored in the embryonic system and thus accelerates and increases their germination rate (García-Hoyos *et al.*, 2011). The mean germination time of *C. pubescens* seeds was 24.18 to 26.22 days (T4 and T1, respectively). There were significant differences (P<0.05) in the speed and germination index of *C. pubescens* seeds. The highest germination index was recorded at T4, followed by T3 and T2. The germination speed in T4 was the highest and significant differences (P<0.05) were determined with the other treatments (Table 2). Table 3 shows the physicochemical characteristics of the substrates used in the germination of *C. pubescens* seeds; in the five treatments the texture was sandy loam.

Several studies have demonstrated the effect of substrate type on seed germination in species of the genus Cinchona, with some variation in results due to climatic, species, and pre-specified methodological factors. For example, Campos *et al.* (2016) on *C. pubescens* seeds

Table 2. Germination results of *C. pubescens* seeds in different substrates.

Treatment	Average germination time (day)	Germination rate (day)	Germination rate (seeds day^{-1})
T1	26.22±0.20 a	7.28±1.28 bc	0.20±0.04 bc
T2	25.54±1.19 a	9.40±1.99 b	0.26±0.05 b
T3	25.38±1.84 a	9.89±1.12 b	0.28±0.01 b
T4	24.18±0.69 a	14.23±0.41 a	0.42±0.01 a
T5	24.88±0.99 a	4.96±0.65 c	0.14±0.02 c

Means with the same letters per treatment indicate no significant differences by Tukey HSD test (P<0.05).

Table 3. Physicochemical properties of the substrates used in the germination of *C. pubescens*.

Treatment	pH (1:2.5)	E.C. (dS m^{-1})	P (ppm)	K (ppm)	N (%)	O.M. (%)
T1	7.63±0.22	1.58±0.09	8.02±2.53	89.29±1.31	0.28±0.00	5.68±0.09
T2	6.64±0.31	0.50±0.37	10.83±3.12	143.73±2.11	0.43±0.01	6.52±0.01
T3	5.71±0.98	0.72±0.36	8.96±2.48	138.72±1.56	0.20±0.00	7.06±0.11
T4	4.82±0.55	0.53±0.09	9.95±1.43	187.42±1.87	0.53±0.01	10.55±0.15
T5	7.70±0.19	0.60±0.15	5.33±1.11	67.71±2.13	0.08±0.00	1.62±0.04

E.C: electric conductivity. O.M: organic matter. N, P, K: macronutrients.

with KNO_3 at 1000 ppm achieved a germination rate of 91%, which is considered high compared to the rate found in this study (88.3%) and according to Conde *et al*. (2017), with 83.33% germination *C. officinalis* on peat substrates. Jäger (2014) showed that *C. pubescens* seeds have germination rates of 50 to 85%, which is the range that includes the results reported in this study. Rodríguez *et al*. (2020) reported 50% germination in sandy textured substrates, a value similar to T2 and T3 in this study. Jeréz (2017) found a germination rate of 70.67% for *C. officinalis* seeds treated with liquid mycorrhizae and in a substrate (20% black soil +60% pine bark +20% rice husk).

Higher germination rates and speed and shorter germination time were reported for *C. pubescens* seeds at T4, which favors sexual propagation of *C. pubescens* and avoids prolonged dormancy of seeds in the germinator that are often affected by pathogen invasion and consequently generate uneven seedling growth. There is no doubt that *C. pubescens* seeds require certain favorable conditions provided by the substrate, including organic matter content, water retention capacity, pH, and adequate amounts of macronutrients (Rodríguez *et al*., 2020).

CONCLUSIONS

It was found that the type of substrate used had a positive influence on the germination *of C. pubescens* seeds; in this sense, it is recommended to use forest soil extracted from areas where there are relicts of *C. pubescens* and avoid combining them with other substrates. Likewise, for the mass propagation of species of the genus *Cinchona*, it is not recommended to use pure sand as a substrate in the germination stage.

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Diagnostic methods of subclinical mastitis in bovine milk: an overview

Métodos de diagnóstico de mastitis subclínica en leche bovina: una descripción general

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ABSTRACT

Bovine mastitis is defined as inflammation of the udder caused mainly by bacterial pathogens and depending on the degree of inflammation it is classified as subclinical and clinical. Particularly in the subclinical form, there are no alterations in milk, udder or animal, but it does affect its components, impairing its use in the dairy industry, and leading to significant economic losses due to discard and decrease in production. Therefore, the detection of subclinical mastitis is based on field and laboratory tests. Currently, there are several methods, mostly based on the measurement of somatic cells present in milk because of the inflammatory process. In this paper, an approach is made on the different methods of detection of subclinical mastitis in milk from conventional or traditional to alternative methods with greater precision.

Keywords:
Bovine mastitis
Diagnostic
Somatic cell count
Ultrasound

RESUMEN

La mastitis bovina se define como la inflamación de la ubre causada principalmente por patógenos bacterianos y dependiendo del grado de inflamación se clasifica en subclínica y clínica. Particularmente en la forma subclínica no se presentan alteraciones en leche, ubre o animal, pero sí afecta sus componentes, impidiendo su aprovechamiento en la industria láctea, y conllevando a pérdidas económicas importantes por concepto de descarte y disminución en la producción. Por ello, la detección de mastitis subclínica se basa en la realización de pruebas en campo y en laboratorio. Actualmente existen diversos métodos, en su mayoría basados en la medición de las células somáticas presentes en leche como resultado del proceso inflamatorio. En el presente artículo se realiza un abordaje sobre los diferentes métodos de detección de mastitis subclínica en leche desde convencionales o tradicionales hasta métodos alternativos de mayor precisión.

Palabras clave:
Mastitis bovina
Diagnóstico
Conteo de células somáticas
Ultrasonido

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INTRODUCTION

Milk is defined as the secretion of the mammary gland of dairy animals obtained by one or more milking without any addition or extraction, intended for consumption in the form of liquid milk or for further processing (FAO, 2011). Milk produced by cows, buffaloes, sheep, goats and camels is used in various parts of the world for human consumption. For much of the world's population, cow's milk represents most of the milk used for consumption, given that it is produced at approximately 86% compared to other dairy species used for the same purpose (FAO, 2020).

In the human diet, milk is one of the most important products to consume, mainly because of its nutritional value, since it provides minerals such as calcium, magnesium, selenium, vitamins, proteins and calories according to the portion consumed. However, this food is highly susceptible to contamination by various pathogenic agents, which alter its quality, often imperceptibly, generating health risks for consumers and in addition, important economic losses in the dairy sector (FAO, 2020).

World milk production in 2020 was estimated at 906 million tons, for South America a total of 82 million tons was reported and 22,592 tons, particularly for Colombia (FAO, 2021). This positions the country as the fourth largest dairy producer in Latin America, in addition to ranking 21st worldwide with daily production margins estimated at approximately 22 million liters (DANE, 2019), which represents a contribution of the dairy sector to the national GDP of 2.3% and to the agricultural sector of 24.3%, among other aspects, due to the generation of employment in the agricultural sector of 17% (Morales and Ospina, 2017).

In this context, regarding diseases that severely affect bovine dairy production, such as mastitis, especially subclinical mastitis, it is essential to know the methods for early detection in order to establish control and prevention measures from the production unit and minimize the impact in terms of treatment costs and milk discard, among others. The objective of this paper was to carry out a bibliographic review of conventional and non-conventional techniques for the diagnosis of subclinical mastitis.

Composition of raw milk

The nutritional composition of milk is a complex mixture of different substances, present in suspension or emulsion and others in solution form, such as water, fat, protein, lactose, vitamins, and minerals, which are called dry extract or total solids. This composition varies according to genetic, nutritional, and herd management conditions. The following is the average composition of bovine milk with its respective ranges of variation: 87.3% water (85.5-88.7%), 3.9% fat (2.4-5.5%), 8.8% non-fat solids (7.9-10.0%), protein 3.3% (3/4 parts casein), lactose 4.6%, minerals 0.8% (Ca, P, citrate, Mg, K, Na, Zn, Cl, Fe, Cu, sulfate, bicarbonate), acids 0.2% (citrate, acetate, lactate, oxalate), vitamins (A, C, D, thiamine, riboflavin) (Arroyave and Naranjo, 2007).

Water: the water content of the milk of different mammalian species can vary from 86 to 90.5%; however, it normally represents 87% of the total milk content. This variation is due to the alteration of any of its other components: proteins, lactose, and mainly, fat. Because of its high-water content, milk allows the distribution of its components to be relatively uniform, and thus any amount of milk, regardless of how small, contains almost all the available nutrients (Badui, 2006).

Fat: Lipids are among the most important constituents of milk and milk products, conferring unique flavor characteristics, nutritional content, and physical properties. Milk fat is a good source of energy and an excellent transport medium for fat-soluble vitamins A, D, E, and K. Carotene, a precursor of vitamin A, gives milk its "cream" color (German and Dillard, 2006).

The fat fraction of milk is in the form of microscopic globules 4.4 µm in diameter. Both total lipid and fatty acid contents can vary considerably in response to changes in diet, breed of animal, and lactation status by 3 to 6%, although typically the fat content can be between 3.5% and 4.7% (Badui, 2006; NOM, 2003). In addition, fat contains mainly triglycerides (about 98%), diacylglycerol (2%), cholesterol (less than 0.5%), phospholipids (about 1%) and free fatty acids (0.1%). On the other hand, saturated fatty acids make up 70% of the total weight of fat, with palmitic acid being the most common, accounting for 30% of milk fat by weight, followed by myristic and stearic acid, which make up 11 to 12% by

weight. Of the saturated fatty acids, 10.9% are short-chain fatty acids. The content of butyric and caproic acid averages 4.4%, and they represent only 2.4% of the total fatty acids (García-Garibay *et al.,* 2012).

Proteins: The primary function of milk proteins is to provide sufficient supply of indispensable amino acids and organic nitrogen for the synthesis and repair of tissues and other biologically important proteins. Cow's milk is considered an excellent source of proteins of high biological value, since it contains the ten indispensable amino acids. The protein fraction of milk regularly corresponds to 3-4% and two main categories can be distinguished, defined by their chemical composition and physical properties: casein, which constitutes 70% of milk proteins, contains phosphorus and coagulates or precipitates at a pH of 4.6; and whey proteins, which represent the remaining 30%, do not contain phosphorus but sulfur and remain in solution in milk at a pH of 4.6 (Badui, 2006).

Caseins: they consist of fractions a, b, k, and g caseins, which are distinguished from each other by their amino acid composition and functional properties. Caseins are suspended in milk through micelles, formed by macromolecular complexes of phosphoproteins and glycoproteins in colloidal suspension. The nutritional role of casein is the supply of amino acids, calcium and inorganic phosphorus (Zhang *et al.,* 2021).

Whey proteins: also known as seroproteins, they are considered soluble proteins and are mainly classified into albumins and globulins, including a-lactalbumins, b-lactoglobulins, immunoglobulins, protease-peptones and other non-specific minority nitrogenous compounds such as lactoferrin and lysozyme. Seroproteins are considered to be high biological value proteins with a broad amino acid profile that includes sulfur amino acids such as cysteine and methionine, branched-chain amino acids, and lysine and tryptophan, thus compensating for casein deficiencies (Zapata *et al.,* 2017).

Lactose: is the main carbohydrate in milk and contains approximately 4.5%. It is 85% less sweet than sucrose or common sugar and contributes, together with salts, to the overall flavor of milk, the amounts of lactose and salts being inversely proportional. Lactose is easily transformed into lactic acid by the action of bacteria. For humans, lactose is the only source of galactose, an important constituent of nerve tissues (Aranceta and Serra, 2004).

Minerals: milk provides essential mineral elements for the human body and is the most important source of bioavailable calcium in the diet. Its good absorption is due to the presence of lactose and vitamin D and to its union with phosphopeptides derived from casein hydrolysis, in addition to the fact that the adequate calcium:phosphorus ratio (greater than the unit) favors its absorption in the human intestine. For this reason, cow's milk is considered to be the best source of calcium both for bone growth in young people and for the maintenance of bone integrity in adults (Aranceta and Serra, 2004).

Fat-soluble vitamins: both milk and dairy products are considered an important food source of vitamin A; this vitamin is involved in functions related to vision, gene expression, embryonic development, growth, reproduction, and immunocompetence. Both vitamin A and its precursors called carotenoids, mainly b-carotene, are present in different amounts in the fat fraction of milk (Miller *et al*., 2007). Vitamin D is involved in the absorption of calcium and phosphorus in the intestine and is essential for the proper maintenance of the skeleton throughout life. It is found in very low concentrations in milk and dairy products to which this vitamin has not been added (Schmid and Walther, 2013). Vitamin E, also called tocopherol, is considered an antioxidant that protects cell membranes from free radical damage. It also participates in the immune response. Some studies even consider it a protective factor against some types of cancer and cardiovascular diseases. This vitamin is present in milk in low concentrations, as is vitamin K (Haug *et al.,* 2007).

Water-soluble vitamins: both milk and its derivatives contain the vast majority of soluble vitamins in varying amounts, although the content of vitamin B2 (riboflavin) and niacin stand out; milk provides lesser amounts of vitamin B1 (thiamine), vitamin B6 (pyridoxine) and folic acid (Haug *et al.,* 2007).

Bovine Mastitis

The importance of milk in human life has already been

discussed, nevertheless, the main disease that afflicts cattle and has a direct impact on milk production is mastitis, a disease that produces inflammation of the mammary gland, due to traumatic, allergic or infectious causes, the latter being the most common caused by pathogenic microorganisms, mainly bacteria and, to a lesser extent, yeasts, fungi and algae (Ruegg *et al.,* 2015).

Traditionally, mastitis-causing pathogens are classified into contagious, environmental and minor pathogens, according to their mode of transmission and reservoir (Ruegg, 2012). Contagious microorganisms are those that are transmitted from cow to cow, therefore, the main reservoir is the animal itself (Fox and Gay, 1993). In this group are *Staphylococcus aureus,* Coagulase-Negative *Staphylococcus* (CoNS)*, Streptococcus agalactiae, Mycoplasma spp* and *Corynebacterium bovis* (Fox and Gay, 1993; Barkema *et al*., 2009). In the case of environmental pathogens, the reservoir is constituted by the habitat of the cow. Gram-negative bacteria such as *E. coli, Klebsiella pneumoniae* are the most common environmental pathogens, but can also be caused by gram-positive pathogens such as *S. uberis* and *S. dysgalactiae* (Smith and Hogan, 1993). Other pathogens include *Pseudomonas aeruginosa, Pasteurella multocida, Prototheca* spp, *Trueperella pyogenes*, *Mycobacterium* spp, Nocardia spp, and some yeasts (Williamson and di Mena, 2007; Tarazona-Manrique *et al*., 2019).

The main route of entry for contagious and environmental pathogens is through the teat orifice, either during milking or between milking (Bradley, 2002). After the bacterial invasion, depending on the invading pathogen, they infect different locations in the mammary gland and cause different symptoms and duration of the infection (Svennesen *et al*., 2019).

In response to the invasion of these pathogens, innate and acquired immune response mechanisms are activated (Medzhitov, 2007). Toxins and virulence factors induce neutrophil migration and secretion of proinflammatory cytokines; subsequently, antigens from invading bacteria are processed in macrophages and B lymphocytes and appear on membranes in association with major histocompatibility complex (MHC) type I or II, so they can be recognized by different types of lymphocytes (Ezzat *et al*., 2014). Thus, they are the lymphocytes that, once activated and proliferated, fight the infection that develops in the mammary gland (Kehrli and Harp, 2001).

According to the signs of inflammation, bovine mastitis is classified as clinical mastitis (where alterations occur in milk, mammary gland, or even systemically) and subclinical (no noticeable signs in milk, mammary gland, or systemically) (Erskine, 2020). Regardless of the form of presentation, the importance of the study, diagnosis, and treatment of mastitis lies, among other things, in the fact that this disease produces severe economic losses within the livestock sector both in the country and in the world (Andrade-Becerra *et al*., 2014).

Subclinical mastitis

In subclinical infection, there are no visible changes in the appearance of the milk, nor are there any manifestations of the disease in the cow, but milk production decreases, and its composition is altered. Its detection is based on somatic cell counts (Blowey and Edmonson, 2010), where values higher than 200,000 cells mL^{-1} are considered positive for intramammary infection. The occurrence of subclinical mastitis has commonly been attributed to contagious pathogens when undetected by the producer, the critical point is the progression to a state of critical point, and as a consequence, in many cases, leading to the discard of the animal (Dohoo *et al*., 2011).

Clinical mastitis

This disease is characterized by the presence of visible alterations in the milk (formation of lumps, changes in color, presence of clots, etc.), changes in the mammary gland (inflammation, pain, heat, tumor, and redness) and, in certain cases, it can reach a systemic involvement with anorexia, fever, and shock (Radostits *et al.,* 2006; Erskine, 2020). According to severity, it is classified as mild (abnormalities in milk), moderate (abnormalities in milk and mammary quarter, based on 6 parameters of alteration of the quarter), and severe (abnormality in milk, signs of systemic disease with or without alterations in the mammary quarters). Regarding the frequency of presentation of clinical mastitis cases according to severity, the majority of cases are mild, followed by

moderate, and, to a lesser extent, severe (Roberson, 2012).

Considering that subclinical mastitis is approximately 40 times more common than clinical mastitis and that, additionally, it does not present visible changes in milk, there is a need to explore and develop new technological tools that, in a sustainable way, contribute to the reduction of losses in dairy herds through the early detection of bovine mastitis and the isolation of sick cows, preventing the spread to healthy cows.

Characteristics of the pathogens causing bovine mastitis

The characteristics and consequences of pathogens on cow health are described below. Gram-positive pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae*, cause infections of prolonged duration in the subclinical phase, with high somatic cell counts (>800,000 cells mL^{-1}), with an affinity for the mammary parenchyma and mucosa of the mammary cistern and ducts. Other gram-positive pathogens such as coagulase-negative *Staphylococcus*, *Streptococcus* spp and *Corynebacterium bovis* cause infections of short to moderate duration with somatic cell counts up to 500,000 cells mL^{-1}, with an affinity for the teat canal and mucosal surfaces. Gram-negative pathogens such as *Escherichia coli, Klebsiella* spp, and *Serratia* spp, cause infections with counts ranging from 500,000 to 1,000,000 cells mL^{-1}, subclinical infections of short to the prolonged course, and clinically 20 to 30% develop systemic signs. Nevertheless, the expectation of spontaneous bacteriological cure is moderate to high (Ruegg *et al.*, 2015).

Other rare pathogens such as *Mycoplasma* spp can cause increases in somatic cell counts up to 500,000 cells mL^{-1}, with prolonged duration periods in the subclinical phase, in addition to the involvement of other organs and low probability of spontaneous bacteriological cure, the main impact of mastitis caused by such an agent (Ruegg *et al.*, 2015).

Effect of mastitis on the compositional and nutritional quality of raw milk

As mentioned above, milk is a product with diverse compositional and nutritional characteristics, being one of the most consumed products worldwide. However, bovine mastitis, mainly of subclinical course, has a negative impact on the quality of milk, causing alterations in it and, as a consequence, the impossibility of its use in the dairy industry.

In addition to the increase in somatic cell count, a parameter that, from the regulatory aspect, is considered in many countries to evaluate milk quality (European Commission, 2020), components such as proteins change dramatically. Casein, the main milk protein, decreases, and whey proteins of lower quality increase, affecting the flavor. Proteins are degraded by the presence of enzymes such as plasmin (Kibebew, 2017; Ismail and Nielsen, 2010). On the one hand, the concentrations of sodium and chloride are increased, in an attempt to maintain osmotic balance. The change in these elements or ions allows monitoring of the evolution of mastitis because they cause an increase in the electrical conductivity of the milk (Fox *et al.*, 2015). On the other hand, it decreases potassium and calcium, since the latter is associated with casein (Calderón-Rangel *et al.*, 2014). Lactose, the milk sugar, is also decreased due to three causes: altered synthesis due to cell damage, loss of lactose in urine, and use of lactose as a substrate by mastitis-causing pathogens (Costa *et al.*, 2019).

Mastitis detection

As mentioned, clinical mastitis presents obvious signs or symptoms in cattle health, so its detection should not be a problem. Nevertheless, subclinical mastitis can only be diagnosed by a series of tests, which will be described below. To make it simpler, the tests will be divided into conventional methods and alternative or non-conventional diagnostic methods.

Conventional methods

Somatic cell count (SCC): Somatic cell count is one of the conventional methods used to detect the presence of mastitis in herds and to assess the sanitary quality of milk. In raw milk, a high somatic cell count value determines not only that the cows have mastitis, but also, information on biochemical changes in the milk, up to production losses (Riveros-Galán and Obando-Chávez, 2021).

Somatic cell counts below 200,000 cells mL^{-1} are considered physiologically normal, while those above

300,000 cells mL^{-1} generally indicate the presence of inflammation. For somatic cell counts in different countries of the European community, the established norms are between 400,000 to 750,000 cells mL^{-1} as the maximum value, while in Colombia, the maximum accepted count is 800,000 cells mL^{-1} (Gómez, 2015).

California Mastitis Test (CMT): it is the most widely used field test in dairy cattle for the diagnosis of subclinical mastitis, it does not count numerical results but categorical results. It consists of adding a detergent to milk, linear alkylbenzene sulphonate, causing the release of DNA from leukocytes present in the udder, and this is converted, in combination with protein agents in the milk, into a gelatinous complex. The categorization of the results is given in several ways, such that it is negative when the reagent and the mixed milk is still watery. When the cell count is higher, the mixture of reagent and milk almost solidifies (Saran and Chaffer, 2000).

The reagent used in the CMT test is characterized because it has a surfactant among its components with the ability to decrease the surface tension of the leukocytes present in mastitis milk. When the surface tension decreases, a burst of leukocytes is immediately produced, which in contact with the reagent, forms the gelatinous complex on the paddle used for the test (Echeverry *et al*., 2010; Moroni *et al.,* 2018). Thus, when there are more cells, a higher concentration of DNA is released and, therefore, the higher the degree of the gelatinous complex, which allows determining the inflammatory response based on the viscosity of the gel formed by mixing the same amount of affected milk with the CMT reagent, the paddle with four compartments evaluates each quarter separately (Moroni *et al.,* 2018; Aguilar and Álvarez, 2019). The CMT was developed in 1957 with the purpose of rapidly detecting abnormalities in milk, the personnel who perform the test only require basic training and coincides with the fact that as the Somatic Cell Count or Leukocyte Count increases, so does the CMT score, making it a reliable field test (Sanford *et al*., 2006).

Wisconsin Mastitis Test (WMT): This test can be used to sample milk from individual cows and milk from cooling tanks. It is characterized by the estimation of somatic cell content. The procedure uses a reagent very similar to that of the CMT, the difference is that the results are measured quantitatively depending on the viscosity, not qualitatively (Philpot and Nickerson, 2000; Bedolla, 2007; NMC, 2016).

Whiteside test: Similar to the California test, this test is based on the increase of leukocytes, where a gelling reaction occurs when mixing mastitic milk with a 4% NaOH solution on a glass plate homogenized with a glass rod for 20 seconds. The result is measured according to milk precipitation as negative, trace, and positive (Hasan and Ahasan, 2013).

Alternative methods

Electrical conductivity: one of the pioneers works carried out for the detection of subclinical mastitis, using non-conventional technology, was that by Nielen *et al*. (1995a), the authors developed a model where they acquired, online and automatically, every 5 seconds, data of variables such as electrical conductivity per mammary quarter, milk temperature and milk production per cow since they concluded that the combination of these parameters would help to improve the detection results in terms of sensitivity (percentage of successfully classified cows with subclinical mastitis) and specificity (percentage of successfully classified healthy cows) (Shoshani and Berman, 1992).

The system, which used neural networks (Nielen *et al*., 1995a), was able to flag a cow or udder quarters when an abnormality was detected through measured parameters, mainly electrical conductivity. They identified that a high somatic cell count coincided with high milk electrical conductivity values. Cows with SCC < 200,000 cells mL^{-1} were considered healthy cows and cows diagnosed with subclinical mastitis were those with SCC > 500,000 cells mL^{-1}. Healthy cows diagnosed with clinical mastitis or those in the range of 200,000 to 500,000 cells mL^{-1} were not taken into account for the study. The results showed that, of the total number of milking, 19.2% were positive for subclinical mastitis, while 80.2% were negative, i.e., healthy milking. The model had a sensitivity of 53% and a specificity of 97% (Nielen *et al*., 1995b). They state that the power of an online system lies in the evaluation of subclinical mastitis from data taken over a long period of time (from 1991 to

1992) and not by data from a few samples. In another study (Paudyal *et al*., 2020), they obtained sensitivity and specificity of 89.9% and 86.8%, respectively, using the same diagnostic technique.

Infrared thermography: any object, material, or body emits radiation, in the form of heat, depending on its temperature. In the field of veterinary medicine, infrared thermography is sensitive enough to perceive changes in skin surface temperature (SST) and relate it to the severity of mammary gland infection. One study measured SST using infrared thermography and one milk sample per quarter in 94 cows using the California Mastitis Test (CMT) and found a strong correlation between SST and CMT (r=0.92), suggesting that thermography is a sensitive technique, as well as being non-invasive for detecting different degrees of mastitis. One of the drawbacks of the technique is that it requires the adaptation of a dark room to perform the measurement using infrared cameras, as well as its calibration, in addition to being expensive and lacking specificity with respect to etiology (Colak *et al.,* 2008). Regarding sensitivity and specificity, other authors report values of 95.6 and 93.6%, respectively (Colak *et al*., 2010). It should also be taken into account that there are other factors that can affect the temperature of the udder skin, such as humidity in the environment, physiological state, and production level of the bovine and aspects related to feeding and milking, which directly influence the measurement (Colak *et al*., 2008).

Piezoelectric sensors: piezoelectric materials are those that have the ability to produce an electrical potential difference when subjected to mechanical deformation and vice versa. From the point of view of sensors with biological applications, piezoelectric can be used to fabricate biosensors (Pohanka, 2018). The operating principle can be explained as follows: an alternating voltage on the faces of the piezoelectric surface produces oscillations in the crystal. A mass (biological sample) on the surface of the piezoelectric, will change the frequency of oscillations in the material. The change in the frequency of oscillations is proportional, among other things, to the mass in contact with the piezoelectric surface; (García-Martínez *et al*., 2011; Zhang *et al*., 2011; Pohanka, 2017).

The application of these devices to evaluate milk quality is related to the monitoring of acetone, lactose, N-acetyl-β-d-glucosaminidase (NAGase), l-lactate dehydrogenase (LDH), and progesterone (Brandt *et al*., 2010). In this regard, several works have been developed to determine lactose concentrations. In these, devices based on the use of enzymes as lactose sensors have been used (Eshkenazi *et al*., 2000). Other studies have focused on the measurement of urea in milk. In this case, they use a pressure biosensor that measures CO_2 from urea hydrolysis. The biosensor is capable of detecting urea concentrations between 2 and 7 mM and can be implemented in online measurement systems (Jenkins and Delwiche, 2002).

Flow cytometry: is a quick technique recognized by the International Dairy Federation (IDF) (Remón-Díaz *et al.,* 2019) that uses laser light for counting cells and other particles in suspension. The technique consists of passing a laser light beam through the sample in solution. The particles, in suspension, interact with the light beam producing two types of signals, one related to light scattering and the other with light emission coming from the fluorochromes present in the cells or particles in suspension. Through the processing and the technique, it is possible to know the characteristics of the cell, such as size, as well as to determine whether or not antigens are present in different parts of the cell, which makes the test specific and sensitive (Barrera *et al*., 2004).

In the area of veterinary medicine, this technique has been used for the detection and identification of bacteria present in milk samples from cattle with mastitis (Langerhuus *et al*., 2013; Gunasekera *et al*., 2003). In the reference paper (Ruiz-García and Sandoval-Monzón, 2018), it is concluded that the correlation between flow cytometry and the somatic cell count is low with a regression or correlation coefficient of 26%.

Ultrasound: milk and its components can be evaluated in a simple and fast way using ultrasound or other waves of the electromagnetic spectrum (Brandt *et al*., 2010). This has encouraged research in ultrasonography to evaluate mastitis, in fact, the reference study has performed in goats (Fasulkov *et al*., 2015). Although it wasn't performed in cattle, the study shows that this ultrasonographic technique allows to evaluate changes in the length of the breast canals and in the thickness of its walls, as well as in the diameter of the ducts through which milk is expelled. It also allows for the visualization

of large hyperechoic areas, which is an indicator of inflammation (Fasulkov *et al*., 2015; Santo *et al*., 2015). With this technique, the progress of the disease can be monitored, and it has been evidenced that, after three days of medical treatment, ultrasonographic images show a normal, anechoic breast. Other investigations have used ultrasonography to study structures with differences in echogenicity, suggesting the presence of mastitis, edema, hematomas, atrophies and fibrosis, and intraluminal obstructions (Porcionato *et al*., 2009, Rambabu *et al.,* 2009). Another ultrasonography study focused on evaluating the correlation between mammary gland biometry and possible alterations such as mastitis and milk production. The gland biometry consisted of measuring udder circumference, width, and quarter height. They concluded that there is no correlation between milk production, mammary gland biometric data, and ultrasonographic changes (Santos *et al*., 2016). As can be seen, ultrasound (ultrasonography) is used to determine, through images, alterations in the mammary gland of cattle and thus diagnose mastitis, however, its use as a non-invasive technique, analyzing the milk instead of the breast, lacks study and application.

Infrared spectroscopy: An alternative method for analyzing fats, proteins, and lactose in milk is mid-field infrared spectroscopy. It is a highly accurate and repeatable method both in the laboratory and in the field. However, due to the limited penetration depth, this spectroscopy is considered unsuitable for analyzing milk in online systems (Brandt *et al*., 2010). On the other hand, near-infrared spectroscopy can be used to analyze milk. In this case, the sensor is cheaper, and this method requires a little sample, even without preparation, making it more attractive for use in online systems (Brandt *et al*., 2010). Some wavelength regions where information related to fats, proteins and lactose is found are known, which are between 1100 and 2500 nm (Brandt *et al*., 2010) and between 600 and 1050 nm. Tsenkova *et al*. (2001) showed that the infrared spectrum of milk changes with high somatic cell count, due to alterations in proteins and changes in electrolytes contained in milk. Finally, spectroscopy in the visible region can be used to detect changes in milk coloration. Wiedemann and Wendl (2004) evaluated milk in the range between 400 and 520 nm. These results were superior compared to colorimetric measurements since the technique ignores the influence of fat content on milk color. The authors were able to correctly classify 85% of healthy quarters with less than 100,000 cells mL^{-1} and 71% of infected quarters with more than 500,000 cells mL^{-1} and a specificity of 95% (Wiedemann and Wendl, 2004).

Accuracy of diagnostic tests

The diagnostic tests CMT, somatic cell count, and electrical conductivity have been shown to have good sensitivity and good specificity for detecting subclinical mastitis in dairy herds (Dego, 2020). Likewise, CMT and somatic cell count have been characterized as correlated and useful in the diagnosis of bovine dairy (Suárez *et al*., 2014). In goat dairy the same situation does not occur due to physiological differences, the standard values for somatic cell count are not shown to be accurate for a good diagnosis of mastitis (Haenlein, 2002).

Signorini *et al*. (2008) conducted a study between May 1999 and August 2007 on "farm-level predictive values of mastitis from individual diagnostic test characteristics and sampling size". The diagnostic techniques analyzed were the California Mastitis Test (sensitivity 0.75 and specificity 0.54), electrical conductivity (sensitivity 0.61 and specificity 0.79), history of clinical mastitis (sensitivity 0.50 and specificity 0.54), and a hypothetical test with proposed sensitivity and specificity of 0.95 for both parameters. They used the statistical package InfoStat (National University of Córdoba) as a regression analysis where the sample size and the sensitivity and specificity of the diagnostic test were used as independent variables. Carpenter and Gardner (1996), analyzed a hypothetical case of the relationship between the sensitivity and specificity of diagnostic tests and the number of animals sampled on the predictive value at the farm level and the sensitivity at the farm level. Both authors agreed that there is an inverse relationship between diagnostic test sensitivity and sensitivity at the farm level for low and medium prevalence and an inverse relationship between specificity and sensitivity at the farm level when prevalence is high.

Dasohari *et al*. (2018) between February and August 2015 conducted a study on subclinical mastitis cases where 115 quarters of 30 cows were analyzed to compare the efficacy of different diagnostic tests such as

the California Mastitis Test (CMT), Somatic Cell Count (SCC), Whiteside Test (WST), among others. It was found that the most sensitive test was CMT (74.6%), followed by SCC (69.5%) and WST (59.3%). However, the best specificity was shown by WST (83.9%). CMT and SCC showed a specificity of 78.6%. The highest probability of having the disease if the test is positive was WST (79.6%) followed by CMT (78.6%) and SCC (77.4%). Overall, CMT is the most reliable field test after laboratory diagnostic test such as SCC.

Economic impact of bovine mastitis

Bovine mastitis is considered to be the disease that causes the greatest economic losses to dairy producers since its presence in the herds is reflected in excessive expenses for the producer and a decrease in income due to a decrease in production, which should generally be perceived within the farm (Hogeveen *et al.,* 2019).

The losses caused by this disease can be grouped as follows: Decreased production, milk discard, cost of medications, veterinary fees, extra work, and loss of genetic potential (Saran and Chaffer, 2000; Halasa *et al*., 2007). It is estimated that a cow with subclinical mastitis decreases her production by 10.9% (Paudyal *et al*., 2020).

Subclinical mastitis is more important and dangerous in dairy cattle because it is not possible to measure its dimension, it is underestimated since it produces chronic productivity losses with imperceptible alterations in the milk, which usually causes measures to be taken against the process when the suppression of productivity is already very large and the procedure to cure it is very expensive (Romero *et al.,* 2018).

Subclinical mastitis, whose frequency is 20 to 50 times higher than clinical mastitis, is nowadays the main problem of the whole pathological complex represented by mastitis. Careful analysis indicates that 80% of milk production losses are due to subclinical mastitis (Romero *et al*., 2018).

The cost attributable to subclinical forms of mastitis amounts to the majority of the total cost, which is between $100 and $150 per cow year^{-1}, or 50 to 80% of the industry's total production losses from mastitis (Burvenich *et al*., 2004), while milk production losses due to subclinical mastitis and cow replacement costs associated with somatic cell counts were estimated at $960 million (Wellenberg *et al*., 2002).

CONCLUSIONS

Subclinical mastitis is a silent disease that has a major impact on the health of bovines on the one hand, and on humans, as well as the world economy on the other. These are some of the reasons why detection methods are important in the diagnosis of the disease. Conventional methods are used worldwide such as CMT, somatic cells count, and WMT because of their practicality and ease to use. However, alternative methods are gaining more attention due to the sensitivity and specificity of the techniques. Besides, state-of-the-art technology allows for early detection of subclinical mastitis and other diseases saving or minimizing the impact it can cause on humans, animals, and milk producers.

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Vacuum impregnation of fresh-cut apples with osmotic solutions containing honey

UNIVERSIDAD NACIONAL DE COLOMBIA

Impregnación por vacío de manzanas frescas cortadas con soluciones osmóticas que contienen miel

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ABSTRACT

Fresh-cut apples were subjected to mild vacuum impregnation (Vacuum pressure=67.7 mbar). M_0: 30 °Brix honey solution (HS); $M_{0.5}$: HS+0.5% citric acid (CA)+0.5% ascorbic acid (AA); and M_1: HS+1% CA+1% AA were used as osmotic solutions. Changes in soluble solids (SS), pH, firmness, color (ΔE^*_{ab}), total phenolic and vitamin C content, and antioxidant capacity were evaluated on days 0 and 7 of storage at 1.5 °C. Mass transfer parameters were calculated. The best results were obtained with the M_1 treatment. Vacuum-impregnated fresh-cut apples gained solids (3.3%) and weight (1.6%) and lost water (1.8%). The following modifications were observed on day 0: an increase of SS (26%), a reduction in firmness (14%), a slight color change (ΔE^*_{ab}=3.3), a great increase in vitamin C content (31 times higher than in fresh-cut fruits) and increases in total phenolic content and antioxidant capacity (27% and 77%, respectively). On day 7 of storage, an additional reduction of firmness was observed, but the other attributes were maintained or increased. The M_1 treatment is an appropriate alternative for processing fresh-cut apples and obtaining improved healthy attributes.

Keywords:
Bioactive compounds
Citric acid
Malus
Vitamin C

RESUMEN

Manzanas frescas cortadas fueron impregnadas por vacío suave (Presión de vacío=67,7 mbar). Se emplearon tres soluciones osmóticas: M_0: solución osmótica de miel a 30 °Brix (HS); $M_{0.5}$: HS+0,5% de ácido cítrico (AC)+0,5% de ácido ascórbico (AA); y M_1: HS+1% AC+1% AA. Se evaluaron los cambios en los sólidos solubles (SS), pH, firmeza, color (ΔE^*_{ab}), fenoles totales, capacidad antioxidante y contenido de vitamina C en el día cero y después de 7 días de almacenamiento a 1,5 °C. Se calcularon los parámetros de transferencia de masa. La mejor alternativa fue el tratamiento M_1. Las manzanas impregnadas ganaron sólidos (3,3%), perdieron agua (1.8%) y ganaron peso (1.6%). El día cero, se incrementaron los SS (26%), se redujo la firmeza (14%), hubo un leve cambio en el color (ΔE^*_{ab}=3,3) y un gran incremento en el contenido de vitamina C (31 veces más que la fruta sin tratar), con un aumento en el contenido de fenoles totales y capacidad antioxidante (27 y 77%, respectivamente). Después de 7 días de almacenamiento, hubo una reducción adicional de la firmeza, pero se mantuvieron o mejoraron otros atributos. El tratamiento M_1 demostró ser una alternativa apropiada para el procesamiento de manzanas frescas cortadas que permite obtener un alimento más saludable.

Palabras clave:
Compuestos bioactivos
Ácido cítrico
Malus
Vitamina C

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Vacuum impregnation is a technique that allows the introduction of food ingredients directly into the product, through its pores, in a controlled way, according to the model of the hydrodynamic mechanism (Andrés *et al.,* 2001; Radziejewska *et al.,* 2014; Zhao and Xie, 2004; Nawirska-Olszańska *et al.,* 2020; Derossi *et al.,* 2021). The potential of this technology to enhance the physical-chemical characteristics, sensory attributes, or health potential of the product depends on the selection of appropriate processing conditions, such as vacuum pressure (VP) and osmotic solution (OS) (Fito *et al.,* 1996). Pressure produces significant changes in the product structure altering the fresh-like quality. Macroscopic properties (optical or mechanical properties) are modified and consequently, the related appearance and textural attributes also (Zhao and Xie, 2004; Cortez-Latorre *et al.,* 2021). Assis *et al.* (2019) and Mejía-Águila *et al.* (2021) worked with apples at 50 mbar and demonstrated the suitability of mild VP. Practically, all the native liquid was removed from the pore structure using VP<400 mbar (Zhao and Xie, 2004). OS composition is also important; for instance, treatments based on citric acid (CA) and ascorbic acid (AA) avoid or reduce enzymatic browning. Honey is a natural anti-browning agent due to its antioxidant activity, which is attributed to the vitamin C, small peptides, flavonoids, and other phenolic compounds, enzymes (glucose oxidase and catalase), and low pH (Jeon and Zhao, 2005). Honey is also a source of antioxidants that reduce the risk of heart disease, cancer, immune system deficiency, cataracts, different inflammatory processes, etc (Boussaid *et al.,* 2018). Concentration of OS is also important. Mujica-Paz *et al.* (2003) found that OS<50 °Brix impregnated massively in the fruit, however, OS>50°Brix are highly viscous and difficult to penetrate the pores of the plant tissue. Regarding storage temperature, Pirovani *et al.* (2015) reported that fresh-cut fruit shelf life is longer stored at 0 °C than those stored at 5 to 10 °C. Cortez-Latorre *et al.* (2021) found increases in vitamin C content, total phenolic content, and antioxidant activity for vacuum impregnated fresh-cut apples storage at 1.5 °C for 7 days.

The high porosity of apple tissue allows vacuum impregnation with hypertonic solutions. In this sense, vacuum and relaxation times are essential processing variables. During the vacuum time, the occluded gas inside the food pores expanded and flows outside the tissue; during the relaxation time, the external OS flows into the pores. Vacuum and relaxation periods can be set in order to attain the desired goals, such as high solids impregnation or high sample dehydration (Paes *et al*., 2007; Cortez-Latorre *et al.,* 2021).

The objective of the present work was to select the adequate OS based on honey, (alone or with the addition of citric and ascorbic acids) to improve the quality attributes of fresh-cut apples by mild vacuum impregnation.

MATERIALS AND METHODS

Fresh-cut apple processing

Apples (Granny Smith cv.) were purchased from a local market and stored at 1.5 °C until processing. Fruits were selected, washed, peeled, cored, and cut into cubes of 1.5 cm side. Vacuum impregnation was carried out using vacuum laboratory equipment composed of a glass vacuum desiccator (6 L volume) connected to a vacuum pump (Bombacio 31 042/169).

The fresh-cut apple processing conditions were selected according to preliminary studies (Faicán *et al.,* 2018), The vacuum impregnation treatments were performed at 22 °C and 67.7 mbar of VP with a weight ratio of 1:10 fruit: OS, during 14 min of vacuum time (VT) and 7.5 min of relaxation time (RT). Three OSs were used: M_0: 30 °Brix honey solution (HS); $M_{0.5}$: HS+0.5% CA+0.5% AA; and M_1: HS+1% CA+1% AA. Finally, apple cubes were placed on a mesh for 1 min to allow OS to be drained from the surface and then on absorbent paper for removal of excess OS. Then, 200 g of apple cubes were packed in 0.42 mm thick PET plastic containers, with a surface area of 0.045 m^2, with permeability for O_2 = $1.62x10^1$ mL mil cm^{-2} hr^{-1} atm^{-1}, and for CO_2=$3.68x10^1$ mL mil cm^{-2} hr^{-1} atm^{-1} (Exama *et al.,* 1993). Samples were analyzed on the day of vacuum impregnation processing (day 0) and after 7 days of storage at 1.5 °C (day 7).

Mass transfer parameters: Solid gain (SG), water loss (WL), and weight reduction (WR)

The mass transfer parameters SG, WL, and WR were determined based on Equations 1, 2, and 3, respectively,

$$SG(\%) = \frac{M_f(100 - H_f) - M_i(100 - H_i)}{M_i} \quad (1)$$

$$WL(\%) = \frac{(M_i \times H_i) - (M_f \times H_f)}{M_i} \quad (2)$$

$$WR(\%) = WL - SG \quad (3)$$

according to da Conceição Silva *et al.* (2012).
Where: M_i=fresh-cut fruit weight (g); M_f=vacuum impregnated fresh-cut fruit weight (g); H_i=fresh-cut fruit moisture content (%); H_f=vacuum impregnated fresh-cut fruit moisture content (%). Determinations were done in triplicate.

Negative values for SG, WL, and WR indicate solid loss, water gain, and an increase in sample weight, respectively.

Soluble solids, pH, and moisture content
To determine soluble solids (SS), pH, and moisture content, samples were crushed in a homogenizer. SS were measured with a PAL-ALFA digital portable refractometer (Atago, Tokyo, Japan). A Cardy Twin B-113 pH meter (Horiba Ltd. Kyoto, Japan) was used for pH determination. Moisture content was determined using a PMR50 moisture analyzer (RADWAG. Poland). Determinations were done in triplicate.

Firmness
Firmness was evaluated using TA. XT Plus texture analyzer (Stable Micro Systems, UK) according to Cortez-Latorre *et al.* (2021). Penetration tests were performed using a cylindrical tip 11 mm long and 4 mm in diameter, with a 50 N load cell. The penetration distance was 8 mm; the test speed was 1 mm s^{-1}. Exponent software was used to determine the maximum force (F) expressed in Newton (N). The F value represents the maximum force exerted by the tip to penetrate each cube. Then, 10 individual cubes were measured, and the mean values of those measurements are reported.

Color
Color was measured with a Minolta CM 508-d spectrophotometer, using an observer angle of 10° illuminant D65, and specular component excluded. L*, a*, b*, h_{ab}, C^*_{ab}, and the total color difference (ΔE^*_{ab}) were the color parameters evaluated. Luminosity varies from L*=100 (white) to L*=0 (black); the parameter Cab* indicates the chromaticity or saturation: h_{ab} is the hue angle, 0° (red), 90° (yellow), 180° (green), and 270° (blue). In total, 10 individual cubes were measured, and the mean value of those measurements was reported (Piagentini and Pirovani, 2017). Total color difference (ΔE^*_{ab}) is defined by equation 4:

$$\Delta E^*abi = \sqrt{(\delta L^*i)^2 + (\delta a^*i)^2 + (\delta b^*i)^2} \quad (4)$$

Where:
$\delta L^*i = L^*_{TFi} - L^*_{FF}$
$\delta a^*i = a^*_{TFi} - a^*_{FF}$
$\delta b^*i = b^*_{TFi} - b^*_{FF}$

Where: FF=fresh-cut fruit, TF=vacuum impregnated fresh-cut fruit, and i=storage day: day 0 or day 7.

Vitamin C
First, 100 g of sample was crushed, and then 12 g was homogenized for 1 min with 25 mL of extraction solvent (30 g L^{-1} metaphosphoric acid and 80 g L^{-1} acetic acid), sonicated for 15 min, and then centrifuged at 4000 g at 4 °C for 20 min. Supernatants were separated and used for vitamin C analysis (Rodríguez-Arzuaga *et al.,* 2021). Aliquots of supernatant were pre-treated with DL-dithiothreitol (DTT) solution (0.005 g L^{-1} prepared in 2.58 mol L^{-1} potassium phosphate dibasic). Extractions were made in duplicate. Total ascorbic acid content was determined by HPLC according to Van de Velde *et al.* (2012). Determinations were performed in triplicate. Results were expressed as mg kg^{-1} of fresh weight.

Total phenolic compounds content
Apple samples (100 g) were crushed in a homogenizer. A mixture of 2.5 g was homogenized with 25 mL extraction solvent (acetone/water 80:20), sonicated for 15 min, and then centrifuged at 4000 g for 20 min at 4 °C. Supernatants were separated and used for the analysis, the determination was performed using the Folin-Ciocalteu reagent, according to Cortez *et al.* (2018). Determinations were performed in triplicate. Results were expressed as mg GAE $Kg^{-1}$of fresh weight.

Antioxidant capacity

Apple cubes (100 g) were crushed in a homogenizer. A mixture of 2.5 g was homogenized with 25 mL extraction solvent (acetone/water 80:20), sonicated for 15 min, and then centrifuged at 4000 g for 20 min at 4 °C. The supernatants were separated and used for analysis. Antioxidant capacity (AC) was measured according to Sánchez-Moreno *et al.* (2003). The anti-radical activity was quantified by measuring the decrease in absorbance of a methanolic solution of the free radical DPPH* (1,1-dipheny-2-picrylhydrazyl) at 517 nm in the presence of aliquots of fruit extracts. Determinations were done in triplicate. Results were expressed as mg AA Kg^{-1} of fresh weight.

Statistical analysis

Results for soluble solids, pH, firmness, color parameters (except ΔE^*_{ab}), total phenolic compounds, vitamin C and antioxidant capacity are expressed as a relative variation percentage for each attribute Q (ΔQi, %), with respect to fresh-cut fruit attribute (Equation 5):

$$\Delta Qi(\%) = \frac{(Q_{TFi} - Q_{FF})}{Q_{FF}} \times 100 \quad (5)$$

Where: Q_{FF}= fresh-cut fruit attribute, Q_{TF}=vacuum impregnated fresh-cut fruit attribute, and i= storage time: day 0 or day 7.

Data were analyzed using ANOVA. The differences among mean values were determined by the Tukey test at $P \leq 0.05$. Statistical analyses were carried out using STATGRAPHICS Centurion XV 15.2.06 (Statpoint Technologies, Inc., Warrenton, VA, USA).

RESULTS AND DISCUSSION

Mass transfer parameters: Solid gain (SG), water loss (WL), and weight reduction (WR)

Table 1 shows the mass transfer parameters in fresh-cut apples subjected to mild vacuum impregnation. There were no significant changes for SG among OSs ($P \geq 0.05$). The average SG was 2.8%.

Table 1. Solid gain (SG), water loss (WL), and weight reduction (WR) in VI fresh-cut apples.

OS	SG (%)	WL (%)	WR (%)
M_0	2.6 a±0.59	0.04 b±0.59	-2.6 b±0.00
$M_{0.5}$	2.5 a±0.09	-0.15 b±0.09	-2.7 c±0.00
M_1	3.3 a±0.15	1.80 a±0.15	-1.6 a±0.00

The same letters within a column indicate no significant differences between treatments determined using the Tukey test ($P \leq 0.05$).
M_0= 30 °Brix honey solution (HS); $M_{0.5}$= HS+0.5% of citric acid+0.5% of ascorbic acid; M_1=HS+1% citric acid+1% of ascorbic acid.

The SG found in the present work was higher than the SG value obtained for fresh-cut apples impregnated at 67.7 mbar with VT=10 min, RT=10 min, and 30 °Brix sucrose OS (2.4%) reported by Faicán *et al.* (2018). This discrepant result may be attributed to the difference in molecular weight between solutes of honey (glucose, fructose, maltose, and other sugars that correspond to approximately 80% of honey composition) and those of sucrose. Bolin *et al.* (1983) found different responses of fresh-cut apples, peaches, and apricots subjected to osmotic dehydration with two OSs, sucrose and high fructose corn syrup, with the latter causing a greater increase in SG. According to those authors, sucrose, a disaccharide, would be expected to migrate slower than fructose, a monosaccharide. The diffusion coefficient of fructose is 32% higher than that of sucrose, consequently, honey OS may have penetrated faster replacing more of the water in the cells. Zhao and Xie (2004) suggested that in most cases, low molecular weight carbohydrates are used in impregnation treatments since this condition allows a faster penetration into the sample. These results are consistent with the findings in the present study, because honey OS penetrated better than sucrose solution and, consequently, SG was higher.

Table 1 shows that OS affected WL ($P \leq 0.05$). No differences between M_0 and $M_{0.5}$ treatments were detected; however, M_1 treatment produced greater WL.

WR was also affected by OS ($P \leq 0.05$). All treatments resulted in a weight gain (indicated by the negative sign of WR). The present results agree with those of Mujica-Paz *et al*. (2003), who reported similar results for fresh-cut Golden Delicious apples due to the high porosity of apples, which facilitates the impregnation of hypertonic solutions (40 °Brix).

Physico-chemical parameters

The soluble solids (SS), pH, firmness, color parameters (L*. hab, C*ab), Total Ascorbic Acid (TAA), Total Phenolic Content (TPC) and Antioxidant Capacity (AC) for fresh-cut fruit are presented in Table 2.

Table 3 shows the relative variation percentage for SS as a function of OS on days 0 (ΔSS_0) and 7 of storage at 1.5 °C (ΔSS_7). No significant effect of OS ($P \geq 0.05$) was observed on either day. SS increased in VI fresh-cut fruit with respect to fresh-cut fruit by approximately 25% and 24%, on days 0 and 7 of storage at 1.5 °C, respectively.

Table 2. Physico-chemical parameters and bioactive properties of fresh-cut apples

Parameter	Mean±sd
Soluble solids (°Brix)	12.6±0.46
pH	3.4±0.07
Firmness (N)	13.1±2.50
L*	71.5±1.75
h_{ab}	95.6±2.62
C^*_{ab}	23.4±2.71
TAA (mg Kg^{-1})	30.2±0.12
TPC (mg GAE Kg^{-1})	404.0±5.30
AC (mg AA Kg^{-1})	1121.8±10.03

(L*. h_{ab}, C^*_{ab})=Color parameters, TAA=Total Ascorbic Acid. TPC=Total Phenolic Content, AC=Antioxidant Capacity. sd=standard deviation

Table 3. Relative variation percentage of soluble solids (ΔSS_i), pH (ΔpH_i), and firmness (ΔF_i) in vacuum impregnated fresh-cut apples on days 0 and 7 of storage at 1.5 °C.

OS	ΔSS_0 (%)	ΔSS_7 (%)	ΔpH_0 (%)	ΔpH_7 (%)	ΔF_0 (%)	ΔF_7 (%)
M_0	23.0^{A}_{a} ±0.8	24.0^{A}_{a} ±0.46	0.0^{A}_{a} ±0.0	0.0^{A}_{a} ±0.0	-12.0^{A}_{a} ±4.16	-67.4^{A}_{b} ±6.32
$M_{0.5}$	26.0^{A}_{a} ±2.0	24.4^{A}_{a} ±0.49	-5.7^{B}_{a} ±0.0	-8.6^{B}_{b} ±0.0	-8.1^{A}_{a} ±4.02	-67.0^{A}_{b} ±3.47
M_1	26.0^{A}_{a} ±1.25	23.0^{A}_{a} ±1.40	-5.9^{C}_{a} ±0.0	-8.8^{C}_{b} ±0.0	-14.0^{A}_{a} ±5.98	-59.0^{B}_{b} ±3.17

The same capital letters in the same column indicate no significant differences among treatments, and the same lower-case letters in the same row indicate no significant differences between days of storage, determined by the Tukey test ($P \leq 0.05$).
M_0= 30 °Brix honey solution (HS), $M_{0.5}$= HS+0.5% of citric acid+0.5% of ascorbic acid, M_1=HS+1% citric acid+1% of ascorbic acid. i=day zero or day seven.

There were significant differences in SS between days 0 and 7 for M_0 treatment ($P \leq 0.05$) (Table 3). Conversely, $M_{0.5}$ and M_1 treatments did not show differences between days 0 and 7 of storage at 1.5 °C. Granny Smith fresh-cut apples impregnated with 50 °Brix honey OS, 700 mbar, VT=10 min, and RT=10 min showed an increase of SS of about 50-70%, remaining stable after 7 days of storage (Rößle *et al*., 2011). They found good adherence of honey OS to fresh-cut fruit, which allows the values to remain stable during storage. Similar results in the $M_{0.5}$ and M_1 treatments.

The ΔpH_0 and ΔpH_7 were affected by OS ($P \leq 0.05$). The application of M_0 treatment did not cause changes in pH. However, pH values decreased in $M_{0.5}$ and M_1 treatments, due to the presence of AA and CA in OSs (Table 2). The present results are consistent with those of Faicán *et al.* (2018), who reported variations in VI fresh-cut Granny Smith

apples under different VPs and treatment times (VP=67.7 and 667.3 mbar, VT/RT=3 min; VT/RT=10 min), with 30 °Brix sucrose OS with the addition of 1% of AA and 1% of CA; the authors obtained negative values of ΔpH, which means a reduction in pH for VI fruits.

No significant differences in pH between days 0 and 7 were observed in M_0 treatment. Nevertheless, for $M_{0.5}$ and M_1 treatments, the ΔpH on day 7 was 1.5 times higher than on day 0. These results agree with those of Cortez-Latorre *et al.* (2021), who reported a decrease in pH, both on days 0 and 7 (7.7 and 5.1%, respectively) for VI fresh-cut apples with an OS of sucrose with an addition of 1% AA and 1% CA, under the same pressure and time conditions as in the present work.

The relative variation percentage of firmness (ΔF_i) was not affected by the OS ($P \geq 0.05$) on day 0 (Table 3). The mean percentage was -11.4%. On the other hand, the firmness after 7 days of storage was influenced by OS used in the vacuum impregnation procedure ($P \leq 0.05$). M_0 and $M_{0.5}$ treatments had a higher reduction in firmness than M_1 (Table 3).

The present results agree with those obtained by Rößle *et al.* (2011), who reported firmness losses of about 20% on day 0 and higher on day 7 (approximately 25%) in vacuum impregnated Granny Smith apples (VT=10 min and VP=700 mbar) with honey OS. This behavior could be due to VT and OS concentration conditions.

According to Rößle *et al.* (2011), the firmness of fresh-cut apples impregnated with high-fructose corn syrup (50%) was significantly reduced, whereas OS with lower concentration (20%) did not show firmness reduction. Zhao and Xie (2004) suggested that with the use of hypertonic solutions, the cells may shrink or shrivel due to water leaving the cell. Hence, the selection of impregnation solutions depends on the purpose of osmotic treatment, i.e., the type of finished product, because the type of OS significantly affects mass transfer during the vacuum impregnation processing, and product deformation and shrinkage might occur.

Andrés-Bello *et al.* (2013) studied the effect of acidification (pH=6.2; 4.4 and 3.9) followed by blanching on the firmness of carrot tissue. Blanching at pH 6.2 caused a firmness reduction ($\approx$70%), and acidification with consequent blanching at pH 4.4 increased the firmness ($\approx$50%). However, blanching and acidifying the tissue to pH 3.9 reduce firmness in the same proportion as that obtained using pH=6.2. Degradation of carrot tissue firmness during heat treatment occurred by two different mechanisms, one at neutral pH and another at pH 3.9 since degradation was much lower at an intermediate pH. The results of the present research agree with the behavior reported by Andrés-Bello *et al.* (2013); indeed, the pH reduction can be one of the reasons for the loss of firmness, since the pH values obtained after the vacuum impregnation process were lower than 3.4. All treatments reduced firmness, with the reduction being higher after seven days. These reductions imply pH values of 3.2 and 3.1.

There were significant differences in firmness between days 0 and 7 ($P \leq 0.05$). The relative variation percentage of firmness on day 7 was approximately six to five times higher than on day 0 (Table 4). Texture breakdown of minimally processed tissue is expected to occur as a response to a wound-induced increase in enzymes targeting cell walls and membranes in response to injury. Huber *et al.* (2001) found that the firmness of light-processed papaya fruit declined more rapidly and extensively than tissue derived from intact fruit stored under identical conditions. In the present work, the processing and storage of VI fresh-cut apples generated tissue degradation, as indicated by Huber *et al.* (2001). Vacuum impregnation with hypertonic solutions generates firmness reduction due to osmotic dehydration of the product with simultaneous loss of turgor and elasticity that alters cellular resistance. The increase in its viscous character generates changes in the air and liquid fractions of the product and also in its shape and size. Loss of turgor pressure is due to plasmolysis or rupture of the tonoplast and plasmalemma of plant cells. The loss of elasticity is due to air-liquid exchange during vacuum operations (Alzamora *et al.*, 1997).

Negative values for the L* relative variation percentage indicate a decrease of the parameter with respect to fresh-cut apples (that is, a darkening of the sample). Positive and negative values in h_{ab} relative variation percentage indicate that the hue of vacuum-impregnated fresh-cut fruits was yellower or redder than fresh-cut fruits, respectively. Positive and negative values in C^*_{ab} relative variation percentage showed that the chroma of vacuum-impregnated fresh-cut fruits was more vivid or duller than that of fresh-cut fruits, respectively (Table 4).

Table 4. Relative variation percentage of color parameters (ΔL^*_i, Δh_{abi} and ΔC^*_{abi}) in vacuum impregnated fresh-cut apples on days 0 and 7 of storage at 1.5 °C

SO	ΔL^*_i (%)		Δh_{abi} (%)		ΔC^*_{abi} (%)	
	0	7	0	7	0	7
M_0	$-4.7^{B}_{a}\pm2.11$	$-22.0^{C}_{b}\pm5.46$	$-1.4^{B}_{a}\pm1.30$	$-5.8^{B}_{b}\pm1.81$	$0.2^{A}_{a}\pm0.10$	$-0.3^{A}_{b}\pm0.48$
$M_{0.5}$	$-2.0^{A}_{a}\pm2.82$	$-17.0^{B}_{b}\pm7.41$	$1.7^{A}_{a}\pm1.18$	$1.0^{A}_{b}\pm1.43$	$4.2^{A}_{a}\pm4.00$	$-0.6^{A}_{b}\pm0.18$
M_1	$-2.0^{A}_{a}\pm1.80$	$-7.0^{A}_{b}\pm4.02$	$1.7^{A}_{a}\pm0.99$	$1.4^{A}_{b}\pm1.08$	$-2.1^{A}_{a}\pm2.08$	$2.1^{A}_{b}\pm2.70$

The same capital letters in the same column indicate no significant differences among treatments and the same lower-case letters in the same row indicate no significant differences between days of storage, determined by the Tukey test ($P\leq0.05$).M_0=30 °Brix honey solution (HS), $M_{0.5}$= HS+0.5% of citric acid+0.5% of ascorbic acid, M_1=HS+1% citric acid+1% of ascorbic acid. i=day 0 or day 7.

On day 0, changes in ΔL^*_0 and Δh_{ab0} were related to the OS ($P\leq0.05$). However, ΔC^*_{ab0} was not affected by OS ($P\geq0.05$). The ΔL^*_0 was always negative, meaning that VI fresh-cut fruits were darker than fresh-cut fruits, probably due to honey color. Small changes in ΔL^*_0 occurred in $M_{0.5}$ and M_1 treatments containing CA and AA (Table 5). The negative value of Δh_{ab} in M_0 treatment indicated that vacuum-impregnated fresh-cut apples were redder than untreated fresh-cut ones. On the contrary, $M_{0.5}$ and M_1 treatments resulted in yellower vacuum-impregnated fresh-cut apples. The ΔC^*_{ab0} resulted in an average value of 0.8%. After 7 days of storage, ΔL^*_7 and Δh_{ab7} were also affected by the OS used for vacuum impregnation ($P\leq0.05$), but the ΔC^*_{ab7} was not, with a mean value of 0.4% (small changes with respect to fresh-cut fruit). As expected, the lowest changes of ΔL^*_7 occurred in the M_1 treatment due to the antioxidant solution. The comparison of color changes (ΔL^*, Δh_{ab}, and ΔC^*_{ab}), for the same treatment, between days, showed significant differences ($P\leq0.05$) (Table 5).

Table 5 Non-treated fresh-cut apples (FF) and fresh-cut apples were subjected to vacuum impregnation with different osmotic solutions at day 0 and after 7 days of storage at 1.5°C

Treatments	Day 0				Day 7			
	L^*	h_{ab}	C^*_{ab}	ΔE^*	L^*	h_{ab}	C^*_{ab}	ΔE^*
FF	71.5	96	22.7		-	-	-	-
								
M_0	68.8	92.9	25.2	4.7	56.3	88.6	25	16.3
								
$M_{0.5}$	69.7	98.4	22.4	3.1	59	97.8	21.3	12.3
								
M_1	69.7	98.4	21	3.3	66.3	98.2	22	5.6
								

M_0=30 °Brix honey solution (HS), $M_{0.5}$=HS+0.5% of citric acid+0.5% of ascorbic acid, M_1=HS+1% citric acid+1% of ascorbic acid.

Our results agree with the behavior reported by Jeon and Zhao (2005), who observed that the use of dark honey generates a reduction of L*. This is a potential problem in light-colored fruits such as apples because the fruit acquires a color similar to that of honey. Browning inhibition in crushed Red Delicious apples with the addition of 1% honey and 0.1% AA is three times higher than in samples obtained only with honey addition (Chen *et al.* 2000).

The total color difference was affected by the OSs on day 0 ($\Delta E^*_{ab\ 0}$) and on day 7 ($\Delta E^*_{ab\ 7}$) ($P \leq 0.05$) (Figure 1). There was a perceptible color difference for M_0 samples with respect to untreated fresh-cut apples and fairly perceptible color differences for $M_{0.5}$ and M_1 on day 0. After 7 days, $\Delta E^*_{ab\ 7}$ values showed strong differences for M_0 and $M_{0.5}$ treatments, but only perceptible differences for M_1 treatment, which had the highest content of AA and CA. In this sense, it is well known that the use of antioxidant compounds contributes to the maintenance of visual attributes, preventing discoloration due to enzymatic browning (Betoret *et al.,* 2015; Rodríguez-Arzuaga and Piagentini, 2018).

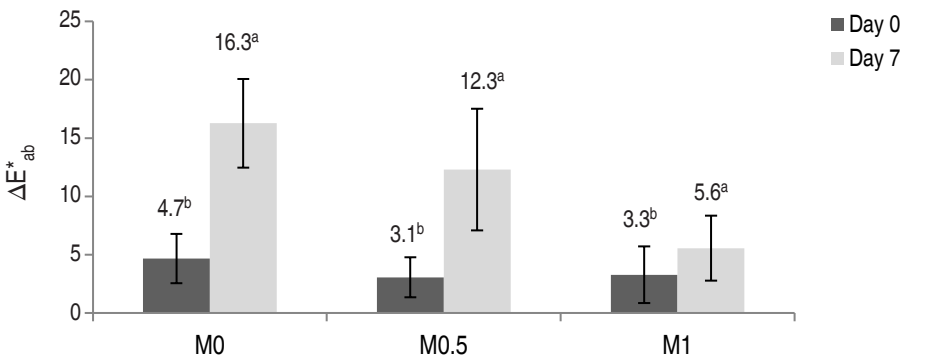

Figure 1. Color difference (ΔE*ab) in vacuum-impregnated fresh-cut apples on days 0 and 7 of storage at 1.5 °C.
M_0= 30 °Brix honey solution (HS), $M_{0.5}$=HS+0.5% of citric acid+0.5% of ascorbic acid, M_1=HS+1% citric acid+1% of ascorbic acid.

Bioactive compounds

The relative variation percentage of total ascorbic acid content (ΔTAA_i) on day 0 was significantly affected by the OS ($P \leq 0.05$). Treatments $M_{0.5}$ and M_1 increased the vitamin C content with respect to fresh-cut fruits on day 0 Treatment M_1 produced a greater increase in total ascorbic acid, 31 times higher than in fresh-cut fruits due to the AA solution. (Figure 2).

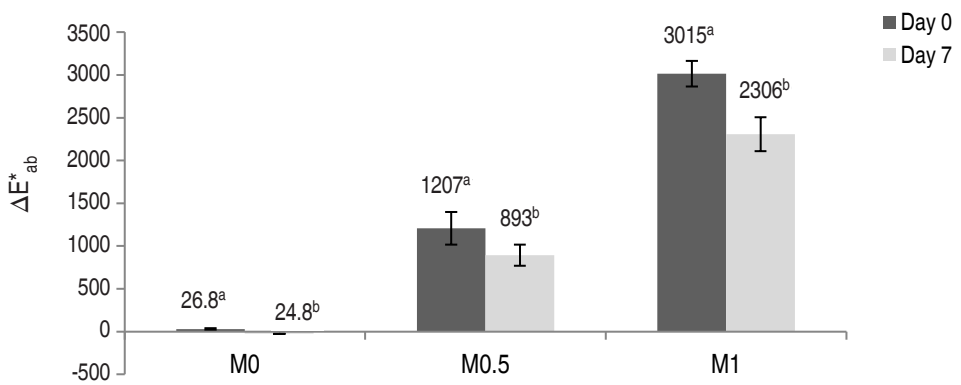

Figure 2. Total ascorbic acid variation (ΔTAA) in vacuum-impregnated fresh-cut apples on days 0 and 7 of storage at 1.5 °C.
M_0=30 °Brix honey solution (HS), $M_{0.5}$=HS+0.5% of citric acid+0.5% of ascorbic acid, M_1=HS+1% citric acid+1% of ascorbic acid.

Cortez-Latorre *et al.* (2021) studied the effect of vacuum impregnation process variables on quality aspects of fresh-cut apple vacuum-impregnated with OS of sucrose with the addition of 1% AA and 1% CA (VP = 67.7 mbar). They determined a high increase (about 5600%) in total ascorbic acid content of treated fruit with respect to fresh fruit when applying the same VT and RT as in the present study. Here, total ascorbic acid content was reduced in all samples during storage (Figure 2). However, the total ascorbic acid content for $M_{0.5}$ and M_1 was still higher than untreated fresh-cut apples at the end of storage. Rodríguez-Arzuaga *et al.* (2021) reported that vitamin C content (197 mg kg^{-1}) of Granny Smith apples treated by immersion (3 min) in a solution with 1.2% of Yerba Mate+0.9 % CA+1% AA at atmospheric pressure increased on day 1 and then decreased up to approximately 50 mg kg^{-1} after 10 days, remaining constant until the end of storage at 2 °C for 18 days and 10 °C for 15 days. Similarly, Cortez-Latorre *et al.* (2021) determined that fresh-cut apples vacuum-impregnated under the same pressure conditions as those in the present work, with a sucrose OS (30 °Brix) with 1% CA and 1% AA, with VT and RT among 1 to 14 min, maintained high values of vitamin C (188-606 mg kg^{-1}) at the end of 7 days of storage at 1.5 °C.

No significant effect of OS on ΔTPC_0 was found ($P \geq 0.05$). However, all the vacuum-impregnated samples increased the phenolic compound content with respect to fresh-cut fruit on day 0 (Figure 3). On the other hand, ΔTPC_7 was affected by OS ($P \leq 0.05$); M_0 treatment lost the phenolic compounds (approximately 19%). Treatment $M_{0.5}$ maintained phenolic compounds similar to fresh-cut apples, and M_1 maintained phenolic compounds uptake (about 20%).

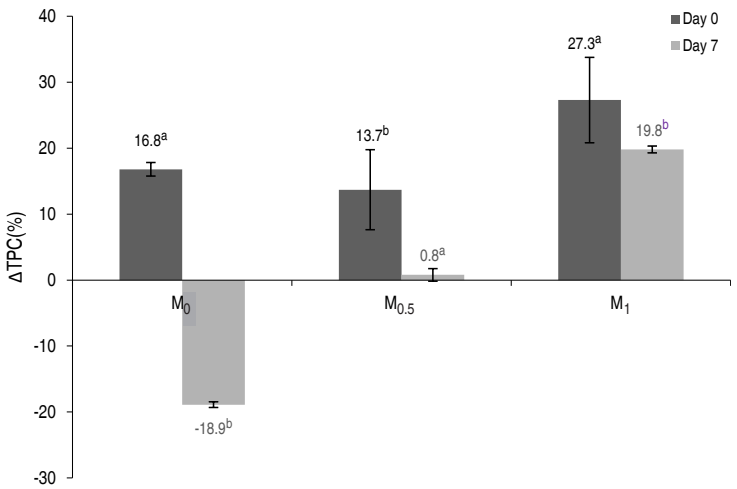

Figure 3. Total phenolic content (ΔTPC) in vacuum impregnated fresh-cut apples on days 0 and 7 of storage at 1.5 °C
M_0=30 °Brix honey solution (HS), $M_{0.5}$=HS+0.5% of citric acid+0.5% of ascorbic acid, M_1=HS+1% citric acid+1% of ascorbic acid.

Oszmianski and Lee (1990) suggested that honey not only inhibits the enzymatic oxidation of polyphenols but also converts part of o-quinones to the original phenols. It is also known that this kind of reaction occurs with AA, which turns the o-quinones into phenols (Robards *et al.*, 1999), and can be the reason for the increase of phenolic compound content after VI treatments in the present study. Nawirska-Olszańska *et al.* (2020) also evaluated the application of vacuum impregnation technology using apple-pear juice as OS in chokeberry fruit as a treatment before the drying process and determined an increase in the phenolic compound content (46.3-63.9%) after the vacuum impregnation process; the phenolic compounds increase obtained herein shows similar behavior.

On the other hand, the phenolic compound content decreased with M_0 treatment without AA and CA after the storage. In this sense, the decrease in polyphenol concentration due to browning was found to be correlated with the degree of enzymatic browning (Murata *et al.*, 1995). Similarly, in this work, apples treated with M_0 showed a high color change after 7 days of storage,

showing a pronounced browning. The absence of AA and CA in M_0 reduces the possibility of delaying enzymatic browning, causing a decrease in phenolic compounds. Like other factors, pH also plays a significant role in the browning of fruits and vegetables. Polyphenol oxidase enzyme activity is inhibited in the presence of acids (Singh *et al*., 2018). This situation can explain the significant differences in total phenolic content between days 0 and 7 ($P \leq 0.05$) for M_0 treatment, with increase of phenolic compounds on day 0 due to honey and losses on day 7 of storage due to enzymatic degradation. The relative variation percentage of antioxidant capacity (ΔAC_i) was influenced by the OS ($P \leq 0.05$). On day 0, the M_1 treatment generated the highest increase of ΔAC_0. On day 7, $M_{0.5}$ and M_1 generated similar increases, which were higher than in M_0 (Figure 4). Similar results were obtained on day 7, with the increase in antioxidant capacity caused by M_1 and $M_{0.5}$ being approximately two times higher than fresh cut apples.

Santarelli *et al*. (2020) evaluated the effect of frozen storage on the content of functional compounds and the AC of vacuum-impregnated apples treated with an organic lemon juice solution with a citric acid content of 0.5% w/v. They found that vacuum impregnation significantly affects the AC of apple products, leading to increases of 10-13%, and attribute this result to the apple uptake of lemon juice and its antioxidant compounds (e.g., flavonoids, citric acid). Similarly, the present work shows that the small AC increase (15 – 23%) in M_0 was due to honey bioactive compounds, whereas higher increases in AC of $M_{0.5}$ and M_1 were related to the uptake of ascorbic and citric acid solution.

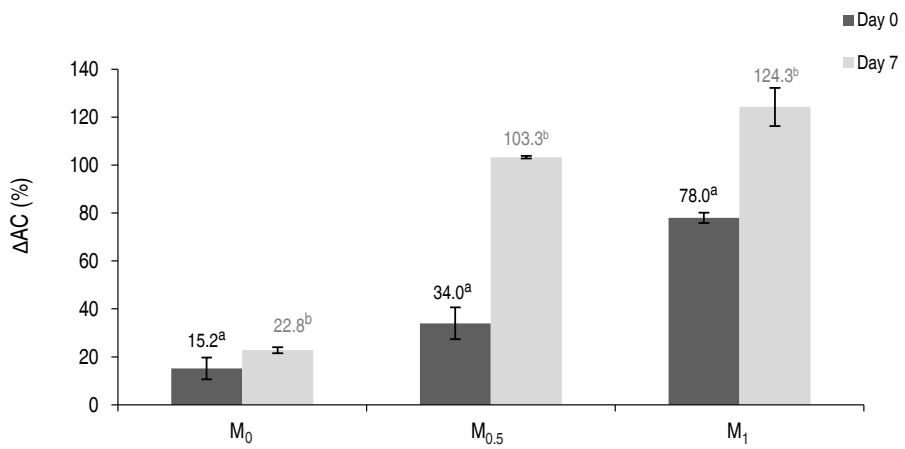

Figure 4. Antioxidant capacity (ΔAC) in vacuum impregnated fresh-cut apples on days 0 and 7 of storage at 1.5 °C.
M_0= 30 °Brix honey solution (HS), $M_{0.5}$= HS + 0.5% of citric acid + 0.5% of ascorbic acid, M_1= HS + 1% citric acid +1% of ascorbic acid.

Significant differences in ΔAC were observed between days 0 and 7 for treatments $M_{0.5}$ and M_1 ($P \leq 0.05$). On day 7, ΔAC increased 3 and 1.6 times in $M_{0.5}$ and M_1 samples, respectively, with respect to day 0. Rodríguez-Arzuaga *et al*. (2021) studied fresh-cut Granny Smith apples by immersion in a solution containing 1.2% of Yerba Mate + 0.9 % CA+ 1% AA at atmospheric pressure and reported an increase in antioxidant activity during the first two days at 2 °C. This antioxidant activity remained stable and was higher than values in the samples without immersion in antioxidant solution until day 7. Similarly, the capacity antioxidant found in the present study increased when using OS with the addition of CA and AA, after 7 days at 1.5 °C.

CONCLUSION

The use of different OSs affected the final quality of the vacuum-impregnated fresh-cut apples. The most adequate treatment was M_1 (30 °Brix honey with 1% AA plus 1% CA), which showed significant increases in vitamin C content, phenolic compound content, and antioxidant capacity. Regarding other quality attributes, there was a slight increase in the soluble solids, a small reduction in firmness, and a fairly perceptible color

difference. After 7 days of storage, an additional reduction of firmness was recorded, with the other attributes being maintained or enhanced. The M_1 treatment was found to be an appropriate alternative for processing fresh-cut apples with improved healthy potential attributes.

ACKNOWLEDGMENTS

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Short article

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Revista Facultad Nacional de Agronomía

Propagation of "Valencia" orange (*Citrus* x *sinensis* Osbeck) by minigraft

UNIVERSIDAD NACIONAL DE COLOMBIA

Propagación de naranja "Valencia" (*Citrus* x *sinensis* Osbeck) por mini injertos

https://doi.org/10.15446/rfnam.v75n3.99339

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ABSTRACT

Demand for citrus (*Citrus* spp) plants for commercial orchards has increased worldwide due to the need for new plantations, renewal of disease-effected crops, and strict regulation for plant production. To evaluate faster and low-cost propagation alternatives for citrus, "Valencia" orange plants were propagated by using two minigrafting techniques (Cleft and inverted T-budding). Rootstocks were raised from "Cleopatra" mandarin seeds, and scions and buds were isolated from 1-year-old grafted plants. For cleft minigrafts, scions were inserted at 5-7 cm height inside of the decapitated rootstocks and covered with Eppendorf® tubes. For T-budding, buds were inserted at 5-7 cm height under the rootstock cortex cut. Unions were fixed with Parafilm®. Grafted plants were maintained under a shade house (50%) with two daily fog irrigation (2 min each). Treatments were distributed with a completely randomized design. Six weeks after grafting, the percentage of success, the shoot length, and the number of leaves per treatment were registered and analyzed with a T test (α=0.05). Cleft minigraft resulted in a higher success percentage and plants with larger shoots. Cleft minigraft could be considered an alternative for citrus propagation in small and medium size nurseries.

Keywords:
Callus
Citrus
Nursery
Rootstock
Scion
Union.

RESUMEN

La demanda de plantas cítricas (*Citrus* spp) para cultivos ha incrementado mundialmente debido a las necesidades de nuevas siembras, reemplazo de árboles enfermos en cultivos establecidos, y las estrictas regulaciones para la propagación de plantas. Con el fin de evaluar alternativas de propagación más rápidas y de bajo costo para cítricos, plantas de naranja "Valencia" fueron propagadas usando dos métodos de mini injertación (Hendidura y T-invertida). Los patrones fueron obtenidos de mandarina "cleopatra" y las yemas fueron aisladas de plantas injertadas de 1 año de edad. Para los injertos de hendidura, las yemas fueron insertadas a 5-7 cm de altura en los patrones o portainjertos decapitados y se cubrieron con tubos Eppendorf®. Para los injertos en T-invertida, las yemas se insertaron a 5-7 cm de altura debajo de la corteza del patrón. Las uniones se fijaron con Parafilm. Los injertos se mantuvieron en una casa malla (59%) con dos riegos diarios (2 min cada uno). Los tratamientos se distribuyeron con un diseño completamente aleatorizado. Seis semanas después de la injertación, el porcentaje de éxito, la longitud de tallos y el número de hojas por tratamiento fueron registrados y analizados con la prueba de T Student (α=0.05). El mini injerto de hendidura resultó en un mayor porcentaje de éxito y tallos más largos. El mini injerto de hendidura puede considerase como una alternativa para la propagación de cítricos en pequeños y medianos viveros.

Palabras clave:
Callo
Cítricos
Viveros
Portainjerto
Yema
Unión

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Citrus plants are one of the most important fruit crops all over the world (Wu *et al*., 2018). Citrus fresh fruits and orange juice are common components of daily diets due to their high levels of vitamin C, folate, flavanones, hesperidin, and naringin, and numerous reported health benefits (Inglese and Sortino, 2019). Production of citrus in the world in 2018 was around 150 million t where oranges accounted for 50%, mandarins 22%, lemons, and limes 12% and other citrus fruits 6%. In Colombia, production in 2018 was estimated at 75000 t distributed in 20% oranges, 13% mandarins, 14% limes, lemons, and 52% other citrus fruits (FAOSTAT, 2020). In the Córdoba department, citrus fruit production, in the same year, was 1150 t, being "Valencia" orange production more than 90% (Agronet, 2021). A worldwide shortage in citrus plant supply is happening due to strict regulations during the propagation process to prevent the spread of diseases such as CTV (Citrus tristeza virus), CEVd (Citrus Exocortis Viroid) and HLB (Huanglongbing) (Wang, 2021; Vashisth *et al*., 2020; Folimonova, 2020; ICA, 2019). Citrus orchards are generally established with grafted plants to combine rootstock and cultivar benefits, to ensure fruit quality and uniformity, and to reduce the time for harvesting. (Talon *et al*., 2020; Barón *et al*., 2019). Minigraft is a clonal propagation technique that uses young rootstocks to be grafted with small size scion/bud parts to obtain younger plants fully adapted to field conditions, avoiding the maintenance of large size plants for scion production and the *ex vitro* acclimatization stage of the micropropagation process (Siqueira *et al*., 2016). Out of the hundreds of grafting techniques, citrus plants are usually grafted by T-budding, a time-consuming process where a bud is removed from the desired cultivar and inserted underneath of the rootstock cortex to promote callus growth and vascular connection between the two parts; the whole process may last for 24-36 months for plants to be ready for field planting (Alves *et al*., 2019; Widaryanto *et al*., 2019). Recent studies on citrus propagation focus on speed up the propagation process and increasing the number of plants produced while complying with the official regulations (Pokhrel *et al*., 2021; Solonia *et al*., 2020). The present research aimed to evaluate two types of minigrafts, cleft and T-budding, their viability and success level on the propagation of "Valencia" orange plants as a way to obtain a faster and cost-effective citrus propagation protocol.

MATERIALS AND METHODS

The experiment was carried during the year 2019-2020 in a shade house of the Institute of Applied Biotechnology for the Caribbean of the Universidad de Córdoba (Monteria, Colombia), located at 8° 31´N and 75° 58´ W with an elevation of 12 masl.

Rootstock growth

Seeds for rootstock production were extracted from horticulturally ripened Cleopatra mandarin (*Citrus reshni* hort. *Ex* Tanaka) fruits harvested from field grown trees at the Universidad de Córdoba – Berastegui Campus (8°40´26" N 75°46´44" W). Fruits were washed twice with distilled water, hand-squeezed and seeds separated with a plastic sieve. The extracted seeds were profusely washed with sterile-distilled water, air-dried overnight on filter paper, and stored in sterile closed glass flasks for 4 weeks in a conventional fridge at 8 °C. Germination occurred after seeds were sown in plastic tube containers (15×5 cm) filed with peat as substrate. Seedlings were maintained under shade house conditions with a 50% saran light for 6 months with fog irrigation twice a day for 1 min each.

Bud and scion selection

Grafting material was isolated from 2-year-old grafted plants of Valencia orange (*Citrus* x *sinensis* Osbeck) obtained from an authorized citrus plant distributor (Reg. ICA 25290-06V). Plants were maintained in a shade house (50%) with fog irrigation twice a day for 1 minute each.

Minigrafting and plant growth

To evaluate grafting success percentage, two types of graft, cleft, and T-budding, were performed on 6-month-old rootstocks and, approximately, 20 cm high. For cleft grafting, the rootstock was decapitated at 5-7 cm high, and a vertical downward cut was done in the center of the decapitated stem using a sterile scalpel. The scion, 2-3 cm long tender tissue containing at least one node, was cut from both sides at the basal end with the scalpel into a gently sloping wedge (~0.5 cm) where the cambium vascular tissue was observed. The scion was properly inserted in the rootstock cut, firmly tied with Nescofilm®, and top covered with a plastic 1.5 mL Eppendorf tube for two weeks. For inverted T-budding, a 1-2 cm vertical downward cut was done with a scalpel

in the rootstock stem at about 5-7 cm high, and down terminated with a perpendicular horizontal cut. The bud was removed from young-tender stem shoots by cutting at about 0.3 cm below the bud with the scalpel and making a slicing cut down under the bud finishing about 0.5 cm beyond the but point. The bud piece was inserted by pushing it upward under the two flaps of the rootstock cut and thereafter firmly tied with Nescofilm®. Two weeks after the grafting, for plants where the scion was viable (green), the rootstock stem was chop-down about 1 cm above the grafting, and once the scion bud began to grow the rootstock stem above de scion was completely removed. Grafted plants were maintained in a shade house (50%) with fog irrigation twice a day for 2 min each. The experiment consisted of a one-way factor with two treatments (Cleft and T-budding minigrafts) and 100 replicates per treatment for a total of 300 experimental units, which were distributed with a completely randomized design. Six weeks after the grafting, for each treatment, the number of successfully grafted plants, the average length of the grafted scion shoot, and the average number of fully expanded leaves were registered and analyzed with a T test (α=0.05).

RESULTS AND DISCUSSION

Minigrafting and plant growth

The success of grafting was observed in plants propagated using cleft and T-budding minigrafts (Figure 1). Valencia orange plants propagated with cleft minigraft showed a 75% success while plants propagated by T-budding minigraft resulted in 38% success; the T-test showed that the number of successful cleft minigrafts was statically higher (P=7.77×10^{-6}) than the number of T-budding successful minigraft. In the same way, plants propagated using cleft minigraft showed a significant (P=0.0483) increase in shoot height, and a statistically (P=0.0005) higher number of leaves, compared to plants propagated using T-budding minigrafts (Table 1). Grafted recovered plants showed no morphological abnormality or deficient growth during the evaluation period.

Grafting is a plant propagation technique used for centuries, especially on evergreen plants (Barón *et al.*,

Figure 1. Grafted plants of "Valencia" orange by cleft (Left) and T-budding (Right) minigrafts.

Table 1. Percentage of success, shoot height and leaf number of Valencia orange plants propagated using T-budding and cleft minigrafts.

Minigraft	Number	Succeeded	Success (%)	Shoot height (cm)	Leaves (Number)
T-budding	300	114	38 b	1.62 b	2.56 b
Cleft	300	225	75 a	2.80 a	4.20 b

*Numbers with the same letter are not different according to T (Student) test (α=0.05).

2019). Massive propagation of citrus plants for crops is based on T-budding 40-50 cm rootstocks with scion buds of specific cultivars, in an 18-24 months process to obtain plant material ready to plant in field crops, after mother plants have been carefully selected (Kamanga *et al*., 2017). Attempts to speed up the process include the use of tissue culture techniques such as rootstock production through micropropagation (Vashisth *et al*., 2020) and *in vitro* micrografting; however, low multiplication rates and time for *ex vitro* plantlet recovery are still a challenge (Chamandoosti, 2020; Sangma *et al*., 2020). The success of grafting is founded on a vascular reconnection between rootstock and scions, a process that involves hormones, molecular factors, and even whole genome transfer at the grafting area (Rasool *et al*., 2020; Gautier *et al*., 2019). Xylem tissue formations, callus proliferation at the graft union, and vascular bundles fiber growth are reported to be regulated by auxins, cytokines, and gibberellins during graft formation (Sharma and Zheng, 2019). An increased accumulation of stilbene metabolites at the graft union as a result of a re programming of the metabolome at the graft interface to support wounding stress, callus cell proliferation and the healing process were observed when grafting grapevine plants (Prodhomme *et al*., 2019). Therefore, tissue regeneration that supports grafting healing, decreases with plant aging due to a lack, or reduced, expression of several transcription factors that promote the expression of products, especially auxins, that contribute to callus formation at the wound site (Ibañez *et al*., 2020).

Demand for citrus plants for orchard plantation is increased worldwide due to difficulties to comply with strict regulations implemented to avoid the spread of diseases or for replanting dead or declining trees (Bhandari *et al*., 2021). In Colombia, legal measures enforce that mother plants to provide bud and scions must be isolated with anti-aphid fabric mesh to avoid incidence of pests (ICA, 2019). These measures significantly increase the cost of plant production leaving middle and small plant propagation operations out of business. Evidence of this situation is the ICA database of nurseries where in the Córdoba department appears only one nursery reported in 2021 (ICA, 2021), indicating that new citrus crops in the area are established with non-locally produced plant material. A lower disease incidence and better field performance are usually reported when crops are planted with locally produced grafted plants (Ramírez-Jiménez *et al*., 2020; Noor *et al*., 2019). Minigrafting has been used to accelerate plant propagation in fruit species (Belmonte-Ureña *et al*., 2020), for the diagnosis of plant diseases (Spano *et al*., 2020), as a strategy for molecular biology studies (Bartusch *et al*., 2020; Tsaballa *et al*., 2021) and as a mechanism for somatic embryos rescue (Raharjo and Litz, 2005). The use of minigraft in the propagation of citrus cultivars has not been previously reported. In the present study, it was observed that propagation of Valencia orange using cleft and T-budding minigraft was possible, with a 75 and 38% of success, respectively; a higher percentage of success in cleft grafting could be the result of a better contact of cambial tissues of the rootstock and the scion compared to the contact between the inserted bud and the inner layer of the rootstock cortex in T-budding; however histological analysis is recommended to completely guarantee it. Percentages of success obtained from this study are prone to increase with some adjustments when massive propagation is implemented. The minigraft technique may provide nurseries and propagators with a suitable mechanism to propagate citrus plants at the local level, reduce costs for mother plant maintenance, and speed up the propagation process by using younger rootstocks; however, it is recommended to evaluate the performance of propagated plants at the nursery and field crop level.

CONCLUSION

The results of the present study of the propagation of "Valencia" orange plants using Cleft minigraft showed a higher percentage of success compared to T-budding, and the plants recovered by this method developed a significant higher number of shoots than plants propagated by T-budding. Cleft minigraft is a viable alternative for plant propagators to produce grafted citrus plants while complying with official regulations.

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Assessing the phenotypic variation, heritability and genetic advance in bread wheat (*Triticum aestivum* L.) candidate lines grown under rainfed semi-arid region of Algeria

UNIVERSIDAD NACIONAL DE COLOMBIA

Evaluación de la variación fenotípica, la heredabilidad y el avance genético en líneas candidatas de trigo harinero (*Triticum aestivum* L.) cultivadas en una región semiárida de secano de Argelia

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ABSTRACT

The genetic improvement of any breeding population largely depends upon the magnitude of genetic variability present. This study was carried out to estimate parameters of the genetic variation among 13 quantitative traits of bread wheat evaluated at INRAA-Setif institute (Algeria) during the 2020–2021 crop season in a rainfed environment. 34 genotypes including four control checks were planted in a randomized complete block design with three replications. Genotypes showed significant variations for almost all the studied traits. Proline content, spikes weight, and grain yield exhibited a high genotypic coefficient of variation along with moderate to high heritability coupled with a high genetic gain, suggesting the implication of additive gene action. The number of spikes, spikes weight, and thousand kernel weight were significantly and positively correlated with grain yield at both phenotypic and genotypic levels. Path analysis results showed that spikes weight is an important route through which most of the measured traits influenced indirectly grain yield. Lines L1, L20, L28, L16, and L18 exhibited a sizeable grain yield advantage, which suggests they are potential candidates for future release and could be incorporated into the wheat breeding programs as parents to improve yield in the rainfed environments of Algeria.

Keywords:
Drought
Genetic correlation
Genetic determinism
Grain yield
Path analysis
Triticum aestivum

RESUMEN

La mejora genética de cualquier población reproductora depende en gran medida de la magnitud de la variabilidad genética presente. Este estudio se llevó a cabo para estimar parámetros de la variación genética entre 13 caracteres cuantitativos de trigo harinero evaluados en el instituto INRAA-Setif (Argelia) durante la temporada de cultivo 2020-2021 en ambiente de secano. 34 genotipos, incluidos cuatro testigos de control, se sembraron en un diseño de bloques completos al azar con tres repeticiones. Los genotipos mostraron variaciones significativas para casi todas las características estudiadas. El contenido de prolina, el peso de las espigas y el rendimiento de grano exhibieron un alto coeficiente de variación genotípico junto con una heredabilidad de moderada a alta junto con una alta ganancia genética, lo que sugiere la implicación de una acción génica aditiva. El número de espigas, el peso de las espigas y el peso de mil granos se correlacionaron significativa y positivamente con el rendimiento de grano tanto a nivel fenotípico como genotípico. Los resultados del análisis de ruta mostraron que el peso de las espigas resultó ser una ruta importante a través de la cual la mayoría de los rasgos medidos influyeron indirectamente en el rendimiento del grano. Las líneas L1, L20, L28, L16 y L18 exhibieron una ventaja considerable en el rendimiento de grano, lo que sugiere que son candidatas potenciales para lanzamientos futuros y podrían incorporarse en el programa de mejoramiento de trigo como progenitores para mejorar el rendimiento en ambientes de secano de Argelia.

Palabras clave:
Sequía
Correlación genética
Determinismo genético
Rendimiento de grano
Análisis de ruta
Triticum aestivum

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Among cereal small grains that dominate the global cultivated area in Algeria, wheat (*Triticum* sp) is the backbone that forms the diet of the local population (Fellahi, 2017). According to the Food and Agriculture Organization (FAOSTAT, 2021), this genus is annually grown in ~2 million hectares with a total production quantity of ~3.9 million t. Even though the county has made great progress in terms of grain production during the last decades, particularly in durum wheat (*Triticum durum* Desf.) species, the national production of bread wheat (*Triticum aestivum* L.) is still far to satisfy the demands of an overgrowing population. In such circumstances, the government still resorts to importing large quantities of wheat with substantial purchases.

Bread wheat is basically grown under rainfed conditions in arid and semi-arid regions of Algeria where drought stress represents the most growth-limiting factor of field crops. This is in addition to subsidiary factors that may restrict its productivity such as the other abiotic (frost, heat, salinity) and biotic stresses (diseases and pests), and technical difficulties related to crop management (irrigation and machinery) (Fellahi, 2017). Drought is a polygenic complex trait that occurs when water loss from the plant surpasses the ability of the plant's roots to absorb water (Mwadzingeni *et al*., 2016). This stress ranks first when compared to other stresses referring to the damages that can occur. Drought affects almost all parts of the plant, which causes serious changes at morphological, physiological, biochemical, and molecular levels (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). These modifications depend both on the severity and the duration of the stress as well as the growth stage of the plant (Araus *et al*., 2002). During the early growth stages, water stress limits the germinability of wheat grain and reduces seedling vigor (Boudiar *et al*., 2019). Likewise, during the flowering and grain filling periods, drought decreases the number of fertile tillers, spikes fertility, grain weight, above-ground biomass, and therefore, grain yield (Pour-Aboughadareh *et al*., 2020).

Improving wheat yield under water stress conditions is a big challenge faced by wheat breeders who are being asked to select potential varieties to satisfy the present and future demands of farmers and consumers. Various traits have been used in wheat breeding programs as indirect selection criteria to improve drought tolerance in a combination with high yield (Chowdhury *et al*., 2021). It was found by Awan *et al.* (2015) that relative water content might be an important trait in screening wheat genotypes for water stress tolerance. Under stressful conditions, water use efficiency demonstrates a negative correlation with transpiration rate and positive associations with photosynthetic rate, relative water content, and stomatal conductance (Sallam *et al*., 2019). An increase of proline content in the leaves may lessen water loss and protect wheat plants against water deficit (Mwadzingeni *et al.,* 2016). According to Awan *et al.* (2015), the most tolerant genotypes possess the capacity to maintain membrane integrity under drought. Selection based on these traits of interest can improve genetic gains for yield and its components as well as drought tolerance enhancement. Understanding the magnitude of genetic variation for desirable secondary traits is important for a successful plant breeding program which enables a breeder to know to what extent the environment affects grain yield (Ene *et al*., 2016). Such good variation for wheat-assessed traits will allow a choice of suitable selection criteria and identification of promising genotypes for breeding and product development purposes (Ngwepe *et al*., 2021). In light of the above background, this study aimed to investigate the amount of genetic variability, heritability, genetic advance, and the association among yield and yield-related traits in advanced wheat (*Triticum aestivum* L.) breeding lines evaluated under semi-arid growth conditions.

MATERIALS AND METHODS

Site, plant materials, and experimental design

The experiment was carried out at the experimental field of the National Agronomic Research Institute of Algeria (INRAA), a research unit of Setif (36°09′ N; 05°22′ E; 981 masl) under rainfed conditions during the 2020–2021 crop season. The plant material consisted of 34 bread wheat genotypes including 30 biparental advanced breeding lines and four control checks named Mawna, Hidhab, Rmada, and $Acsad_{1135}$ (Table 1). The first three control checks are released varieties that are widely cultivated in Algeria (Fellahi, 2017). The experiment was set up on December 22, 2020, in a randomized complete block design (RCBD) with three replications using an experimental seeder type Hege 80. The wheat crop cycle extended from December 22, 2020 to June 30, 2021. All genotypes were headed during the first third of May.

Table 1. Code, name/pedigree, and origin of investigated bread wheat genotypes.

No	Code	Genotype#	Origin	No	Code	Genotype#	Origin
1	L1	$Acsad_{1135}$ × Rmada	INRAA	18	L15	Ain Abid × Hidhab	INRAA
2	**C1**	**Mawna**	ACSAD	19	L16	Mawna × Rmada	INRAA
3	L2	Djanet × Hidhab	INRAA	20	L17	Ain Abid × Rmada	INRAA
4	L3	$Acsad_{1069}$ × Hidhab	INRAA	21	L18	$Acsad_{1069}$ × Hidhab	INRAA
5	**C2**	**Hidhab**	CIMMYT	22	L19	$Acsad_{1069}$ × Hidhab	INRAA
6	L4	$Acsad_{1069}$ × EL-Wifak	INRAA	23	L20	$Acsad_{1135}$ × Mahon-Demias	INRAA
7	L5	Djanet × Rmada	INRAA	24	L21	Mawna × Mahon-Demias	INRAA
8	L6	Djanet × Hidhab	INRAA	25	L22	Ain Abid × Hidhab	INRAA
9	L7	Djanet × EL-Wifak	INRAA	26	L23	Djanet × Rmada	INRAA
10	L8	Djanet × EL-Wifak	INRAA	27	L24	$Acsad_{1069}$ × EL-Wifak	INRAA
11	L9	$Acsad_{1069}$ × EL-Wifak	INRAA	28	L25	Djanet × Rmada	INRAA
12	L10	Ain Abid × EL-Wifak	INRAA	29	L26	Ain Abid × Rmada	INRAA
13	L11	$Acsad_{1069}$ × EL-Wifak	INRAA	30	L27	$Acsad_{1135}$ × Rmada	INRAA
14	L12	$Acsad_{1069}$ × Rmada	INRAA	31	L28	$Acsad_{1069}$ × Hidhab	INRAA
15	L13	$Acsad_{1069}$ × Mahon-Demias	INRAA	32	L29	$Acsad_{1069}$ × Hidhab	INRAA
16	**C3**	**Rmada**	ACSAD	33	L30	$Acsad_{1135}$ × Rmada	INRAA
17	L14	Djanet × Rmada	INRAA	34	**C4**	**$Acsad_{1135}$**	ACSAD

Genotypes with similar pedigrees are sister lines, control checks are indicated with bold letters, INRAA: National Agronomic Research Institute of Algeria, ACSAD: Arab Center for the Study Arid Zones and Dry Lands, CIMMYT: International Maize and Wheat Improvement Center.

The plot dimension was 6 m^2, which included 6 rows, each 5 m long, spaced 0.2 m apart. The soil is calcareous (Calcisol), with a silty clay texture (44% clay, 44% silt, 12% sand) and organic matter content of 1.05% (Internal soil analysis report). The experimental plots were fertilized with 100 kg ha^{-1} of mono-ammonium phosphate (52% P_2O_5 + 12% N) before sowing and 80 kg ha^{-1} Urea (46% N) at the tillering stage. Weeds were controlled chemically and manually when necessary. Recorded monthly rainfall distribution, temperature (max, min, and mean), and relative humidity during this crop season are displayed in Table 2. The amount of monthly rainfall recorded during the 2020–2021 crop season, from September 1 to June 30, reached 320.24 mm, which is below the long-term average.

Table 2. Mean monthly rainfall, temperature, and relative humidity during the 2020-2021 crop season (Tutiempo Network, 2021).

Month	Rainfall (mm)	Temperature (°C)			Relative humidity (%)
		Min	Max	Mean	
September	25.14	13.80	26.30	19.80	55.10
October	7.12	7.30	21.20	14.20	49.70
November	66.00	6.20	16.70	8.60	66.00
December	55.10	2.00	10.20	6.00	80.90
January	16.00	1.00	11.00	6.10	67.00
February	8.13	3.30	15.70	9.50	52.10
March	44.95	3.40	14.40	9.00	59.70
April	9.91	7.10	19.50	13.30	53.10
May	81.54	11.60	25.50	18.70	48.20
June	6.35	17.80	32.60	25.40	31.10

Data recorded

A total of 13 phenological, physiological, and agronomic traits were evaluated in this experiment. Days to 50% heading (DH, days) were recorded as the number of days from January 1 until 50% of the heads in a plot. The traits relative water content (RWC, %), electrolyte leakage from injured cells (Inj, %), canopy temperature (CT, °C), proline content (PC, µg g^{-1}), leaf chlorophyll content (CC, SPAD), flag leaf area (FLA, cm^2) and specific leaf weight (SLW, mg cm^{-2}) were taken on the flag leaves. The RWC was determined at the heading according to the procedure described by Pask *et al.* (2012). Five flag leaves were collected and weighed to get the fresh weight (FW). The leaves were placed into test tubes containing 10 mL of distilled water and kept in dark for 24 h before being weighed to get the turgid weight (TW). The leaf samples were oven dried at 80 °C for 48 h to record the dry weight (DW). RWC was calculated as: RWC (%) = [(FW – DW)/(TW – DW)] × 100. The electrolyte leakage from injured cells (Inj) was performed on the fully expanded flag leaves according to Bajji *et al.* (2001). Two leaves were randomly collected for each genotype, washed with tap water then, distilled water to remove adherent dust, and cut into 1 cm length segments. Ten leaf pieces were placed in test tubes containing 10 mL of double-distilled water. The test tubes were placed at the ambient temperature of the laboratory, and periodically and manually agitated.The first reading of their electrical conductivity was recorded after 24 h (EC1) using a laboratory conductivity meter (HI 2300 EC/TDS/NaCl Meter, Hanna Instruments). The tubes were then placed in a boiling water bath at 100 °C for 1 h and their conductivity was recorded (EC2). The Inj was calculated as follows: Inj (%) = (EC1/EC2) × 100. The CT measurements were taken between 12:00 and 14:00 hours on a sunny day using a portable infrared thermometer (Fluke Corporation, Everett, WA, USA). Three measurements were taken per plot at approximately 0.5 m distance from the plot edge. The PC was determined according to the procedure described by Monneveux and Nemmar (1986). The CC was determined for three random flag leaves in each plot with a SPAD-502 chlorophyll meter (Minolta Co. Ltd., Tokyo, Japan). The FLA was determined from a 5-leaf sample. Leaf length (L) and wide (l) were measured and the area was determined according to Spagnoletti-Zeuli and Qualset (1990) using the following formulae: FLA (cm^2) = 0.607 (L × l). The specific leaf weight (SLW) was obtained after drying the leaf samples as SLW (mg cm^{-2}) = DW/FLA (Amanullah, 2015), wherein DW is the fresh weight and FLA is the flag leaf area of the sample. Plant height (PH, cm) was recorded just before harvest, from the ground to the tip of the main spike, awns excluded. Grain yield (GY, g m^{-2}), spikes weight (SW), and the number of spikes (NS) were determined according to one linear meter for each genotype and then converted to square meter. Thousand kernel weight (TKW, g) was obtained after threshing of the spikes from the mass of 250-grains sample per plot.

Statistical analysis

Data recorded for measured traits were subjected to a one-way analysis of variance (ANOVA) to test the differences among genotypes at a 5% probability level. The phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad-sense heritability (h^2_{bs}), genetic advance (GA), and genetic advance as a percentage of the mean (GAM) were calculated for each trait based on the mean square values obtained from the ANOVA according to the equations given by Johnson *et al.* (1955) and Acquaah (2012) as follow:

$$PCV = \frac{\sqrt{\sigma_p^2}}{\mu} \times 100$$

$$GCV = \frac{\sqrt{\sigma_g^2}}{\mu} \times 100$$

$$h^2_{bs} = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

$$GA = k \times \sigma_p \times h^2_{bs}$$

$$GAM = \frac{GA}{\mu} \times 100$$

Where, σ_p^2 is the phenotypic variance, σ_g^2 is the genotypic variance, μ is the grand mean of the trait and k is the efficacy of selection, which is 2.06 at 5% selection intensity.

Phenotypic and genotypic correlation coefficients for each pair of traits were computed as described by Mansouri *et al.* (2018). Path analysis based on genotypic correlation coefficients was also done to calculate the

direct and indirect path coefficients according to Dewey and Lu (1959). GY was considered a response variable, while other traits as causal/independent variables. All statistical analyses were performed using OPSTAT software.

RESULTS AND DISCUSSION

Analysis of variance, means, phenotypic and genotypic variability

In crop breeding programs aimed at producing new varieties with improved yield potential, the efficiency of selection mainly depends upon the amount of genetic variability present in the plant population. The results of ANOVA revealed highly significant differences among the studied genotypes (except for plant height), suggesting the presence of a considerable amount of variability among the plant material investigated (Table 3). Bendjama and Ramdani (2022) reported the existence of genetic variation among wheat genotypes. The same findings were shown for other crops from the same family such as durum wheat (*Triticum durum* Desf.) (Mansouri *et al*., 2018), barley (Hordeum vulgare L.) (Dinsa *et al*., 2018), and rice (*Oryza sativa* L.) (Tiwari *et al*., 2019) regarding various traits including CC, PC, days to heading, days to maturity, PH, spike length, NS, number of grains per spike, GY, TKW, and harvest index.

Table 3. Analysis of variance (ANOVA) for measured traits in evaluated bread wheat genotypes.

Traits	Source of variation		
	Replication (df=2)	Genotype (df=33)	Error (df=96)
Date to heading	2.72	7.40**	0.72
Relative water content	22.63	18.60*	10.20
Cell injury	0.24	27.49**	4.58
Canopy temperature	16.68	3.88**	1.53
Proline content	4.69	4.57**	1.16
Chlorophyll content	7.98	17.18**	6.49
Flag leaf area	3.86	5.80**	1.35
Specific leaf weight	0.07	0.60**	0.15
Plant height	107.60	31.02^{ns}	23.93
Number of spikes m^{-2}	2.93	5.80**	2.63
Spikes weight	663.97	5.34**	1.03
Thousand kernel weight	85.54	44.68*	25.19
Grain yield	329.23	939.05**	331.88

ns, * and **: non-significant and significant effects at 5% and 1% probability levels, respectively. df= degree of freedom.

Estimates of means and ranges of average values observed for wheat traits evaluated are presented in Table 4. Mean values for days to heading varied from 123.00 (L7, L11, L24, L26, L27) to 129.16 (L16) with an overall mean of 124.42 days. For RWC, these figures were 67.19 (L27) and 76.72% (L10). The min, max, and average values of cell injury, were 85.00 (L22), 97.13 (L8), and 92.22%, respectively; of CT were 25.87 (L18), 30.23 (L1), and 27.82 °C; of PC were 5.52 (L12), 178.91 (L3), and 62.43 µg g^{-1}; and CC varied from 41.53 (L6) to 49.83 (C4) with an overall mean of 45.65 SPAD. The mean values for FLA were 9.76 (L25) to 15.30 (L17) and 11.93 cm^2. For specific leaf weight, minimum, maximum, and average values were 5.12 (L28), 6.81 (L8,) and 5.88 g cm^{-2}, respectively. Similarly, PH mean values were 38.67 (L30), 52.67 (L10), and 44.73 cm. These values were 123.33 (C4), 371.67 (L1), and 179.04 spikes for the number of spikes m^{-2}; 53.33 (L7), 308.33 (L1), and 96.47 g m^{-2} for SW; 20.00 (L5, L15, L22, C2), 33.33 (L1, L21) and 26.96 g for TKW; mean values characterizing GY values were 25.00 (L7), 120.00 (L1) and 47.72 g m^{-2} (Table 4).

Table 4. Estimation of means, ranges, phenotypic coefficient of variation, genotypic coefficient of variation, broad-sense heritability, genetic advance, and genetic advance as percent of mean of measured traits in bread wheat genotypes.

Traits	Mean	Range	PCV (%)	GCV (%)	h^2_{bs} (%)	GA	GAM
DH	124.42	123.00–129.33	1.37	1.20	75.69	2.68	2.15
RWC	72.99	67.19–76.72	4.94	2.29	21.54	1.60	2.19
Inj	92.22	85.00–97.13	3.79	2.99	62.49	4.50	4.88
CT	27.82	25.87–30.23	5.47	3.18	33.93	1.06	3.82
PC	62.43	5.52–178.91	76.74	54.01	49.54	48.88	78.30
CC	45.65	41.53–49.83	6.94	4.14	35.45	2.32	5.07
FLA	11.93	9.76–15.30	14.11	10.21	52.35	1.82	15.22
SLW	5.88	5.12–6.81	9.30	6.54	49.49	0.56	9.48
PH	44.73	38.67–52.67	11.47	3.44	8.99	0.95	2.12
NS	179.04	123.3–371.67	33.91	18.17	28.72	35.92	20.06
SW	96.47	53.33–308.33	51.45	39.29	58.31	59.62	61.79
TKW	26.96	20.00–33.33	20.88	9.46	20.51	2.38	8.82
GY	47.72	25.00–120.00	48.44	29.81	37.88	18.04	37.79

DH: Days to heading (days), RWC: Relative water content (%), Inj: Injured cells (%), CT: Canopy temperature (°C), PC: Proline content (µg g^{-1}), CC: Chlorophyll content (SPAD), FLA: Flag leaf area (cm^2) SLW: specific leaf weight (mg cm^{-2}), PH: Plant height (cm), NS: Number of spikes m^{-2} (Spikes m^{-2}), SW: Spikes weight (g), TKW: Thousand kernel weight (g), GY: Grain yield (g m^{-2}), PCV: Phenotypic coefficient of variation, GCV: genotypic coefficient of variation, h^2_{bs}: Broad-sense heritability, GA: Genetic advance, GAM: Genetic advance as percentage of the mean.

According to the presented results, it was observed that among all the material studied, line L1, showed elevated values for all the agronomic traits such as the NS, SW, TKW, and GY that give opportunities for wheat breeders to exploit its potential for breeding purposes. Lines L20 and L28, and to a lesser extent L16 and L18 also appeared as desirable entries as they exhibited better yield performances when compared to other wheat genotypes including control checks (Figure 1). These five promising lines also surpassed the rest of the lines and check varieties for other desirable traits such as NS, SW, and TKW; and proved better than control checks for stress tolerance-related traits including RWC, Inj, and CT.

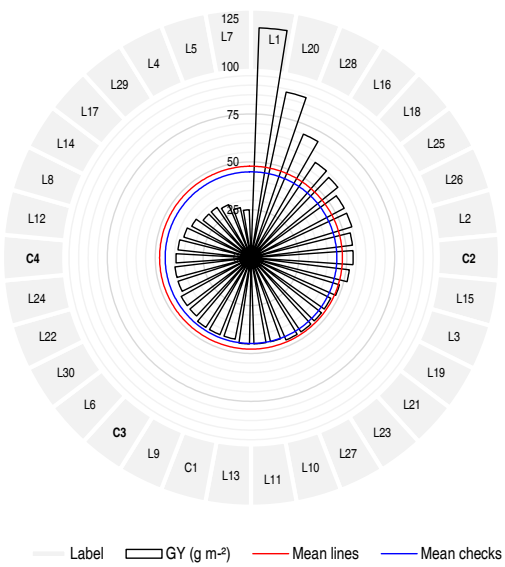

Figure 1. Mean values for grain yield (g m^{-2}) of bread wheat genotypes.

Studying heritable and non-heritable factors in the total variability facilitates the breeding procedure, giving meaningful and specific details about the population under evaluation. The estimates of the phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad-sense heritability (h^2_{bs}), genetic advance (GA), and genetic advance as percentage of mean (GAM) for 13 assessed traits are given in Table 4. The PCV ranged from 1.37 to 76.74%, whereas, the GCV ranged from 1.20 to 54.01%. PCV and GCV estimates were categorized as low (0–10%), moderate (10–20%), and high (>20%) values as indicated by Sivasubramanian and Menon (1973). The highest PCV was observed in PC (76.74%), followed by SW (51.45%), GY (48.44%), number of spikes (33.91%), and TKW (20.88%). Moderate values of PCV were obtained in FLA (14.11%) and PH (11.47%), and low PCVs were found in the remaining recorded traits. The estimates of GCV were high for PC (54.01%), SW (39.29%), and GY (29.81%); moderate for the number of spikes per unit area (18.17%) and FLA (10.21%); and low for the rest of the attributes suggesting small exploitable genetic variability and less potential for favorable advance in selection. These results agree with the conclusions of Mekaoussi *et al.* (2021) who reported a relatively low genotypic variability among physiological traits such as RWC, flag leaf CC, CT, and membrane stability index assessed in a set of 25 durum wheat genotypes under south Mediterranean conditions. These authors demonstrated high environmental coefficients of variation (CV_e) and explained their results by the lack of precision and/or the environmental effects on the expression of recorded traits. Among the yield attributing traits, the PCV values were, in general, greater than the GCV, which reflects the influence of the environment in the expression of these evaluated characteristics. These findings were in agreement with those of Din *et al.* (2018) and Regmi *et al.* (2021).

According to Regmi *et al.* (2021), PCV and GCV only indicate the amount of total variability available in an assessed trait and do not split it into heritable (genotypic variance) and non-heritable (environmental variance) fractions. Thus, the heritability parameter is largely used to separate genetic and environmental components of this phenotypic variation. Under this context, breeders try to minimize the influence of the environment as much as possible to identify the genes of interest for their efficient performance on agronomic traits. Moreover, estimates of heritability have a role to play in determining the effectiveness of selection for a such trait. They were previously classified as low (0–30%), moderate (30–60%), and high (>60%) values as indicated by Robinson *et al.* (1949). The highest heritability (h^2_{bs}) estimated was exhibited by days to heading (75.69%) followed by cell injury (62.49%), indicating a higher contribution of the genotype in the expression of the phenotype rather than the environment. These results are consistent with the finding reported by Farooq *et al.* (2011) who also revealed high heritability for days to heading and relative cell injury percentage in wheat under both normal and heat stress conditions, suggesting that selection for these traits is highly desirable. Moderate heritability estimates were observed for CT (33.93%), PC (49.54%), CC (35.45%), FLA (52.35%), SLW (49.49%), SW (58.31%) and GY (37.88%). The lowest heritability values were found in RWC, PH, NS m^{-2}, and TKW with estimates below 30%. Low heritability can be explained by the high environmental influence over the expression of these traits and therefore, selection will not be worthwhile (Acquaah, 2012).

Genetic advance as percentage of mean (GAM) indicates the predicted genetic gain for a particular trait under selection cycles and measures the extent of its stability under selection intensity. This parameter depends upon the genetic coefficient of variation and heritability and selection differential. Johnson *et al.* (1955) categorized the GAM into low (0–10%), moderate (10–20%), and high (>20%). The greatest GAM of 78.30 was observed in PC followed by 61.79 in SW, 37.79 in GY, and 20.06 in NS m^{-2}. GAM was found just intermediate in the FLA (15.22) and low in the remaining traits. The obtained results are consistent with those of Seyoum and Sisay (2021) for PH, Hossain *et al.* (2021) for CT, Fellahi *et al.* (2019) for heading date and FLA, Din *et al.* (2018) for TKW, and Regmi *et al.* (2021) for the number of fertile tillers and GY.

Heritability in conjunction with genetic advances was reported to be more useful than heritability value alone in selecting individuals with desired characteristics (Johnson *et al.*, 1955). Accordingly, high heritability (75.69 and 62.49%) coupled with low GAM (2.15

and 4.88) were recorded in the days to heading and cell injury, respectively. As a result, it appears that these characteristics were primarily influenced by the interaction of genetic and environmental factors. Thus, it is not feasible to select wheat individuals based on the genotypic values of these two recorded traits. Similar findings have been reported in wheat by Fellahi *et al.* (2013) and Hossain *et al.* (2021). PC, SW, and GY showed moderate heritability (49.54, 58.31, and 37.88%) coupled with high GAM (78.30, 61.79, and 37.79), respectively. This reflects the additive gene action in the expression of these traits and selection could bring desirable changes over the population mean. These findings corroborate earlier results stated by Regmi *et al.* (2021) for yield attributing traits including grain weight per spike, thousand-grain weight, GY, and harvest index. In the FLA moderate heritability (52.35%) coupled with moderate GAM (15.22) but with low genetic advance (GA) of 1.82% were observed. This finding implies that FLA-based selection would not be successful. Fellahi *et al.* (2019) also found similar results for this morphological trait in F_2 bread wheat progenies. Moderate heritability (33.93, 35.45, and 49.49%) accompanied by low GAM (3.82, 5.07, and 9.48) was explored by CT, CC, and SLW, respectively. A low heritability estimate (28.72%) joined with high GAM (20.06) was observed for NS m^{-2} and low heritability and its simultaneous GAM were recorded in RWC (21.54% and 2.19), PH (8.99% and 2.12), and TKW (20.51% and 8.82) reflect the occurrence of non-additive gene effect and high influence of the environment in the expression of these traits. Thus, selection based on these traits would not be desirable. This finding agrees with the report of Salmi *et al.* (2019) who found that expected responses to selection were low for RWC and just moderate for PH and TKW.

Phenotypic and genotypic correlations among assessed traits

As a result of the substantial and positive correlation between the traits, selection for one feature would have a direct impact on the expression of another trait, facilitating selection and advancement in the breeding program. The degree and direction of the relationships between assessed traits are measured by correlation coefficient analysis. Phenotypic (r_p) and genotypic (r_g) correlation coefficients among wheat recorded traits in this study are given in Table 5. Days to heading possessed a negative and significant correlation with SLW and TKW at phenotypic (r_p=–0.272* and r_p=–0.215*) and genotypic (r_g=–0.382* and r_g=–0.660*) levels. DH also showed significant genotypic associations, negative with GY (r_g=-0.208*), and positive with CT (r_g=0.310*), PC (r_g=0.201*) and PH (r_g=0.247*). The correlation of RWC with NS m^{-2} (r_p=0.283* and r_g=0.726*) was found positive and significant at both levels. However, RWC had highly significant genetic correlations with CT (r_g=–0.259*), PC (r_g=0.466*), CC (r_g=–0.300*), FLA (r_g=–0.687*), PH (r_g=–0.658*), SW (r_g=0.441*) and GY (r_g=0.315*). On the contrary, it revealed a non-significant phenotypic correlation with these traits as presented in Table 5.

Correlation of cell injury with FLA (r_g=–0.302*) and PH (r_g=–0.375*) at the genotypic level was found significant and negative. However, their respective phenotypic correlations were observed negative but non-significant. Similarly, CT revealed significant and positive phenotypic (r_p=0.262*) and genotypic correlation (r_g=0.424*) with FLA. Besides this, relation of this trait with PC (r_g=0.243*), SLW (r_g=–0.268*), PH (r_g=–0.236*), NS (r_g=0.451*), SW (r_g=432*), TKW (r_g=266*) and GY (r_g=0.439*) at genotypic level was found significant with different directions. PC demonstrated positive genotypic relationship with NS (r_g=0.429*) and SW (r_g=0.220*); whereas CC exhibited positive phenotypic association with TKW (r_p=0.215*) and significant correlations at genotypic level, negative with FLA (r_g=–0.389*) and positive with PH (r_g=0.322*). There was a significant and positive correlation between FLA and SLW (r_p=0.259* and r_g=0.432*). However, only genotypic correlation was observed as significant and negative with TKW (r_g=–0.302*). The genotypic correlation between SLW and PH was negative and significant (r_g=–0.758*). This last trait also presented significant genotypic relations, negative with TKW (r_g=–0.266*) and positive with GY (r_g=0.522*). In addition to its significant negative relationship with days to heading and positive associations with RWC, CT, and PH, GY demonstrated positive and highly significant correlations with NS, SW, and TKW at phenotypic (r_p=0.692*, r_p=0.849* and r_p=0.257*) and genotypic (r_g=0.972*, r_g=1.000* and r_g=0.457*) levels, respectively. NS also exhibited positive and significant phenotypic and genotypic

correlations with SW (r_p=0.874* and r_g=0.994*) and TKW (r_p=0.219* and r_g=0.332*). Besides, these last two traits registered a strong positive association with each other in both phenotypic (r_p=0.265*) and genotypic (r_g=0.298*) levels. These results are consistent with the previous findings by Fellahi *et al.* (2013) who reported that GY showed positive and significant correlations, positive with CC, PH, spike length NS plant^{-1}, TKW, and harvest index, and negative with days to heading and number of grain per spike at both phenotypic and genotypic levels. Simultaneous improvement of all these yield component traits is therefore feasible. The highly significant and negative correlation observed between days to heading and GY indicated that selection of earliness would be a very crucial key for the enhancement of yield under rainfed conditions among the plant material under selection. Mansouri *et al.* (2018) also encouraged this selection approach since early genotypes selected showed above-average spikes in number, grain, and biological yields and greatly avoided terminal heat stress. Improvements in RWC and PH will lead to an increase in the GY. The above statement is fully supported by the findings of Al-Ashkar *et al.* (2021) describing the situation that selection based on RWC along with CT, green leaves area, and leaf area index might improve genetic gain for GY in stressful environments.

Table 5. Phenotypic (lower triangle) and genotypic (upper triangle) correlation coefficients among measured traits in evaluated bread wheat genotypes.

Traits	DH	RWC	Inj	CT	PC	CC	FLA	SLW	PH	NS	SW	TKW	GY
DH		0.176	0.159	0.310	0.201	–0.127	0.103	–0.382	0.247	–0.056	–0.106	–0.660	–0.208
RWC	0.014		0.064	–0.259	0.466	–0.300	–0.687	0.178	–0.658	0.726	0.441	–0.184	0.315
Inj	0.106	0.091		0.093	–0.109	–0.175	–0.302	0.151	–0.375	–0.140	–0.013	–0.086	0.059
CT	0.069	–0.058	–0.050		0.243	–0.094	0.424	–0.268	–0.236	0.451	0.432	0.266	0.439
PC	0.110	0.026	–0.012	0.025		0.098	–0.070	–0.170	0.039	0.429	0.220	0.128	0.042
CC	–0.077	0.048	–0.163	0.031	0.160		–0.389	0.011	0.322	0.156	0.110	0.106	0.057
FLA	–0.010	–0.002	–0.126	0.262	–0.073	–0.029		0.432	–0.024	–0.108	–0.006	–0.302	–0.060
SLW	–0.272	0.114	0.149	–0.070	–0.018	0.074	0.259		–0.758	0.032	0.052	–0.083	–0.149
PH	0.058	–0.013	–0.144	–0.163	–0.091	–0.039	–0.030	–0.142		–0.063	0.186	–0.266	0.522
NS	–0.052	0.283	0.052	0.115	0.188	0.017	–0.024	0.015	–0.018		0.994	0.332	0.972
SW	–0.077	0.190	0.057	0.180	0.138	0.086	0.052	0.043	–0.048	0.874		0.298	1.000
TKW	–0.215	–0.089	–0.040	0.009	0.121	0.215	–0.060	–0.006	–0.126	0.219	0.265		0.457
GY	–0.100	0.026	0.100	0.106	0.043	0.140	–0.079	–0.021	–0.027	0.692	0.849	0.257	

DH: Days to heading (days), RWC: Relative water content (%), Inj: Injured cells (%), CT: Canopy temperature (°C), PC: Proline content (µg g^{-1}), CC: Chlorophyll content (SPAD), FLA: Flag leaf area (cm^2) SLW: specific leaf weight (mg cm^{-2}), PH: Plant height (cm), NS: Number of spikes m^{-2} (Spikes m^{-2}), SW: Spikes weight (g), TKW: Thousand kernel weight (g), GY: Grain yield (g m^{-2}), Significant correlation coefficients at 5% probability level are indicated with bold numbers.

Path analysis

Path coefficient analysis subdivides the correlation coefficient into direct and indirect effects, which allows determining which attribute influences substantially the dependent variable. The direct and indirect effects of evaluated phenotypic traits on GY as a response variable are presented in Table 6. TKW (0.130) had the highest positive direct effects on GY. On the other hand, the strongest negative direct effect was exhibited by NS m^{-2} (–0.538) followed by RWC (–0.167), CC (–0.159), SLW (–0.156), CT (–0.135) and FLA (–0.135). Conversely, the causal variables days to heading, cell injury, and PH showed negligible direct effects on yield.

RWC (0.743) followed by CT (0.729), TKW (0.503), PC (0.372), PH (0.314) and CC (0.185) showed important and positive indirect effects on GY through SW. The indirect effect of days to heading on yield through SW was negative (–0.179). In addition, SW (–0.535), PC (–0.231), and TKW (–0.178) had consequent negative

indirect effects on GY by total NS m^{-2}. FLA and PH had high significant indirect positive effects of 0.115 and 0.110 on GY, respectively, through RWC, whereas NS m^{-2} acted on yield negatively (–0.121) through it. PH depicted a high indirect positive effect of 0.118 on GY through SLW. The residual effect appeared to be non-negligible (–0.138), indicating that other possible independent/causal variables (secondary traits) which were not included in the study could significantly influence the dependent variable (main trait or yield).

Table 6. Direct (diagonal) and indirect (off-diagonal) genotypic effects of yield contributing traits on grain yield of wheat genotypes evaluated.

Traits	DH	RWC	Inj	CT	PC	CC	FLA	SLW	PH	NS	SW	TKW	r_g
DH	**0.050**	–0.029	–0.004	–0.042	–0.008	0.020	–0.014	0.059	–0.006	0.030	–0.179	–0.086	**–0.208**
RWC	0.009	**–0.167**	–0.002	0.035	–0.018	0.048	0.093	–0.028	0.015	–0.390	0.743	–0.024	**0.315**
Inj	0.008	–0.011	**–0.025**	–0.013	0.004	0.028	0.041	–0.023	0.009	0.075	–0.023	–0.011	0.059
CT	0.016	0.043	–0.002	**–0.135**	–0.009	0.015	–0.057	0.042	0.006	–0.242	0.729	0.035	**0.439**
PC	0.010	–0.078	0.003	–0.033	**–0.038**	–0.016	0.010	0.026	–0.001	–0.231	0.372	0.017	0.042
CC	–0.006	0.050	0.004	0.013	–0.004	**–0.159**	0.053	–0.002	–0.008	–0.084	0.185	0.014	0.057
FLA	0.005	0.115	0.008	–0.057	0.003	0.062	**–0.135**	–0.067	0.001	0.058	–0.011	–0.039	–0.060
SLW	–0.019	–0.030	–0.004	0.036	0.006	–0.002	–0.059	**–0.156**	0.018	–0.017	0.087	–0.011	–0.149
PH	0.012	0.110	0.009	0.032	–0.002	–0.051	0.003	0.118	**–0.024**	0.034	0.314	–0.035	**0.522**
NS	–0.003	–0.121	0.004	–0.061	–0.016	–0.025	0.015	–0.005	0.001	**–0.538**	#	0.043	**0.972**
SW	–0.005	–0.073	0.000	–0.058	–0.008	–0.017	0.001	–0.008	–0.004	–0.535	#	0.039	**1.000**
TKW	–0.033	0.031	0.002	–0.036	–0.005	–0.017	0.041	0.013	0.006	–0.178	0.503	**0.130**	**0.457**

DH: Days to heading (days), RWC: Relative water content (%), Inj: Injured cells (%), CT: Canopy temperature (°C), PC: Proline content (µg g^{-1}), CC: Chlorophyll content (SPAD), FLA: Flag leaf area (cm^2) SLW: specific leaf weight (mg cm^{-2}), PH: Plant height (cm), NS: Number of spikes m^{-2} (Spikes m^{-2}), SW: Spikes weight (g), TKW: Thousand kernel weight (g), GY: Grain yield (g m^{-2}), r_g: genotypic correlation coefficient of grain yield with other assessed traits, Residual effect = –0.138, # denote the value of direct/indirect path coefficient over 1.

Path analysis procedure was largely used by researchers in wheat breeding under various growth conditions. According to Mohammadi *et al.* (2012), PH, days to maturity, agronomic score, and TKW exerted the highest positive direct effect on GY and could be reliable selection criteria for selection under drought tolerance conditions. Shamsi *et al.* (2011) revealed that TKW was the most important yield component, which agrees with the results of the present study. Breeding to enhance the potential yield of wheat genotypes through this trait has been reported as a promising approach both in dryland and irrigated conditions (Mohammadi *et al.*, 2012). In their studies, Rabti *et al.* (2020) and Mekaoussi *et al.* (2021) showed that the GY of durum wheat depended on biomass, NS, TKW, number of kernels per spike, and harvest index under rainfed environments. Their results also pointed out that physiological traits had negligible direct effects and small indirect effects on GY by biomass, NS, and harvest index. But the morpho-agronomic traits measured did not express any notable indirect effects on GY through physiological traits which are in agreement with the findings of the present investigation.

CONCLUSIONS

Mean squares for all measured agronomic traits, PH excluded, showed significant differences among all bread wheat genotypes evaluated. The estimated PCV values were high for PC, SW, NS, TKW, and GY. The remaining traits recorded moderate to low PCV estimates. Similarly, GCV values were high for PC, SW, and GY; and moderate to low for the rest of the measured variables. The PCV values were higher than GCV estimates for all traits studied. It was found that PC, SW, and GY had moderate to high heritability along with high genetic advance as a percent of mean together with wide genetic variation and lower environmental influence indicating the involvement of additive genetic effects in the inheritance of these traits which might be effective for the selection under stressful environment. Under these growth conditions, positive and highly significant

correlations were found both in phenotypic and genotypic levels between GY and NS, SW, and TKW. Moreover, a high positive direct effect at the genotypic level was exhibited by the characters SW and TKW. The highest positive indirect effects were demonstrated by RWC, CT, TKW, PC, PH, and CC through SW. The results of mean performance showed that there might be some promising breeding lines such as L1, L20, L28, L16, and L18 which were adapted to semi-arid conditions of the Eastern High plateaus of Algeria and had the ability to perform better even under water stress.

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Salicylic acid, cinnamaldehyde, and thymol incorporated into cassava starch coatings for mango preservation

Ácido salicílico, cinamaldehído y timol incorporados a revestimientos de almidón de yuca para la conservación del mango

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ABSTRACT

Mango is a fruit consumed all over the world. There are some methods used during storage such as modified atmosphere, hot steam, irradiation, wax coating, and immersion in hot water to extend mango shelf life. However, heat treatment accelerates maturation and reduces organoleptic quality. Edible coatings are also used to extend the shelf life of food. Edible coatings are layers made of proteins, polysaccharides, lipids, antimicrobial components, antioxidants, or a mixture of them. Additives with antimicrobial, antioxidant, or other properties, are added to coatings to improve their functionality. Coatings improve food quality by slowing down unwanted changes and extending shelf life. Knowing that starch is not the best material for preparing edible coatings, in the present work, it was modified by adding salicylic acid or a cinnamaldehyde-thymol mixture to the cassava starch coating-forming solution. Cassava starch or chitosan coatings were applied to Tommy Atkins mangoes. Total soluble solids, titratable acidity, weight loss, and instrumental texture (firmness) were determined over four weeks of storage at 12 °C and 90% of relative humidity. Mangoes coated with cassava starch containing salicylic acid (SSA) had the highest weight loss, while fruits coated with starch-cinnamaldehyde-thymol (SCT) had the lowest weight loss during the whole storage time. The titratable acidity showed a decrease throughout the four weeks of storage. However, in the third week of storage, mangoes coated with chitosan, SSA, and SCT samples ripened more slowly, as indicated by higher acidity than uncoated samples. The SSA and chitosan-coated mangoes showed statistically similar penetration forces that were also higher than the SCT and uncoated samples. Cinnamaldehyde-thymol improved the hydrophobic characteristics of the starch coatings and therefore, it reduced the weight loss of mango during storage.

Keywords:
Instrumental texture
Shelf life
Titratable acidity
Tommy atkins
Total soluble solids

RESUMEN

El mango es una fruta que se consume en todo el mundo. Para prolongar su vida útil durante su almacenamiento, se usan atmósferas modificadas, vapor caliente, irradiación, recubrimientos con cera o inmersión del mango en agua caliente. Sin embargo, el tratamiento térmico acelera la maduración y reduce la calidad organoléptica. Los recubrimientos comestibles también se usan para prolongar la vida útil de los alimentos. Los recubrimientos son capas hechas de proteínas, polisacáridos, lípidos, componentes antimicrobianos, antioxidantes o una mezcla de ellos. Aditivos con propiedad antimicrobiana, antioxidante u otra propiedad, se añaden a los recubrimientos para mejorar su funcionalidad. Los recubrimientos mejoran la calidad de los alimentos al ralentizar los cambios no deseados y prolongar su vida útil. Conociendo que el almidón no es el mejor material para preparar recubrimientos comestibles, en el presente trabajo, se modificó agregando ácido salicílico o una mezcla de cinamaldehído-timol a las soluciones formadoras de recubrimientos de almidón de yuca. Se aplicaron recubrimientos de almidón de yuca o quitosano a mangos Tommy Atkins a los cuales se les determinó sólidos solubles totales, acidez titulable, pérdida de peso y textura instrumental (firmeza) a lo largo de cuatro semanas de almacenamiento a 12 °C y 90% de humedad relativa. Los mangos recubiertos con almidón de yuca que contenían ácido salicílico (SSA) presentaron la mayor pérdida de peso, mientras que los frutos recubiertos con almidón-cinamaldehído-timol (SCT) presentaron la menor pérdida de peso a lo largo de todo el tiempo de almacenamiento. La acidez titulable mostró una disminución a lo largo de las cuatro semanas de almacenamiento. Sin embargo, en la tercera semana de almacenamiento, los mangos recubiertos con muestras de quitosano, SSA y SCT maduraron más lentamente, como lo indica una mayor acidez que las muestras sin recubrir. Los mangos recubiertos con SSA y quitosano mostraron fuerzas de penetración estadísticamente similares que también fueron más altas que las muestras SCT y sin recubrimiento. El uso de revestimientos a base de quitosano o almidón de yuca, este último conteniendo ácido salicílico o cinamaldehído-timol retrasó la maduración del mango. El cinnamaldehído-timol mejoró las características hidrófobas del revestimiento de almidón y, por lo tanto, redujo la pérdida de peso del mango durante el almacenamiento.

Palabras clave:
Textura instrumental
Tiempo de vida
Acidez titulable
Tommy atkins
Sólidos solubles totales

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Mango is among the five most consumed tropical fruits worldwide (Caballero *et al*., 2015). Tommy Atkins is the variety of mango most exported from Ecuador. To be exported, modified atmosphere, hot steam, irradiation, wax coating, and immersion in hot water are used. Mango is normally immersed in hot water to extend its shelf life (National Mango Board, 2020). However, heat treatment accelerates maturation and reduces organoleptic quality.

The short shelf life of mango fruits, susceptibility to chilling injury and postharvest diseases are common postharvest problems that need to be considered for expanding the international mango trade (Singh *et al*., 2013). Edible coatings become a technological alternative that may reduce mango deterioration during storage. Edible coatings are made from proteins, polysaccharides and lipids. Starch is the most important material used in the formulation of biodegradable films and edible coatings (Chiumarelli *et al*., 2010). Plasticizers and other additives are utilized to modify the physical and functional properties of edible coatings (Shah *et al*., 2016).

The use of edible coatings along with essential oils (EOs) has importance because of EOs extend the shelf life of food (Sung *et al*., 2013). EOs and their active components extracted from aromatic and medicinal plants have antibacterial and antifungal properties (Maurya *et al*., 2021). Some examples of the EO constituents are carvacrol (found in thyme and oregano), carvone (dill seed), cinnamaldehyde (cinnamon), citral (lemongrass), p-cimene, eugenol (clove), limonene (citrus fruits), menthol (peppermint) and thymol (thyme and oregano) (Kawacha *et al*., 2021). EOs have been used together with starch coatings to preserve fruits: cassava starch and cinnamaldehyde-thymol in fresh-cut mango (Santacruz, 2021), cassava starch and thyme in apples and persimmons (Sapper *et al*., 2019), and sweet potato starch and cumin essential oil in pears (Oyom *et al*., 2022). In addition to EOs, salicylic acid (SA) can be used in the formulation of edible coatings to delay fruit ripening. SA has been used to control fruit decay of guava (Lo'ay and El Khateeb, 2011), apricot (Ezzat *et al*., 2017) and papaya (Castro *et al*., 2017). Edible coatings based on chitosan (Chien *et al*., 2007), arabic gum (Khaliq *et al*., 2015) and alginate (Robles-Sánchez *et al*., 2013) have been used in mango, however, no studies on the use of edible coatings based on cassava starch containing SA, cinnamaldehyde or thymol on Tommy Atkins mango have been performed.

In recent years, chitosan has had considerable interest in the industry due to its biodegradability, non-toxicity (Wang *et al*., 2020a), and antimicrobial properties (Wang *et al*., 2020b). Studies of chitosan coatings to preserve mango have been performed (Yin *et al*., 2019; Tavassoli-Kafrani *et al*., 2020). However, the low availability and high cost of chitosan in the Ecuadorian market (Salas, 2011) compared to other materials may reduce its application. Therefore, essential oils and salicylic acid together with cassava starch could be a good choice for the formulation of edible coatings thanks to its availability and relatively low price (Souza *et al*., 2012).

In the present work, Tommy Atkins mangoes were coated with either chitosan or cassava starch. Starch coatings together with SA or a mixture of cinnamaldehyde-thymol were utilized. A comparison of total soluble solids, titratable acidity, weight loss, and instrumental texture (firmness) of coated fruits with the two coating materials along four weeks of storage at 12 °C was done.

MATERIALS AND METHODS

Chitosan, degree of deacetylation 95% and Mw of 149 kDa, was donated by Universidad Pública de Navarra, Spain. Cinnamaldehyde, thymol, tween®-20, glycerol, and glucose were obtained from Merck (Germany). Cassava starch (La Pradera, Ecuador) and Tommy Atkins mangoes were obtained from a local market in Manta, Ecuador. Mangoes with a degree of ripening of two (Báez, 1998) were selected according to the size and without any injuries. Afterward, 48 mangoes were washed with water (Santacruz, 2021) and 12 fruits were used for each treatment (Table 1). The coated mangoes were obtained by immersing the fruits in three different solutions (Table 1), followed by drying at room temperature (approx. 25 °C) and storage for four weeks at 12 °C and an RH of 90%. Analyses of weight loss, total soluble solids, titratable acidity, and instrumental texture were performed in triplicate every week.

Table 1. Composition of coating forming solution

Treatment	Chitosan (%, w/v)	Cassava starch (%, w/v)	Salycilic acid (mmol L^{-1})	Cinnamaldehyde (%, w/v)	Thymol (%, w/v)
Uncoated	-	-	-	-	-
Chitosan	1	-	-	-	-
SSA[1]	-	0.5	2	-	-
SCT[2]	-	0.5	-	0.15	0.15

[1]Starch + salicylic acid; [2]Starch + cinnamaldehyde + thymol

Coating forming solution preparation
Chitosan coating forming solution was prepared by dissolving 1% (w/v) of chitosan in 1% (v/v) acetic acid solution. 1% (w/v) of Tween 20, 0.5% (w/v) glycerol and 0.5% (w/v) of glucose were added before the solution was homogenized by using an ultraturrax (Polytron, Switzerland) at 11000 rpm for 4 min.

Starch coating forming solution was prepared according to Santacruz *et al.* (2015). A 0.5% (w/v) cassava starch suspension in water was heated from room temperature up to 90 °C, under stirring, where it was kept for 5 min. 1% of Tween®-20, 0.5% (w/v) glycerol, and 2 mmol L^{-1} of SA were added before cooling. Once the solution was cooled to room temperature, glucose (0.5%, w/v), 0.15% (w/v) cinnamaldehyde (>95%) and 0.15% (w/v) thymol (98.5%) were added. Finally, the coating forming solution was homogenized by using an ultraturrax (Polytron, Switzerland) at 11000 rpm for 4 min.

Weight loss, titratable acidity, and total soluble solids of coated mangoes along storage time
Weight loss was calculated by the following equation.

$$\%WL = \frac{(W0 - Wt)100}{W0}$$

Where: WL: Percentage of weight loss, W0: Fruit weight at time zero, Wt: Fruit weight at any storage time.

Titratable acidity was determined by titration with 0.01 M NaOH solution according to the AOAC method (1990), the results of three measurements were reported as a percentage of citric acid. Total soluble solids were determined according to the AOAC method (1990). Three fruits were disintegrated using a household blender and the obtained juice was filtered with textile and analyzed with a refractometer (Atago, Japan). The results of three measurements were reported as °Brix.

Instrumental texture analysis
Puncture tests were performed in triplicate at the central part of three fruits according to Castro *et al.* (2014). 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 was inserted 15 mm into the fruit at a speed of 10 mm s^{-1} and the maximum penetration force was recorded.

Statistical analyses
All measurements were performed in triplicate. ANOVA and Tukey test with a significance level of 5% were run using InfoStat statistics software (Infostat version 2014, Argentina).

RESULTS AND DISCUSSION

Weight loss, titratable acidity, and total soluble solids of coated mangoes along storage time. Mangoes coated with cassava starch containing salicylic acid (SSA) showed the highest weight loss, whereas fruits coated with starch-cinnamaldehyde-thymol (SCT) showed the lowest weight loss throughout the whole storage time (Table 2). SCT mangoes showed a weight loss that varied between 4.4 and 15.7%, which was statistically different than SSA samples. After the first week of storage, no difference in weight loss was found among uncoated mangoes and mangoes coated with either chitosan or SCT. The hydrophilic nature of starch may contribute to losing water in a higher amount in mangoes coated with SSA compared to uncoated samples. The use of cinnamaldehyde-thymol together with starch (SCT) led to lower weight loss compared to

SSA. The hydrophobic properties of cinnamaldehyde-thymol (Man *et al*., 2019) may be the reason for such behavior. The incorporation of EOs into the coating-forming solution can confer water-resistance properties to coatings because this oil has a hydrophobic nature (Sánchez-González *et al*., 2011).

Table 2. Weight loss and total soluble solids of coated mangoes. Coatings based on either chitosan or cassava starch and stored for 28 days at 12 °C and 90% relative humidity.

Treatment	Days of storage (week)									
	0		7 (1)		14 (2)		21 (3)		28 (4)	
	WL (%)	SS (°Brix)	WL (%)	SS (°Brix)	WL (%)	SS (°Brix)	WL (%)	SS (°Brix)	WL (%)	SS (°Brix)
U	0	6.4±0.3 a	5.0±0.2 ab	6.0±0 a	9.6±0.1 a	10.8±0 b	13.8±0.1 a	14.5±1.8 a	17.8±0 a	14.3±0.1 b
C	0	6.4±0.4 a	4.9±0.6 ab	7.1±0.3 a	9.3±1.1 a	12.9±0.4 c	13.3±1.5 a	11.2±2.3 a	17.2±1.9 a	10.2±0.3 a
SCT	0	6.4±0.3 a	4.4±0.2 a	5.8±2.3 a	8.4±0.3 a	13.8±0.3 c	12.0±0.5 a	13.5±0.9 a	15.7±0.7 a	11.2±1.9 a
SSA	0	6.4±0.3 a	6.3±0.6 b	5.4±0.3 a	12.3±0.8 b	9.6±0.8 a	17.5±1.1 b	10.4±0.8 a	22.4±1.4 b	9.5±0.1 a

The same capital letters in a column indicate no statistically significant difference (P<0.05). U=Uncoated, C=Chitosan, SCT=Starch+cinnamaldehyde+thymol, SSA=Starch+salicylic acid, WL=Weight loss, SS=Soluble solids

Titratable acidity showed a decrease along the four weeks of storage. Biochemical changes, e.g., ascorbic acid content on mangoes during ripening may lead to a reduction of titratable acidity (Pandarinathan and Sivakumar, 2010). There was no difference in titratable acidity among samples up to the second week of storage (Table 3). However, on the third week of storage, mangoes coated with chitosan, SSA, and SCT samples, ripened more slowly as indicated by higher acidity than uncoated samples. On the fourth week of storage, only mangoes coated with SSA showed lower acidity than the uncoated sample. A decline in acidity demonstrates advancement of maturation (Maftoonazad *et al*., 2008), thus the coated fruits contributed to delaying the fruit maturation/ripening. Higher acidity in coated fruits may be the result of the formation of carboxylic acid by dark fixation of CO_2 (Maftoonazad *et al*., 2008).

Table 3. Titratable acidity and maximum force of coated mangoes. Coatings based on either chitosan or cassava starch and stored for 28 days at 12 °C and 90% relative humidity.

Treatment	Days of storage (week)									
	0		7 (1)		14 (2)		21 (3)		28 (4)	
	TA (%)	MF (N)	TA (%)	MF (N)	TA (%)	MF (N)	TA (%)	MF (N)	TA (%)	MF (N)
U	3.5±0.2 a	27.8±1.8 a	2.2±0 a	26.9±0.9 a	2.2±0 a	24.7±3.6 ab	0.8±0.2 b	9.4±0.4 c	1.7±0.4 a	7.1±0.9 b
C	3.5±0.2 a	27.9±1.4 a	2.5±0.3 a	27.6±0.2 a	2.4±0.3 a	24.7±0.1 ab	1.4±0 a	15.5±3.3 ab	1.1±0.1 ab	11.6±0.9 a
SCT	3.5±0.2 a	27.9±1.2 a	2.3±0.4 a	27.3±0.2 a	2.9±0.2 a	21.0±1.3 b	1.4±0 a	13.5±0.7 bc	1.2±0 ab	7.1±0.9 b
SSA	3.5±0.2 a	27.7±1.2 a	2.4±0.4 a	27.5±0.2 a	2.7±0.9 a	27.3±0.6 a	1.4±0.1 a	19.1±1.2 a	1.0±0 b	10.0±0.7 a

The same letters a column indicate no statistically significant difference (P<0.05). U=Uncoated, C=Chitosan, SCT=Starch+cinnamaldehyde+thymol, SSA=Starch+salicylic acid, TA=Titratable acidity (percentage of citric acid), MF=Maximum force (Newtons)

The four treatments showed an increase in total soluble solids along the storage time. Total soluble solids had no differences among samples up to the first week (Table 2), however, an increase for the four samples during the second week of storage was noticed. At the end of storage, the uncoated sample reached the highest value of total soluble solids (approximately 14 °Brix), which was statistically different than the three coated mangoes (total soluble solids between 9 and 11 °Brix). No statistical difference was found among total soluble solids for coated mangoes. The low value of mangoes coated with SSA suggests that SA may reduce the rate of ripening. This

ripening reduction may be probably through inhibition of ethylene biosynthesis (Yin *et al*., 2013). In fact, Lo'ay (2017) found that an exogenous supply of SA delays the ripening of grapes.

Texture analysis

Results of puncture tests showed that the maximum force of penetration decreased during the storage time for all samples. Mangoes coated with SSA and chitosan showed statistically similar forces of penetration which were also higher than SCT and uncoated samples (Table 3). SSA and chitosan coatings led to mangoes with low total soluble solids and low penetration forces which may be the result of a low respiration rate (Cissé *et al*., 2015) and an inhibition of ethylene production by the presence of SA (Hayat *et al*., 2007). Chitosan coating caused substantial delays to some processes involved in ripening most notably weight loss, total soluble solids, and texture of the fruit. These effects may be linked to the reduced rates of ethylene production and respiration that might be the result of lower internal oxygen levels (Jitareerat *et al*., 2007).

CONCLUSIONS

The use of coatings based on either chitosan or cassava starch containing either salicylic acid or cinnamaldehyde-thymol delays changes in weight loss, soluble solids, titratable acidity, and firmness of mango along four weeks of storage at 12 °C. Cinnamaldehyde-thymol improves the hydrophobic characteristics of the cassava starch coating compared to salicylic acid and therefore reduces the weight loss of stored mango. For practical applications, the use of starch coatings with salicylic acid together with cinnamaldehyde-thymol to control the ripening of mango could be analyzed in future research.

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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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- Si hay varios trabajos de un autor publicados en un mismo año, se citarán con una letra en secuencia alfabética de los títulos, adosada al año. Ejemplo: (Gómez, 2000a, 2000b, 2000c)

- En el caso de citas con tres o más autores, es necesario mencionar en el texto el apellido del primero y reemplazar los demás por la expresión latina abreviada *et al.* (en cursiva) que significa y otros; en la referencia se deben poner los apellidos e iniciales de todos los autores. Ejemplo: (García *et al*., 2004).

- Cuando se hace referencia al autor dentro del texto, sólo se encierra el año entre paréntesis y se omite la coma que separa al autor del año. Ejemplo: (1) De acuerdo con Castañeda (2000), …; (2) Concorde con los resultados de Poveda *et al.* (2018) …

- Cuando es una cita de una cita se ponen la información de los autores citados y los autores citantes. Ejemplo: Magalhaes *et al.* (1979) expone que … (as cited in Gómez, 2004).

- Organizaciones se citan por sus siglas, en caso de no tener se cita con su nombre completo. Ejemplo: (1) (FAO, 2015), (2) (Ministerio de Agricultura y Ganadería, 2019)

Referencias

Sólo se listan las referencias bibliográficas mencionadas en el texto. No se aceptan notas de clase o artículos en preparación, o cualquier otra publicación de circulación limitada.

Las referencias bibliográficas se deben ordenar alfabéticamente por el apellido del primer autor, sin numeración y sin sangría. Para citar varias publicaciones del mismo autor, se debe seguir el orden cronológico creciente; si son del mismo año, se debe seguir el orden alfabético de los títulos.

Las referencias deberán contener todos los datos que permitan su fácil localización. Las referencias se citan en el lenguaje de publicación.

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.

Cita de cita, sólo se referencia la fuente consultada. Ejemplo: Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia.

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.

The Journal receives and publishes, without any cost, research articles, reviews, revisions, letters to the editor and editorials written in the English language.

The Journal is a four-monthly publication at national and international level. Its aim is to publish original, unpublished, and peer-reviewed articles of a scientific nature which respond to specific questions and provide support and testing of a hypothesis, related to agronomy, animal husbandry, forestry engineering, food and agricultural engineering, and related areas that contribute to the solution of the agricultural constraints in the tropics.

Taking into account Colciencias (Administrative Department of Science, Technology and Innovation of Colombia) criteria, the journal welcomes papers of the following types:

Research papers in science and technology: A document presenting in detail the original results of completed research projects. The structure generally used contains four main parts: Introduction, methodology (materials and methods), results and discussion, and conclusions. The maximum extension must be 5200 words; excluding figures, tables, references. The maximum number of bibliographic references suggested is 30. This type of article is peer-reviewed and indexed.

Review articles: Documents resulted from a completed research systematizing, analyzing, and integrating the published or unpublished research findings, on a field of science or technology, in order to report the progress and development trends. It is characterized by a careful review of the literature of at least 50 references. The maximum length must be 6000 words; excluding figures, tables, references. This type of articles is arbitrated and indexed.

Short articles: short paper presenting original preliminary or partial results of a scientific or technological research, which usually require a quick diffusion. In all cases 60% of references must come from articles published in the last ten years.

Articles must be submitted in accordance with the guidelines set forth in “Instructions to Authors”; those who violate the rules will not initiate the basic editorial process. Shall be filled the form “Authorization for Release of Works and Economic Rights Assignment”, which will be provided by the Journal. This document is explicit in mentioning that all authors are informed and agree with article submitted for consideration to the Journal, that there is no conflict of interest between them, and also state that the manuscript has not been and will not be submitted for publication to another Journal.

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INSTRUCTIONS TO AUTHORS

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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.

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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)

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- 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)

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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.

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Examples:

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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.

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Beral H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

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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.

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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.

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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

Black, William, Rodolfo Russo, y David Turton. «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes». Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

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[3] William Black, Rodolfo Russo, y David Turton, «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes», Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

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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.

References

Black, William, Rodolfo Russo, y David Turton. «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes». *Physics Letters B* 694, n.º 3 (noviembre de 2010): 246-51.

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