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INHERITANCE OF SEED SIZE IN WATERMELON / DAMAGE CAUSED BY *CAPULINIA LINAROSAE* ON VENEZUELAN GUAVA CROPS / ESTIMATION AND CORRELATION SPAD IN *P. GUAJAVA* L. / STUDY OF CHOLUPA PHENOLOGY / PASSIFLORACEAE SPECIES TOLERANT TO WATER-SALT STRESS / SOIL FERTILITY AND PLANTAIN NUTRITION WITH BACTERIAL DISEASES IN CUNDINAMARCA / PHYSIOLOGICAL MATURITY OF PITAYA FRUITS / THE ECOPHYSIOLOGY OF CAPE GOOSEBERRY / ROLE OF HORMONES IN SEED-FRUIT RELATIONSHIPS / LOGISTIC GROWTH MODEL TO YIELD TRAITS OF LETTUCE / CHARACTERIZATION OF BEGOMOVIRUSES FROM WEEDS IN VALLE (COLOMBIA) / SELECTION OF SUPERIOR YELLOW DIPLOID POTATO GENOTYPES WITH BLUP / PACLOBUTRAZOL APPLIED TO SEED TUBERS

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EDITORIAL NOTE

HELBER ENRIQUE BALAGUERA-LÓPEZ
Chief editor
Revista Colombiana de Ciencias Hortícolas

Volume 14, number 1 of 2020, of the Revista Colombiana de Ciencias Hortícolas is an edition that I will fondly remember because it marks my start as an editor-in-chief of a scientific journal, after having acted as the co-editor for the 2 previous issues. I shall fulfill my duties with the greatest responsibility for what I consider to be one of the the best horticulture journals in Colombia.

This edition will also be remembered because it was published in the midst of the largest epidemiological crisis the world has experienced in recent years, the Covid-19 pandemic. The Revista Colombiana de Ciencias Hortícolas decided to actively continue its work, as have many other scientific, academic journals and institutions, in order to send a message to our readers, authors, reviewers and society in general that, in the midst of so much difficulty, we must continue to contribute to the development and dissemination of scientific information.

On behalf of the two editorial institutions Sociedad Colombiana de Ciencias Hortícolas (SCCH) and the Universidad Pedagógica y Tecnológica de Colombia (UPTC), I thank the Universidad Francisco de Paula Santander (UFPS) and its co-editor Marlon Hans Rodríguez for contributing to the journal for several years, ending in 2020. Their participation was very important and decisive for the growth of the journal in recent years.

We will continue to work hard to maintain the frequency, quality and impact that define the journal; we are also preparing to participate in the "Convocatoria para indexación de revistas científicas colombianas especializadas - Publindex 2020" (Call to index specialized Colombian scientific journals - Publindex 2020), with the aim of maintaining the category B award from 2018.

The digital format of the journal in English will remain, with continuous publication of accepted manuscripts that can be changed with final, diagrammed versions. In this issue, there are 13 articles, nine of which are in the Fruit Tree Section with research results on the inheritance of seed size in watermelon populations, the conventional and alternative management of *Capulinia linarosae* in guava, the estimation of the nitrogen nutritional level through the measurement of chlorophyll in guava, a phenological study on the cholupa (*Passiflora maliformis* L.), the tolerance to water-saline stress in some promising species from the Passifloraceae family, the relationship between soil fertility and incidence of plantain bacterial diseases, and, finally, three reviews of the pitaya fruit quality (*Hylocereus undatus*), the ecophysiology of cape gooseberries and the seed-fruit relationships in fleshy fruit trees.

The vegetable section has four articles that report results on the adjustment of a logistic model for productive characteristics in lettuce cultivars under summer conditions, the partial molecular characterization of begomoviruses isolated from weeds collected in tomato crops, the use of BLUP (Best Linear Unbiased Predictors) analysis in advanced stages of selection in creole potatoes, and the growth and partition of potato dry matter as influenced by paclobutrazol applied to seed tubers.

We want to take the opportunity to thank Dr. Diego Miranda Lasprilla for directing the journal during 2019. I also want to highlight the valuable collaboration of all the reviewers who anonymously contributed to the improvement of the scientific quality of the journal. Finally, the journal thanks all the authors who contributed to this issue and invites its national and international readers to submit their works on horticulture issues for publication.

Dr. Helber Enrique Balaguera-López

Editor-in-chief

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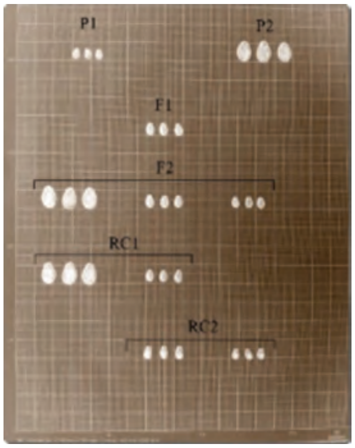
Inheritance of seed size in watermelon populations

Herencia del tamaño de la semilla en poblaciones de sandía



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Seed phenotype in the studied populations.

Photo: T.L. Nascimento

ABSTRACT

Seed size is an important agronomic trait and is applicable to different abilities. Small seeds guarantee the greater use of the pulp, while larger seeds facilitate sowing. However, there is little work on the genetic control of this characteristic in watermelon. The objective of this work was to study the seed size inheritance in watermelon populations by crossing contrasting genotypes, seeking to gain information to provide technical support during the selection of seed size for the development of new watermelon genotypes. The seed lengths of six populations, P_1, P_2, F_1, F_2, BC_1 and BC_2, were measured using the GENES software segregating and nonsegregating generations procedure. This trait is controlled by two genes with incomplete dominance. In addition, depending on the populations studied, inheritance for the characteristic in question may behave differently. Nevertheless, the selection of superior individuals within populations can be performed based on this phenotype, which allows the exploitation of these individuals within breeding programs to develop lines or hybrids.

Additional key words: *Citrullus lanatus*; seed characteristics; crop improvement; selection; incomplete dominance.

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RESUMEN

El tamaño de las semillas es un rasgo agronómico importante, pudiendo utilizarse para diferentes habilidades. Mientras que las semillas pequeñas garantizan un mayor aprovechamiento de la pulpa, semillas más grandes facilitan la siembra, sin embargo, existen pocos trabajos sobre el control genético de este carácter en la sandía. El objetivo de este trabajo fue estudiar la herencia del tamaño de las semillas en poblaciones de sandía cruzando genotipos contrastantes, buscando proporcionar soporte técnico durante la selección del tamaño de la semilla en el desarrollo de nuevos genotipos de este fruto. Fue medida la longitud de la semilla de seis poblaciones P1, P2, F1, F2, RC_1 y RC_2 utilizando el procedimiento generaciones segregantes y no segregantes (GENES), por medio del cual fue posible concluir que la herencia del tamaño de semillas en las poblaciones estudiadas es controlada por dos genes con dominancia incompleta. Además, dependiendo de las poblaciones estudiadas la herencia del carácter en cuestión puede comportarse de manera diferente. La selección de individuos superiores dentro de las poblaciones puede ser realizada con base en el fenotipo, lo que posibilita la exploración de estos individuos dentro de los programas de mejoramiento para componer líneas o híbridos.

Palabras clave adicionales: *Citrullus lanatus*; características de la semilla; mejoramiento de cultivos; selección; codominancia incompleta.

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INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] is one of the highest yielding fruit crops in Brazil, and according to the 2016 world ranking, the country was ranked as the 4th largest watermelon producer, with 2,090,432 t of fruit, second only to China (79,043,138 t), Turkey (3,928,892 t) and Iran (3,813,850 t) (Fao, 2018). The development of new genotypes with commercial attributes different from those already on the market, such as the size of the seeds, can make the production of Brazilian watermelon more expressive. Moreover, the consumer market has increasingly been demanding nutritious and healthy fruits (Farias *et al*., 2014).

For fresh consumption, the smaller the seed size, the easier it becomes to consume the fruit, while for the production of fruit and sowing in the field, the larger the seed size is, the greater the production and the easier the planting. Thus, understanding the genetic control of traits is important for the development of genotypes aimed at having the most diverse characteristics (Li *et al*., 2018). However, it is worth mentioning that information about the genetic behavior of watermelon characteristics is scarce. Among these reports, those studying the size of the seeds, besides being rare, are quite old, which characterizes it as being one of the main obstacles for breeders.

In previous studies, Poole *et al*. (1941), cited by Li *et al*. (2018), described that the genetic control of seed size was controlled by two genes, 's' and 'l', with 's' being epistatic to 'l'. A few years later, the 'Ti' genes for tiny seeds and 'ti' genes for tomato seeds were described (Tanaka *et al*., 1995; Li *et al*., 2018). According to Yong *et al*. (2009), watermelon seed size can be classified as giant, large, medium, small, micro and tiny. In addition to seed size, inheritance studies of other characteristics in watermelon have been reported, including fruit yield (Kumar and Wehner, 2013), seed mass (Adjouman *et al*., 2016), resistance to the PRSV-W virus (Alves *et al*., 2014), and egusi seed (Gusmini *et al*., 2004). This suggests that before performing a characteristic improvement, it is necessary to understand the genetic control mechanisms within the populations of interest.

Thus, the objective of the present work was to study the inheritance of seed size characteristics in watermelon populations through crossing of contrasting genotypes.

MATERIALS AND METHODS

Obtaining the populations

The experiment was conducted from 2017 to 2018 at the Bebedouro Experimental Station at Embrapa Semiárido in Petrolina-PE (Brazil). Two watermelon lines, with contrasting seed size, were used line 31715.001 (“P_1”) (small seed) and lineage 31717.005 (“P_2”) (large seed), to obtain F_1, F_2, BC_1 and BC_2 populations.

As such, crosses were carried out via controlled hand-pollination (CHP) according to the methods used by Nascimento *et al.* (2018). Regarding the CHP, in the flowering stage, male and female flowers of the lineages and the F_1 hybrids were isolated at preanthesis using aluminum sachet bags. On the following day, the open male flowers were detached from the plants, their petals were removed to expose their anthers, and then pollen grains were gently deposited on the stigmas of the female flowers. After this procedure, the pollinated flowers were identified with cardboard tags containing information on the parental crossings, plant type, and CHP date. Afterward, the flowers were isolated again for a period of 72 h. Between 30 and 35 d after pollination, the fruits were harvested, and their seeds were extracted, washed and left to dry in the shade.

To obtain the F_1 population, between January and April 2017, seeds were sown in polystyrene trays containing proper commercial substrate and then kept in a greenhouse for 12 d. After that period, the seedlings were transplanted to the field.

In the following generation, five seeds from the parents (P_1 and P_2) and from the F_1 population were sown during the months of May to August 2017, and using CHP, the F_1 plants were self-fertilized and simultaneously crossed with the parents, P_1 and P_2, which produced the seeds of F_2, BC_1 and BC_2 populations. All populations obtained were then evaluated in the field, from May to August 2018, under open pollination.

Fertilization was carried out according to a soil analysis and as recommended by Mendes *et al.* (2010), with 30 kg ha^{-1} of N, 80 kg ha^{-1} of P_2O_5, and 30 kg ha^{-1} of K_2O, plus 15 kg ha^{-1} of $ZnSO_4$ and 10 kg ha^{-1} of $CuSO_4$. Fertilizers were top-dressed with irrigation water containing 50 kg ha^{-1} of N in the form of $Ca(NO_3)_2$ and 40 kg ha^{-1} of K_2O using KCl, between 50 to 60 d after sowing.

The water was applied by drip irrigation at a daily depth that varied according to the plants’ needs depending on climatic conditions, monitored by a weather station located near the experimental area. The crop coefficients (Kc values) used to calculate crop evapotranspiration (ETc) were those obtained by Freitas and Bezerra (2004) in Canindé, CE, Brazil, for Crimson Sweet watermelon, whose corresponding values were 0.46 to 0.70 at the vegetative stage, 0.89 to 1.22 at the flowering stage, and 1.14 to 0.74 at the fruiting stage.

Phytosanitary treatments were carried out with agrochemicals registered by the Brazilian Ministry of Agriculture, Livestock and Supply, and weeds were removed manually.

Phenotypes of seed size in watermelon populations

During the months of April to August 2018, seeds from all the populations were germinated in expanded polystyrene trays containing commercial substrate for vegetables. At 12 d after sowing, the seedlings were transplanted into the field at the Bebedouro Experimental Station in accordance with a completely randomized design, with each plant considered an experimental unit and with a varied number for each population, in soil previously prepared with gray plastic mulching sheets, with 1 m spacing between plants and 2.5 m between rows. After transplanting, the seedlings were covered with an agrotextile blanket until the flowering period, at which point it was removed for pollination. The seeds of each population came from a single fruit.

At 70 d after planting, fruits were harvested. The seeds were subsequently extracted, washed and left to dry in the shade. Eight, 10, 7, 271, 95 and 94 plants from the P_1, P_2, F_1, F_2, BC_1 and BC_2 populations, respectively, were evaluated.

To investigate seed size inheritance, seed length was measured, as this characteristic is highly correlated with width and weight (Hawkins and Dane, 2001). The lengths of 10 seeds were measured with a digital caliper (Stainless Hardened: JL-YB5474) following the method used by Nascimento *et al*. (2018), with seeds randomly chosen from one fruit of each plant.

The mean and variance [P_1 ($\sigma^2_{f(P_1)} = \sigma^2_{m(P_1)} = \sigma^2_{P_1}$), P_2 ($\sigma^2_{f(P_2)} = \sigma^2_{m(P_2)} = \sigma^2_{P_2}$), F_1 ($\sigma^2_{f(F_1)} = \sigma^2_{m(F_1)} = \sigma^2_{F_1}$); F_2 ($\sigma^2_{f(F_2)} = \sigma^2_{g(F_2)} + \sigma^2_{m(F_2)}$), BC_1 ($\sigma^2_{f(BC_1)} = \sigma^2_{g(BC_1)} + \sigma^2_{m(BC_1)}$) and BC_2 ($\sigma^2_{f(BC_2)} = \sigma^2_{g(BC_2)} + \sigma^2_{m(BC_2)}$)] of the populations were then estimated.

The mean degree of dominance (MDD) of the characteristic was estimated based on the variances and in accordance with the model MDD = $2\,\bar{F}_1 - (\bar{P}_1 + \bar{P}_2)/\bar{P}_1 - \bar{P}_2$. Broad-sense ($h^2_a$) (1) and narrow-sense ($h^2_r$) (2) heritability were estimated as follows:

$$h^2_a = \frac{\sigma^2_{g(F_2)}}{\sigma^2_{f(F_2)}} = \frac{\sigma^2_{f(F_2)} - \left(\sigma^2_{P_1} + 2\sigma^2_{F_1} + \sigma^2_{P_2}\right)/4}{\sigma^2_{f(F_2)}} \qquad (1)$$

$$h^2_r = \frac{\sigma^2_a}{\sigma^2_{f(F_2)}} = \frac{2\sigma^2_{f(F_2)} - \left[\sigma^2_{f(BC_1)} + \sigma^2_{f(BC_2)}\right]}{\sigma^2_{f(F_2)}} \qquad (2)$$

Additionally, the values of k1 (based on variance) and k2 (based on the mean) were estimated, where k = 0 indicates the absence of dominance (k=0=additive), 0 ≥ k ≤ 1 indicates incomplete dominance, k>1 indicates over dominance, and k=1 indicates complete dominance.

Additionally, the minimum number of genes (MNG) controlling the characteristic was estimated by the formula $n = (\bar{P}_1 + \bar{P}_2)^2 / 8\ [\sigma^2_{g(F2)}]$. In terms of computer resources, the "segregating and non-segregating generations" procedure of the GENES software program was used (Cruz, 2016).

Afterward, the characteristic mean was subjected to the chi-square (X^2) test at a 0.05 significance level for verification of the mendelian pattern within the F_2 population according to the following model (3):

$$x^2 = \sum_{i=1}^{n} \left[\frac{(Obs_i - Esp_i)^2}{Esp_i}\right] \qquad (3)$$

where X^2 is the chi-square value calculated and Obs_i and Esp_i are the observed and expected frequencies of the i-th phenotypic class (i = 1, 2, ..., n), respectively (Schuster and Cruz, 2013).

RESULTS AND DISCUSSION

The mean, variance and standard deviation of the seed length of the parents of the segregating populations (Tab. 1) were different, which indicated the existence of genetic variability between them.

The results are in line with the assumption of Cruz *et al.* (2014), who stated that better precision in genetic analyses requires parents whose studied characteristics contrast. According to those authors, genetic parameter estimates allow the identification of the nature of the action of the genes involved in characteristic control. Thus, estimates can be used as an advantageous auxiliary tool in breeding program management.

The variance expressed by the parents and the hybrids showed low values, while the F_2 and RC_2 populations presented high values. Similar results were reported by Souza *et al.* (2006), who, when studying seed size inheritance in watermelon generations, observed high values of variance in the F_2 and BC_2 populations. The high values in both studies possibly occurred because gene segregation within these populations is higher than in the other populations.

Although watermelon is an allogamous species, the low values of variance in the P_1, P_2 and F_1 populations of the present study are due to environmental factors, since the parents used to develop the segregating populations were generated by seven cycles of self-fertilization. Thus, each of the plants of these generations is considered to correspond to the same genotype. However, expecting higher values of the

Table 1. Number of plants, means, variance and standard deviations of seed size, as evaluated for six watermelon populations.

Generation (seed size)[1]	Number of individuals	Mean	Variance	Standard deviation
P_1 (small)	8	5.90	0.0286	0.17
P_2 (largest)	10	11.63	0.0934	0.31
F_1 (medium)	7	6.89	0.0214	0.15
F_2 (¼ small, ½ medium and ¼ largest)	271	7.71	5.4044	2.32
BC_1 (½ small and ½ medium)	95	6.24	0.2474	0.50
BC_2 (½ medium and ½ largest)	94	9.68	5.4711	2.34

[1] Seed size in populations.

variance of F_2 populations and backcross generations due to the interaction of genetic and environmental factors influences the phenotypic expression of traits (Cruz *et al.,* 2014).

The F_2 population presented the fourth lowest mean. According to Zewdie and Bosland (2000), depending on the interaction of a trait, the mean for this population may increase or decrease, indicating that parents are contributing alleles to increase or decrease the trait under study. Based on this information, the mean value of the F_2 population may have occurred because most seeds were classified as medium and short, which together represent approximately 75% of the total amount.

According to the genetic parameter estimates for the F_2 population (Tab. 2), in comparison with the environmental variance, the genetic variance had the greatest influence. Nascimento (2017) found similar results for watermelon seed size, with a higher genetic variance than environmental variance. These results show that the environment has little influence on the phenotypic expression of the evaluated characteristic and that the phenotype observed was mostly the result of genetic influence.

Table 2. Genetic parameters related to seed size in a watermelon F_2 population obtained by crossing contrasting varieties.

Parameter	Value
Phenotypic variance (σ_p^2)	5.404
Environmental variance (σ_e^2)	0.041
Genotypic variance (σ_g^2)	5.363
Additive variance (σ_{ad}^2)	5.090
Variance of dominance deviation (σ_d^2)	0.273
Broad-sense heritability (h_a^2)	99.237
Narrow-sense heritability (h_r^2)	98.189
Mean degree of dominance (MDD)	0.327
K values	k1 (0.33) and k2 (0.66)
Minimum number of genes controlling the characteristic (MNG)	1.82
Maximum value	12.8
Minimum value	4.2

The estimates of the phenotypic, genotypic, additive and environmental variances showed that the contribution of the additive-effect genes to the dominance genes in terms of seed size was significant (Tab. 2), indicating additive allelic interaction. Corroborating the results of the present study, Tanaka *et al.* (1995), when examining the inheritance of fruit shape and seed size in two contrasting watermelon populations, also observed that seed size was governed predominantly by additive allelic interaction, with the small-seed phenotype being an easily improved characteristic.

However, Adjoumani *et al.* (2016), when studying the genetics of seed traits resulting from the intraspecific crossbreeding of distinct watermelon varieties, observed that the effects of genes for the characteristic in question showed dominant allelic interactions. The differing results reported by the above authors can be attributed to the genetic differences of the populations evaluated in each study.

It is worth emphasizing that the predominance of additive allelic interaction facilitates the selection stage, since superior individuals also show superior descent (Cruz *et al.,* 2014). Regarding dominant allelic interactions, breeding programs can benefit from developing hybrids.

Within the F_2 population, one superior individual was observed for the smallest seed size (4.2 mm) (Tab. 2). This individual can be further investigated and give rise to a small-seed open-pollinated cultivar and can also produce a small-seed-size variety, which can be used for hybrid combinations that may result in reduced-seed-size genotypes. It is worth noting that small seed size is dominant over large seed size, and medium size is dominant over small and large sizes (Guner and Wehner, 2004).

The use of transgressive genotypes to develop hybrids with reduced seed size can be very promising, as producers may be able to acquire hybrid seeds of relatively large size, making planting easier, whereas the fruits of these hybrids will have relatively small seeds, thus making watermelon consumption more agreeable and providing a market advantage over the other genotypes.

In the present study, individuals from the F_2 population presented the following approximate phenotypic percentages of seed size: small (25%), medium (50%) and large (25%). Previous inheritance studies on watermelon seed traits such as weight showed that monogenic segregation at a 3:1 ratio did not occur as expected but was close enough to suggest that the smallest seed mass was monogenic and dominant over the largest seed mass (Weetman, 1937).

Similar results were observed in the present study for seed size, where the smallest size, although not in accordance with the expected 3:1 ratio, was quite close to a ratio of 1:2:1, thus suggesting that the characteristic is controlled by two genes.

In addition, the seed size characteristic showed high broad-sense and narrow-sense heritability (Tab. 2). Similar results were observed by Amburani (2018), who, when studying the heritability of morphoagronomic traits in 30 watermelon genotypes, obtained heritability estimates of more than 80% for seven of the 13 traits evaluated, including 100-seed weight and number of seeds per fruit. According to Cruz *et al.* (2014), narrow-sense heritability represents the variance of the additive type fixed in the populations as a result of the advancement of generations. Based on this information, the correlation between phenotypic values and genetic values is considered high for the characteristic in question, and it is possible to select individuals with relatively small seed sizes based on their phenotype.

To infer the mean degree of dominance and determine the best strategy to improve various characteristics, the values of k1 (0.33) and k2 (0.66) (Tab. 2) were calculated and showed mutual agreement, indicating the existence of incomplete dominance for seed size in the studied populations. Adjoumani *et al.* (2016), when studying the genetics of seed traits resulting from the intraspecific crossing of distinct watermelon varieties, obtained the same results. In both studies, these results reinforce the efficiency of genotype selection of individuals of interest based on the phenotype.

The minimum value for the number of genes involved in the control of the studied characteristic was 1.82 (Tab. 2). This information is important for estimating the type of inheritance that controls a given characteristic (Lobo *et al*., 2005). In the present study, seed size is controlled by two genes, indicating that simpler breeding methods, such as mass selection, can be used to obtain small-seed-size cultivars.

When we performed the chi-square test on the populations (Tab. 3), there were no significant differences between populations for observed and expected frequencies associated with phenotypic segregation of the BC_1 population, in which all individuals should present the same seed size. However, it was observed that the seeds of the individuals were divided into small and medium sizes, confirming the hypothesis by which two genes control the characteristic under incomplete dominance.

Similarly, Poole *et al.* (1941), cited by Prothro *et al.* (2012) studied seed length inheritance by evaluating crosses between plants that produce small and large, small and medium, and medium and large seeds and proposed that this characteristic is controlled by two genes because medium-size seeds were dominant over small- and large-size seeds. Those authors proposed that the recessive *l* and *s* genes afforded large- and small-seed phenotypes, respectively. In addition, among the evaluated genotypes, the *s* gene was epistatic for *l*, and *LL SS* was assigned to medium-size seeds; *ll SS*, large-size seeds; and *LL ss* or *ll ss*, small-size seeds.

On the other hand, Tanaka *et al.* (1995) proposed that, in a Sweet Princess watermelon population, tiny seed size was monogenic, controlled by the *Ti* gene and dominant over medium seed size; thus, the

Table 3. Chi-square test results of the watermelon populations to investigate the segregation of two loci based on the 1:2:1 ratio.

Generation	Observed			Expected			X^2	DF	P
	Small	Medium	Large	Small	Medium	Large			
P_1	8	0	0	8.0	0	0	-	-	-
P_2	0	0	10	0	0	10.0	-	-	-
F_1	0	7	0	0	7.0	0	-	-	-
F_2	64	130	49	67.75	135.5	67.75	5.62^{ns}	2	0.05
BC_1	45	50	0	47.5	47.5	0	0.263^{ns}	1	0.05

^{ns} not significant at the 0.05 significance level (P) by the chi-square test (X^2); DF: degrees of freedom.

tiny seed size characteristic was possibly governed by simple inheritance.

The differences observed in the results of the present study possibly show that, for the characteristic in question and depending on the cultivars involved in the crosses, inheritance may behave differently with respect to the populations under study, so it is not possible to extrapolate these results to other populations. Furthermore, the method adopted here for seed size measurement was quantitative, expressed in millimeters, while the study by Polle *et al.* (1941) categorized seed size into classes; that is, they performed a qualitative assessment, disregarding minimal size differences and limiting the population.

The results obtained by means of the predicted gain from selection (Tab. 4) reinforce that, for the improvement of the characteristic under study, the use of simpler breeding methods can be effective, since the gain from the selection was 59% for relatively large seeds and a decrease of just under 30% for relatively small ones. In other words, it is possible to select and recombine individuals that produce large seeds and small seeds, seeking to develop hybrid combinations that produce seeds of intermediate size; this is in addition to the possibility of exploiting these individuals to develop new cultivars for different purposes.

Table 4. Predicted gain from selection for seed size in a watermelon F_2 population obtained by crossing contrasting varieties.

Parameters	Selection	
	Highest values	Lowest values
MSI	12.577	5.285
S	4.870	-2.422
GS (%)	59.516	-29.605
PM 1° S	12.294	5.425

MSI: mean of selected individuals; S: selection differential; GS (%): gain from selection (in percentage), and PM1°S; predicted mean for a selection cycle.

CONCLUSIONS

In the present work, inheritance of the smallest seed size trait in the studied populations is controlled by two genes with incomplete dominance;

Depending on the population under study, the inheritance behavior for the seed size characteristic may behave differently.

Selection of superior individuals for the smallest seed size within an F_2 population can occur based on phenotype;

Increased numbers of individuals can be exploited within breeding programs to develop lines or hybrids.

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Damage levels caused by *Capulinia linarosae* Kondo & Gullan (Hemiptera: Eriococcidae) on guava crops under two agronomic management practices in the south of Lake Maracaibo, Venezuela

Niveles de daño causados por *Capulinia linarosae* Kondo & Gullan (Hemiptera: Eriococcidae) en cultivos de guayaba bajo dos prácticas de manejo agronómico en el sur del lago de Maracaibo, Venezuela

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Young colony of *Capulinia linarosae* on a principal guava stem, Agropecuaria Aranzazu, El Moralito, State of Zulia, Venezuela.

Photo: J.F. Redondo-Méndez

ABSTRACT

The objective of the present study was to test the hypothesis of no difference in the level of damage caused by the guava cottony scale *Capulinia linarosae* (Hemiptera: Eriococcidae) in two guava crops under different agronomic management practices; a crop with organic management practices (PU_A) was compared with another subjected to chemical methods (PU_B). Forty plants on each farm were selected randomly to estimate damage on the stems, branches, leaves, and fruits. The levels of damage were statistically different ($P<0.0001$) between the methods for the stems and branches but not for the leaves or fruits ($P>0.05$). It was concluded that the magnitude of damage was greater in the crop under chemical control and that agronomic management was a critical factor. Excess insecticides and incorrect pruning applications cause damage to plant bark and favor insect proliferation. While the use of a sulfur-calcium broth in PU_A regulated pH, it favored the

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* Article based on the thesis "Incidencia de la mota blanca (*Capulinia* sp.) del guayabo en dos unidades de producción del municipio Colón, Parroquia El Moralito que difieren en el manejo agronómico".

effect of other products and promoted the growth of green algae, which covered bark crevices and inhibited cottony scale establishment. The excessive use of agrochemicals for weed control in PU_B caused soil nudity and killed natural enemies, whereas, in PU_A, the grass layer was clipped at 20 cm from the soil, maintaining moisture and creating a favorable habitat for natural enemies.

Additional key words: control methods; *Psidium guajava*; cottony scale; alternative agriculture; integrated pest management.

RESUMEN

El objetivo del estudio fue someter a prueba la hipótesis de que no hay diferencias en el nivel del daño causado por *Capulinia linarosae* (Hemiptera: Eriococcidae) en dos cultivos de guayaba sometidos a prácticas de manejos agronómicos diferentes: se comparó un cultivo cuyas prácticas de manejo tienden a orgánicas (UP_A) con otro sometido a métodos químicos (UP_B). En cada cultivo se seleccionaron 40 plantas al azar para estimar el daño en tallos, ramas, hojas y frutos. Se encontraron diferencias estadísticamente significativas ($P<0,0001$) entre los dos métodos de control para tallos y ramas, pero no para hojas ni frutos ($P>0,05$). Se concluye que la magnitud del daño es mayor en el cultivo sometido a control químico y que el manejo agronómico es un factor crítico. El exceso de insecticidas y la aplicación incorrecta de podas en la UP_B dañan la corteza de las plantas y favorecen la proliferación del insecto. Mientras que el uso de caldo Sulfo-cálcico en la UP_A regula el pH, favorece el efecto de otros productos y promueve el establecimiento de algas verdes que cubren las grietas dificultando el establecimiento del insecto. El exceso de agroquímicos para el control de malezas en UP_B deja el suelo desnudo, matando sus enemigos naturales, mientras que en la UP_A se corta el estrato herbáceo a unos 20 cm del suelo, manteniendo su humedad y creando un hábitat favorable a los controladores biológicos.

Palabras clave adicionales: métodos de control; *Psidium guajava*; mota blanca; agricultura alternativa; manejo integrado de plagas.

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INTRODUCTION

The guava (*Psidium guajava* L.) is a tree in the Myrtaceae family that originated in the tropical Americas although it is now distributed throughout the planet. Global production of guava fruits is close to 31 million tons, with India, China, and Thailand contributing more than 75% of this total. Mexico produces 5%, Brazil produces almost 4%, and the rest is provided by other countries, including Indonesia, Philippines, Bangladesh, and Nigeria, among others (Mendes *et al.,* 2017; Reddy, 2018).

The production of guava in Venezuela began in the ’80s and quickly became one of the most important fruit crops. The varieties present in the country are mainly: ‘Criolla Roja’, ‘San Miguel’ and ‘Rio Chiquito’ (Aular and Casares, 2011). By 1992, there were about 4,000 ha of crop production that were almost entirely located on the Maracaibo plain (Cermeli and Geraud-Pouey, 1997). By 1996, the cultivated area was 3,000 ha, reaching 3,500 ha in 2008. The average annual yield has been between 15 and 20 t ha^{-1}, and the total national production has varied between 50,000 and 60,000 t (Quijada and Matheus, 1997).

Since its appearance in Venezuela at the beginning of 1993, the guava cottony scale (*Capulinia linarosae* Kondo & Gullan, 2016, Figs. 1A and 1B), has caused severe damage (Geraud-Pouey *et al.,* 2001a; Camacho *et al.,* 2002; León *et al.,* 2014; Kondo *et al*., 2016) and, more recently in Colombia (Kondo *et al*., 2016; Ramos Portilla, 2018), has become the principal pest in this crop. The damage is caused mainly by nymphs and adult females that feed on the sap they extract with their sucking mouth apparatus, which is introduced through the cortex (Cermeli and Geraud-Pouey, 1997). Adult females lay about 300 eggs, and populations can multiply more than 150 times per generation (43.2 d). This reproductive potential explains the occurrence of population outbreaks observed in the field. The rapidly growing colonies cover trees that, according to Chirinos *et al.* (2004), in extreme cases appear to be covered by snow.

Colonies usually begin development on the most shaded stem-parts and branches, covering it completely (Chirinos *et al*., 2004). As the colony grows, a necrotic patch of internal tissues occurs, causing the bark to crack, forming a kind of canker (Chirinos *et al*., 2004). The necrotic areas impede the normal circulation of sap, producing leave-yellowing and later a generalized withering (Chirinos *et al*., 2004). As a result, the plant looks like it has been burned (Chirinos *et al.,* 2004).

In the south of Lake Maracaibo (SLM), problems generated by *C. linarosae* are considerable. Large crop areas are affected, and, in some cases, attacks have caused mortality in most of the plants. Several alternatives have been used to control this insect, with chemical control being the most used (Chirinos *et al.,* 2004). In the last decade, some farmers have adopted more organic agronomic management practices; however, these applications are still limited. It should be noted that a large part of the SLM is, by decree, a Special Zone of Sustainable Development; for this reason, agricultural systems established there must be based on agro-ecological methods that allow sustainability. This requires a reduction in the use of agrochemicals and increased use of organic methods.

It is believed that the damage caused by *C. linarosae* on guava plants is influenced by the methods and techniques applied to control pests but there is no agreement on the effectiveness of organic or conventional chemical methods. This study tested the hypothesis that there is no difference in the magnitude of damage caused by the guava cottony scale on plants subjected to different control methods: organic and use of chemicals.

MATERIALS AND METHODS

This research was conducted on two farms or production units (PU): Agropecuaria Aranzazu (PU_A, 8°48'27" N and 71°46'48" W) and Agropecuaria El Toro

Figure 1. A. Young colony of *Capulinia linarosae* on a branch. B. Young colony of *C. linarosae* on a principal stem. Photos: J. Redondo-Méndez.

(PU_B, 8°48'30" N and 71°46'03" W), both located next to the Santa Barbara-El Vigia highway, near El Moralito, Municipality of Colon, State of Zulia, Venezuela. PU_A had a guava orchard of 5 ha, and PU_B had 2 ha.

The study area has an annual average rainfall of more than 2,000 mm. The annual distribution of rainfall is bi-seasonal, with a short dry period of 3 to 4 months between December and March, and a rainy season of 8 to 9 months, between April and November. The average annual temperature is 28°C, with monthly minimums and maximums that range between 21.4 and 34.2°C (Seijas, 1984). The ecological unit in the study area was tropical humid forest (Ewel *et al.,* 1976).

Each plantation was divided into 30 × 30 m plots, and one of these plots was selected at random for observations in a sub-sample of 40 plants/PU; the subsample plants were also randomly selected using a table of random numbers. The damage was evaluated in (a) the entire main stem; (b) a secondary branch, (c) 20 leaves and (d) all the fruits present on the branch. The fifth main branch was selected (using the oldest branch as the first), and, on that branch, the first secondary branch was chosen. The leaves were counted starting from the apical end of the selected secondary branch.

To estimate the magnitude of damage caused by *C. linarosae,* the methodology proposed by Güerere and Quiroz (2000) was modified as follows: zero (0): no observable damages; one (1): slight damage, the surface of stems and branches with up to 20% depressions, incipient necrosis, brown lesions also present on the leaves and fruits; two (2) slight damage: the surface of stems and branches with up to 40% depressions, slight necrosis, brown lesions also present on the leaves and fruits; three (3) moderate damage, the surface of stems with generalized depressions up to 60%, moderate necrosis on the leaves, branches and fruits, mainly brown to blackish lesions; four (4) minor damage: the surface of stems with generalized depressions up to 80%, not very severe necrosis on the leaves, branches and fruits, mainly black lesions; and five (5) very severe damage: surface of stems with generalized depressions of up to 100%, very severe necrosis on the leaves, branches and fruits: black lesions.

SPSS IBM® version 20 was used to process the data with a one-way analysis of variance. The variables included the damage level on the stem, secondary branch, leaves, and fruits, with a significant effect at 0.05 and 0.001 probability level.

RESULTS

Description of the control methods of *Capulinia linarosae*

In PU_B, the control method used organophosphates, such as clorpyrifos, that caused damage to the plant bark. The excessive application of chemical products manifested psoriasis (Fig. 2A) or burn and subsequent stem-necrosis (Fig. 2B).

On the contrary, PU_A had an integrated control of *C. linarosae*; chemical products (i.e. profenofos: 0-4-bromo-2-clorofenil-0-etil-S-propilfosforotioato) were applied only when (following the farmer's criteria) there were "severe population outbreaks" of the insect. On the other hand, a sulfur-calcium liquid mixture was applied every 15 d, controlling *C. linarosae* and promoting the establishment of green algae (Fig. 2C). Additionally, to control the insect, PU_A used a product consisting of 2 kg of urea, 1 L of vinegar and 1 kg of detergent powder, dissolved in 200 L of water, that was applied every six months with a motor sprinkler. According to the farmer, this product gave satisfactory results.

Description of the weed control methods

PU_B used systemic (glyphosate) and contact herbicides (paraquat and diquat). As a consequence of these applications, the soil lost its vegetative layer (Fig. 3A). When weeds reappeared, they were removed with a scythe, leaving a vegetative layer with a height of about 10 cm.

In PU_A, the weed control was carried out only with a scythe, cutting at 20 cm or more above the ground (Fig. 3B).

Description of fertilization methods

The fertilization in PU_B was done with a complete formula (N, P, K), alternating it with a mix of Poliverdol® (16-16-12); foliar fertilizer, insecticides and fungicides were applied by helicopter. PU_A used an organic fertilizer prepared *in situ*, known locally as "guarapita", applied every 3 months; it consisted of 40 kg of fresh cow dung, 20 L of whey, 20 kg of molasses, 4 kg of ash, 4 bledo plants (*Amaranthus* sp.) and 5 spoons of yeast; all dissolved in 200 L of water.

Figure 2. A. Exfoliation of the stem bark. B. Stem necrosis. C. Green algae on the stem of a plant treated with the sulfured-calcium mixture. Photos: J. Redondo-Méndez.

Figure 3. A. Soil without vegetative layer after weeding with Paraquat. B. Soil with 20 cm or more of vegetation cover after manual weed removal. Photos: J. Redondo-Méndez.

Additionally, PU_A used a product consisting of 2 kg of urea, 1 L of vinegar and 1 kg of detergent powder, dissolved in 200 L of water, applied every 6 months with a motor sprinkler. According to the farmer, this product gave satisfactory results.

Description of pruning methods in the guava plants

In PU_B, the pruning was not frequent, so cracks in the cortex remained that facilitated the presence of the insect.

PU_A used moderate pruning to limit the vertical growth of the plants. Every time the plants became sick (the branches, leaves, and fruits were necrosed), phytosanitary pruning was performed, and the pruned material was piled close to the plant.

Damage levels

The insects caused little damage in the leaves and fruits but instead were established mainly in the most shaded parts, especially in the main and secondary stems that covered them completely. The average damage level was greater in the PU where a chemical control was applied (Fig. 4).

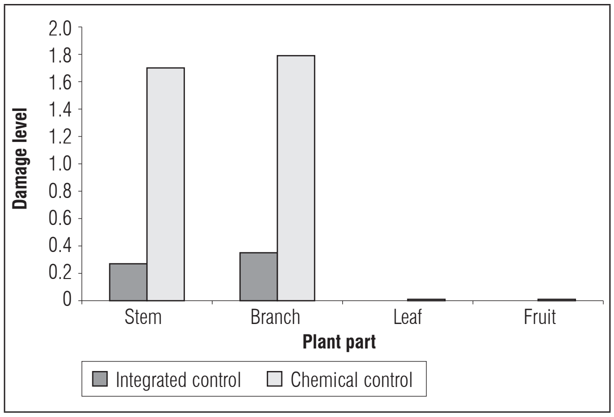

Figure 4. Damage levels on the studied farms.

Statistically significant differences were found ($P<0.0001$) between the control methods for the stems and branches (Tab. 1) but not for the leaves or fruits ($P>0.05$), so the hypothesis that there is no difference in the magnitude of damage estimated for the two crops was rejected.

Table 1. The exit of analysis of variance.

Plant part	F	Sig.
Stem	67.703	0.000***
Branch	93.329	0.000***
Leaf	1.101	0.296
Fruit	1.101	0.296

** significant at $P<0.001$.

DISCUSSION

In PU_B, the chemical methods to control *C. linarosae* altered the stem-bark texture and, therefore, favored the establishment and proliferation of the guava cottony scale.

Organophosphorus insecticides have a prolonged residual effect on guava leaves and stems. According to Ettiene *et al.* (2010), its persistence is greater on stems than on leaves because stems are protected from direct exposure to sunlight, while, on leaves, it is volatilized and photo-degraded, which explains the scant damage caused by insects in that part of the plant. On the other hand, detailed studies have suggested that in plants with less bark exfoliation tend to occur fewer infestations (Geraud-Pouey *et al.,* 2001b).

Chemical insecticides represent a useful alternative to control agricultural pests although their use must be minimized because of the negative effects on agrosystems, mainly as the result of their lethal impact on beneficial insects (Devine *et al.,* 2008; Chirinos and Geraud-Pouey, 2011; Grogan, 2014) but also because of the detrimental effect on human health (Devine *et al.,* 2008).

In many cases, farmers are unaware that there are insects that are natural predators of pests, which inhibits appropriate management decisions; they opt for the most immediate methods, such as the use of broad-spectrum insecticides. This decision can worsen the problem because these products are more expensive (Grogan, 2014). Many coccinellid beetles are recognized as natural enemies of *C. linarosae* (Romay *et al*., 2016), and some parasitoid wasps, such as Hymenopteran *Metaphycus marensis* are potential natural enemies (Chirinos and Kondo, 2019).

The application of profenofos in PU_A did not seem to affect the plants but it prevented the establishment and proliferation of the insect. Moreover, the application of a sulfur-calcium liquid mixture controlled *C. linarosae* since it regulates the soil pH, favors the effect of chemical or organic products, and promotes the establishment of beneficial microorganisms, such as some green algae, that, when established, cover cracks in plants, making insect establishment difficult.

The application of glyphosate (a systemic herbicide) and paraquat and diquat (contact herbicides) caused a reduction in the total disappearance of epigeous fauna and the alteration of soil organism communities, which, together, can lead to an imbalance in the agroecosystem, promoting this pests (Altieri and Nicholls, 2003; Lavelle *et al.,* 2004).

On the other hand, the weed control method carried out in PU_A allowed the farmer to maintain enough moisture in the soil during the dry season. This practice favored the colonization of the herbaceous stratum by natural enemies of *C. linarosae*.

Paraquat and diquat are biologically very active and highly toxic to plants and animals, especially to humans (Singh *et al.,* 2014), moderately toxic to birds, toxic to some fungi and bacteria and increases the populations of some soil pathogens. Field observations indicate that *C. linarosae* colonizes guava plants through the soil, so this insect, upon frequent contact with this herbicide, may have developed some resistance. In addition to the fact that a lack of a vegetation layer causes the disappearance of natural enemies, this creates conditions that are favorable for increased population sizes of this pest.

The fertilization methods used in both PUs were different. Despite the application of fertilizers with a complete formula, the applications mixed with insecticides and fungicides from a helicopter in PU_B did not improve the condition of the plants because the helicopter released an excessive quantity of insecticides, causing damage on the stem-bark; it can also cause physiological stress in plants and favor this insect. In contrast, the organic fertilizer used in PU_A allowed the farmer to have more vigorous and more scale-resistant plants.

Some authors (for example Silva *et al*., 2015) have pointed out that organic fertilizers positively affect the growth of plants and minimize the advancement of insect pests and diseases. The relocation of minerals in plants can affect oviposition, growth rates, survival and reproduction of insect pests, while promoting increased populations of spiders and coleopterans, among others (Hodgson and Miller, 2010). However, García (2009) clarified that the responses of pests to organic farming methods, as compared to conventional methods, are divergent and that landscape heterogeneity is important because it benefits natural enemies.

In PU_B, pruning was not performed frequently, so cracks in the cortex were maintained, which facilitated the presence of *C. linarosae.* To reduce the damage caused by this insect, natural cracks and those caused by injuries must be prevented in the bark (Geraud-Pouey *et al.,* 2001b). In PU_A, moderate pruning was carried out frequently, which reduced the availability of cracks where insects can establish and eliminated dead plant parts where pathogenic microorganisms can proliferate.

The diversification of crops and the adoption of organic practices increase the population of several natural enemies, which improve the biological control of pests (Minh and Heong, 2005; Poveda *et al.,* 2008; Godhani *et al.,* 2009; Mates *et al.,* 2012; Monteiro *et al.,* 2013; Potgieter *et al.,* 2015).

There is abundant evidence that agronomic management exerts a marked effect on the incidence of pests (Ndolo, 2004; Mates *et al.,* 2012; Rush *et al.,* 2013; Veromann *et al.,* 2013; Silva *et al.,* 2015). For example, Oso and Falade (2010) investigated the effects of a variety of spatial arrangements on a maize crop on the incidence of the Lepidopteran *Maruca vitrata* and the Thysanopteran *Megalurothrips sjostedti* and found that the plantation pattern is a determining factor for insect infestations.

On the other hand, there are increasing indications that simplified land uses associated with heavy dependence on agrochemicals reduce environmental quality, threaten biodiversity and increase pest populations. The implementation of agronomic practices that maximize ecosystem services such as biological control of insect pests will surely increase sustainability. Therefore, the use of pesticides must be reduced and replaced with the use of innovative organic products (Garrat *et al*., 2011; Mates *et al.,* 2012; Rush *et al.,* 2013).

In short, the sustainability of an agroecosystem depends on several natural services but can also be affected by elements that occur naturally or that are man-induced, such as herbivory, which decreases productivity and increases production costs. For these reasons, the natural regulation of pests is the most important ecosystem service, maintaining a high level of biodiversity on farms (Rusch *et al.,* 2010).

CONCLUSIONS

The magnitude of damage was greater on the farm subjected to chemical control, and agronomic management was a critical factor. Excessive pesticide use and pruning damaged the bark and favored the proliferation of the guava cottony scale in PU_B. The use of a sulfur-calcium based mixture in PU_A favored the effect of other products and promoted algae establishment, which covered bark crevices, impeding insect establishment. The excess agrochemicals used for weed control in PU_B caused soil nudity and killed natural enemies, whereas, in PU_A , the grass layer was clipped at 20 cm above the soil, maintaining moisture and creating a favorable habitat for natural enemies.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Estimation and correlation of chlorophyll and nitrogen contents in *Psidium guajava* L. with destructive and non-destructive methods

Estimación y correlación de contenidos de clorofilas y nitrógeno en *Psidium guajava* L. mediante métodos destructivos y no destructivos

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***P. guajava* L. cv. 'Regional Roja' fruit.**

Photo: V.C. Pulido-Blanco

ABSTRACT

Estimating photosynthetic pigments, such as chlorophylls, as well as the foliar nitrogen content is very important for the evaluation of the nutritional and physiological status of a plant. However, producers cannot easily carry out these estimations because they involve analyses in laboratories that are costly and time-consuming. The SPAD-502 total chlorophyll index meter is efficient at rapidly estimating total chlorophyll contents in situ, along with its relationship with the total nitrogen concentration in tissues. The objective of this research was to validate a non-destructive in situ method (SPAD) for use in place of destructive estimation methods for chlorophylls a, b and total, as well as the foliar nitrogen concentration in guava plants (*Psidium guajaba* L.), cv. Regional Roja, in three phenological stages under the conditions of the municipality of Vélez-Santander (Colombia). The data obtained with the SPAD-502 and the content of chlorophylls a, b and total were adjusted to a second-degree polynomial with correlation coefficients (r) greater than 0.91 for the three phenological stages, while the concentration of foliar nitrogen was adjusted to a linear type model with determination coefficients (R^2) higher than 0.90, indicating a highly linear correlation between the non-destructive method and the destructive methods used in the present study.

Additional key words: SPAD; photosynthetic pigments; mineral nutrition; portable meter; nutritional status.

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RESUMEN

La estimación de pigmentos fotosintéticos como las clorofilas, así como el contenido de nitrógeno foliar son muy importantes para la evaluación del estatus nutricional y fisiológico de la planta; sin embargo, los productores no pueden acceder de forma fácil a estos, debido a que implican el análisis en laboratorios que en ocasiones generan costo y demora en la obtención de los resultados. El medidor de índice de clorofila total tipo SPAD-502 es un equipo que ha demostrado su eficiencia en cuanto a la estimación rápida de los contenidos totales de clorofila in situ, así también, su relación con la concentración del nitrógeno total en tejido. El objetivo de la investigación fue el de validar el método in situ, no destructivo (SPAD) frente a los métodos de estimación destructivos de clorofilas a, b y total, así como la concentración de nitrógeno en tejido foliar en plantas de guayaba (*Psidium guajaba* L.) cv. Regional Roja en tres estadios fenológicos, bajo condiciones del municipio de Velez-Santander (Colombia). Los datos obtenidos con el SPAD-502 y el contenido de clorofilas a, b y total, se ajustaron a un polinomio de segundo grado con coeficientes de correlaciones (r) mayores a 0,91, en los tres estadios fenológicos evaluados, mientras que la concentración de nitrógeno foliar se ajustó a un modelo de tipo lineal con coeficientes de determinación (R^2) superior al 0,90, esto indica la existencia de una alta correlación lineal entre el método no destructivo y los métodos destructivos empleados en el presente estudio.

Palabras clave adicionales: SPAD; pigmentos fotosintéticos; nutrición mineral; medidor portátil; estatus nutricional.

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INTRODUCTION

The mineral nutrition of crops is of great concern for producers, who currently use diagnostic tools, such as soil and/or tissue analysis that are costly, time-consuming and inhibit quick decision-making. For tissue or photosynthetic pigment analyses, destructive methodologies are used that, although important in the evaluation of the physiological status of plants, are expensive and inaccessible for producers (Amarante *et al*., 2009). Furthermore, because of the importance of nitrogen as a primary element in tissue formation (Marschner, 2012), methodologies used for its quantification in laboratories are very costly and require the transport of samples and a lot of time, delaying the implementation of corrective measures for deficiencies of this element.

The early detection of the chlorophyll or nitrogen content in plants facilitates decision-making for dealing with element deficiencies, such as nitrogen (N) and magnesium, which is why diagnosis in a timely manner is essential for producers. Traditionally, farmers use visual parameters such as the greenness of the leaves to determine the need for N. However, this indicator is highly subjective, which is why methods that guarantee a rapid and quantitative diagnosis are needed.

The measurement of variables, such as the chlorophyll index, with non-destructive methods is a widely used technique for different species (Castañeda *et al*., 2018; Mendoza-Tafolla *et al*., 2019), including the commonly used SPAD total chlorophyll index type meter. This portable device indirectly, with a non-destructive method, evaluates the total chlorophyll index in leaves by means of light transmitted through the leaf, with the wavelengths 650 and 940 nm, providing an excellent approximation of the total chlorophyll content and N nutritional status in various crops (Zotarelli *et al*., 2003).

Several studies have indicated the existence of a direct relationship between the chlorophyll content and the N content in leaves because this element is needed for the synthesis of the chlorophyll molecule and is linked to the light phase of the photosynthetic process (Taiz and Zeiger, 2010). Studies carried out by Zotarelli *et al*. (2003) indicated the importance of using indirect methods, such as the SPAD-502® chlorophyll meter (Konica Minolta, Osaka, Japan), for monitoring the availability of N. Ramírez-Builes *et al*. (2012) proposed the use of portable chlorophyll meters as a tool for the early detection of N deficiencies and yield decreases associated with stress conditions.

The SPAD-502® chlorophyll meter has been used successfully in the quantification of nitrogen content in crops such as vine apples (Callejas *et al*., 2014; Castañeda *et al*., 2018), corn (Castillo and Ligarreto, 2016), basil (Fenech-Larios *et al*., 2009), and tomatoes (Rodríguez *et al*., 1998; Hurtado *et al*., 2017), among others, because this method is easy, fast, and non-destructive.

Therefore, the present study aimed to estimate the concentrations of chlorophyll and nitrogen in guava leaves (*Psidium guajava* L.) in different phenological stages in order to observe their correlation with the non-destructive SPAD method under field conditions.

MATERIALS AND METHODS

This study was carried out on the San Diego farm, village of Aco de Peña Blanca, which is located in the municipality of Vélez-Santander, with longitude and latitude coordinates: 73°39'30'' W and 5°57'49'' N; 1,920 m a.s.l. The study area was 1 ha, which was georeferenced to fully identify the area in terms of its geographical characteristics and the trees it contained. Four hundred eight trees in the *P. guajava* cv. Regional Roja were identified, which were 5 years old, in the productive stage, established with a planting distance of 5 m between plants and 5 m between rows and traditionally managed, of the 408 trees, 44 trees were selected that belonged to the area, where, historically, this crop has shown good growth and development.

For the quantification of the SPAD total chlorophyll index, the a, b and total chlorophyll contents and the nitrogen content in the tissue, three sampling times were established in the 44 selected plants in order to measure these variables at points where the plants see greater absorption of elements such as nitrogen: E2: visible flower buds, H1: fruit set and I1: fruit filling (80% of total formation), selected according to the phenological scale proposed by Salazar *et al*. (2006) and carried out with general pruning of the evaluated plants.

To determine the total chlorophyll index in the field, 20 leaves were selected from the branches located in the middle third of the plants, which were fully expanded, in order to quantify the index using a SPAD-502® (Konica Minolta, Osaka, Japan), obtaining the average value per sample unit.

The a, b and total chlorophyll contents were estimated in a laboratory with a spectrophotometer, Thermo Scientific™ Genesys™ 10 series, following the methodology proposed by Solarte *et al*. (2010). For this, 0.5 cm radius leaf discs were taken from the middle part of the leaves (between the central rib and the margin) until reaching 5 g (6 leaves), which were placed in a mortar with 14 mL of 80% (v/v) acetone previously cooled to -10°C, macerated for 5 min, vortexed for 2 min in order to ensure complete contact of the plant material with the acetone, and centrifuged for 10 min at 4000 rpm. The supernatant was removed, placed in a 25 mL volumetric balloon, which was covered with aluminum foil, and titrated with previously cooled 80% (v/v) acetone. The entire process was carried out with low illumination. Finally, an absorbance reading was taken for each sample at a 663 nm wavelength for chlorophyll a and 647 nm for chlorophyll b. The analytical blank was 80% acetone (v/v). The chlorophyll a, b and total contents were calculated with the data using the equations proposed by Lichtenthaler (1987) for 80% acetone (v/v) (Equations 1, 2 and 3):

$$\text{Chl a} = 12.25A_{663.2} - 2.79A_{646.8} \tag{1}$$

$$\text{Chl b} = 21.50A_{646.8} - 5.10A_{663.2} \tag{2}$$

$$\text{Chl total (a+b)} = 7.15A_{663.2} - 18.71A_{646.8} \tag{3}$$

The tissue nitrogen content was determined with the Kjeldahl method using 150 g samples (15 leaves) of plant material, quantifying the SPAD chlorophyll index. The samples were covered with absorbent paper to eliminate moisture and packed in labeled paper bags, which were stored in a polystyrene refrigerator. The analyses were carried out in the ASAYMA laboratory, Palmira-Colombia.

The data obtained for the chlorophyll a, b and total contents (mg g^{-1} fresh weight) and the nitrogen content in the leaf tissue (%) were correlated with the data obtained from the total chlorophyll index (SPAD) in order to obtain the respective graphs and regression equations. The correlation analyses were done with the statistical software R 3.6.2, free version.

RESULTS AND DISCUSSION

The data analysis showed the linear relationship between the total chlorophyll index (SPAD) and the chlorophyll a, b and total contents obtained with spectrophotometry for the three evaluated stages (E2, H1, I1). According to the results of the Pearson method correlation analysis, a linear association was observed between the SPAD method and the chlorophyll a, b and total tissue contents, with positive and significant correlation coefficients for all stages with values that ranged from 0.91 to 0.97 (Fig. 1).

The total chlorophyll index was between 29.0 and 51.9 SPAD units for the evaluated stages, while the total chlorophyll (Chla + Chlb) for stage E2 presented a minimum value of 0.91 and a maximum of 3.45 (Fig. 2); stage H1 presented a minimum value of 0.56 and a maximum of 2.76 (Fig. 3) and stage I1 presented a minimum value of 0.97 and a maximum of 2.86 (Fig. 4).

The above indicated that the quantification of the chlorophyll content, either directly or indirectly, was influenced by the phenological stage. The SPAD method was an important tool for determining the tissue chlorophyll contents, quickly and reliably, in the guava plants under the study conditions and in the evaluated stages.

The results indicated that the SPAD method was highly correlated with the content of photosynthetic pigments, such as chlorophyll a, b and total. According to the polynomial model, an R^2 value greater than or equal to 0.90 ($n = 44$; $P \leq 0.0001$) was obtained in all evaluated stages, indicating that there was a highly significant and positive correlation between the destructive method and the SPAD method (Fig. 2, 3, 4).

These results agree with those of studies on various species (Zotarelli *et al*., 2003; Torres Netto *et al*., 2005; Hurtado *et al*., 2017). The selection of leaves from the middle third was successful since there was no evidence of high variation in terms of data; similar results were described by Castillo and Ligarreto (2016), who, when evaluating the SPAD method in corn plants, concluded that the best area for sampling is the middle third of the plant.

The results agree with the report by Torres Netto *et al*. (2005), who indicated that, in coffee leaves, polynomial equations generate the best correlation between the SPAD method and the destructive method. Donnelly *et al*. (2020) observed that the quadratic model showed the best fit when correlating the SPAD method and the chlorophyll contents extracted from leaves of different tree species. However, the results differed when compared to the behavior of other species with respect to the mathematical adjustment of the studied relationships, such as in tomatoes and cotton, in which the relationships between SPAD readings and the concentrations of photosynthetic pigments were linear (Rodríguez *et al*., 1998; Neves *et al*., 2005; Jiang *et al*., 2017).

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Notably, method validation should be carried out for each crop as far as possible under real production conditions (field or greenhouse) because different variations can be generated, including variations in the morphological type, such as an increase or decrease in the leaf thickness, in the physiological type, such as a greater or lesser content of photosynthetic pigments, in the environmental type, such as greater or lesser exposure to light, and in the different genotypes of a species (Jifon *et al*., 2005; Uddling *et al*., 2007).

The results for the correlation between the percentage of nitrogen and the SPAD units were similar to those found for the chlorophyll a, b and total contents although it differed in terms of the mathematical model since, in this case, it was adjusted to linear equations. The chlorophyll index values ranged between 34.0 and 51.7 for the SPAD units and 0.7 and 2.46% for the nitrogen content in the leaf tissue (Fig. 5A, B and C).

According to the linear model, coefficient of determination values (R^2) greater than or equal to 0.90 ($n = 44$; $P \leq 0.0001$) were obtained in all evaluated stages (Fig. 5), indicating the existence of a high correlation between the foliar nitrogen content and total chlorophyll index (SPAD). The results agree with the observations of Mendoza-Tafolla *et al*. (2019), who indicates that readings with a SPAD type meter presented a high correlation ($P=0.01$) with the foliar nitrogen concentration in *L. sativa* plants. Which were adjusted to a simple linear regression, with a positive correlation coefficient of 0.95 and a coefficient of determination (R^2) of 0.90. On the other hand, Yue *et al*. (2020), when performing a correlation analysis of the SPAD method and the nitrogen concentration in wheat plants, indicated adjustment to a second-degree polynomial in different phenological stages and at different times of evaluation.

The nitrogen content in the soil or nutrient solution influenced the SPAD readings and their correlation with the percentage of nitrogen in the leaves. In the present study, the correlation between the methods was positive, connected to the amount of data used and the selection of suitable leaves for the measurement (Figure 5A, B and C). The significance of the correlation depended on the position of the leaf, indicating that, in the middle third, there was high N mobility in the plants since it moves more easily to organs with high transpiration and active photosynthetic processes, as compared to leaves in the upper third, in which said processes are incipient, or leaves in the lower third, in which said processes can be affected by senescence processes (Malavolta *et al*., 1997). Since the purpose of the SPAD method is to quickly diagnose element deficiencies without visual symptoms, the evaluation of middle-third leaves is more accurate over time (Azia and Stewart, 2001).

CONCLUSION

In the present study, there was a significant correlation between the measurement of the total chlorophyll index (SPAD) and the concentration of chlorophyll a, b and total extractable and the percentage of nitrogen in the foliar tissue, quantified in leaves from the middle third of the guava plants in three phenological stages of the reproductive phase, cultivated under the conditions of the municipality of Vélez-Santander. Linear regression equations were obtained for the nitrogen content in the leaf tissue, and second-degree polynomials were obtained for the chlorophyll a, b and total extractable from the SPAD units.

This study confirmed the usefulness and convenience of using the SPAD technique for the non-destructive determination of chlorophyll and nitrogen contents in foliar tissue in guava plants under field conditions since it is an easy, fast and efficient method; however, the sample used must be representative and sufficient for generating a good correlation.

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Progress in the study of cholupa (*Passiflora maliformis* L.) phenology in producing areas of Colombia

Avances en el estudio de la fenología de la cholupa (*Passiflora maliformis* L.) en áreas productivas de Colombia

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Cholupa fruits followed phenologically.

Photo: J. Ocampo-Pérez

ABSTRACT

The cholupa or stone granadilla (*Passiflora maliformis* L.) is one of the eight cultivated species in the genus *Passiflora* L. However, the phenological development of this species has not been investigated. This study aimed to assess some phenological aspects of cholupa growth in the vegetative and reproductive phases in the town of Rivera (Colombia). The plant phenology during the vegetative and reproductive phases of growth was evaluated with weekly measurements of the length and number of stem nodes, primary and secondary branches, and longitudinal and transverse fruit diameters in commercial crops. The curves described the dynamics of growth in the main stem and primary and secondary branches and their respective equations using the curve fitting technique, which was fit to sigmoidal-logistic models with high statistical reliability. The time from anthesis to fruit maturity was between 50 and 60 days; the final longitudinal and transverse diameters averaged 54 and 53 mm, respectively. The phenological BBCH scale was established for the major phenological stages (E1: leaf development of the main stem, E3: stem elongation, E5 and E6: appearance and development of floral organs and flowering, and E7: fruit development). These aspects of cholupa development were similar to reports on other *Passiflora* species, providing very useful results for defining agronomic practices in cultivation and breeding programs.

Additional key words: growth curves; development stages; BBCH; stone granadilla; Passifloraceae.

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RESUMEN

La cholupa o stone granadilla (*Passiflora maliformis* L.) es una de las ocho especies cultivadas del género *Passiflora* L. Sin embargo, el conocimiento sobre el desarrollo fenológico de esta especie no se ha investigado. Este estudio tuvo como objetivo evaluar algunos aspectos de la fenología del crecimiento de cholupa en las fases vegetativa y reproductiva en la ciudad de Rivera (Colombia). Para esto, se evaluó la fenología de las plantas durante las fases vegetativas y reproductivas del crecimiento mediante mediciones semanales de la longitud y el número de nudos del tallo, ramas primarias y secundarias, y el diámetro longitudinal y transversal del fruto en cultivos comerciales. Se obtuvieron las curvas que describen la dinámica de crecimiento del tallo principal y las ramas primarias y secundarias y sus respectivas ecuaciones mediante la técnica de ajuste de curvas, que se ajustó a modelos sigmoidales-logísticos con alta confiabilidad estadística. El tiempo desde la antesis hasta la madurez del fruto fue entre 50 y 60 días; los diámetros longitudinales y transversales finales promediaron 54 y 53 mm, respectivamente. La escala fenológica BBCH se estableció para las principales etapas fenológicas (E1: desarrollo de la hoja del tallo principal, E3: alargamiento del tallo, E5 y E6: apariencia y desarrollo de los órganos florales y floración, y E7: desarrollo del fruto). Estos aspectos del desarrollo de la cholupa fueron similares a los reportados para otras especies de Passiflora y son resultados muy útiles para definir las prácticas agronómicas en los programas de cultivo y reproducción.

Palabras clave adicionales: curva de crecimiento; estados de desarrollo; BBCH; stone granadilla; Passifloraceae.

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INTRODUCTION

The cholupa or stone granadilla (*Passiflora maliformis* L.) is one of the eight cultivated species in the genus *Passiflora,* such as yellow passion fruits (Ocampo *et al.*, 2015a), sweet granadillas (Ocampo *et al.*, 2015b), and others with agronomic potential (Hurtado-Salazar *et al*., 2020). This species is native to northern Ecuador, Colombia, Venezuela and the Antilles and was introduced to Europe as an ornamental plant in greenhouses. The flower is hermaphrodite with a high degree of self-incompatibility (95%), and is cross-pollinated (allogamy) mainly by insects of the genus *Xylocopa* spp. (Ocampo *et al*., 2015a). The fruits are characterized by a high content of total phenols (277.00 mg gallic acid equivalent/L fresh weight-FW) and the total antioxidant activity (1,685.00 μmol Trolox/L FW) (Fischer *et al*., 2018). The cultivation of the cholupa has become a line of economic and social importance in the Department of Huila, Colombia, because of its high profitability and the generation of rural jobs, which can reach 648 wages per hectare for a three-year cycle. Cholupa is grown commercially in 14 municipalities with about 200 ha, Rivera being the principal one. In Huila, around 1,200 t of fruit were produced in 2018 (Agronet, 2019), of which 10% were pre or post-harvest losses, and 90% were destined for consumption in the regional market as fresh fruit, mainly in the preparation of juices. The production behavior throughout the year is continuous, with two marked harvest times: the first is October to January, the last two months having the highest production, and the second is from the first week of April to the end of May.

The effect of physical, chemical and biotic environments on the physiological mechanisms of a plant is known as plant ecophysiology (Larcher, 2003). The cholupa is cultivated in Colombia between 600-1,000 m a.s.l. (Ocampo *et al.,* 2015a), with temperatures between 26 and 32°C, rainfall between 1,200 and 1,450 mm, relative humidity between 60 and 70%, and 8-11 h light/d (Fischer *et al*., 2018). These ecological conditions greatly affect the duration of the phenological stages of the plant (Fischer *et al*., 2009).

The study of biological events and their causes as a function of biotic and abiotic factors, as well as their relationship between the phases characterized by these events, within one or more species, is known as phenology (Silva *et al*., 2007). Phenological characterizations through the stages of the phenophases (vegetative and reproductive) provide greater detail in the description of plant cycle, helping in the prediction of seed collection for seed production and in the conservation and breeding programs of the species (Rego *et al*., 2006). Furthermore, the phenological identification of growth stages of mono- and dicotyledonous plant species uses the extended BBCH scale, an acronym of Biologische Bundesanstalt, Bundessortenamt und Chemical Industry (Meier, 2001). The scale has 10 main stages or plant development stages that are clearly identifiable and visible, starting with budbreak (stage 0) and ending with the latency period (stage 9); intermediate values include leaf development, buds, flowers, fruit development and subsequent ripening; secondary stages are also listed from 0 to 9 and are related to ordinal or percentage values of development (Mayor, 2011). Another useful tool to identify the physiological behavior of fruit species uses growth curves and development events, which are genetically determined, hormonally regulated and can be modified by environmental conditions (Garriz *et al*., 2005). They strengthen knowledge on a system (López *et al*., 2005), evaluate possible management strategies, and provide an approach for the potential yield (Cañizares *et al*., 2003). Among the non-linear models used to characterize growth and/or development as a function of time, the logistic, the exponential, and the monomolecular models stand out (García, 2008; Moreno-Medina *et al*., 2016; Almanza-Merchán *et al*., 2017).

Although there is an empirical knowledge from cholupa producers on the duration and the time (in the year) where the most interesting phenological events take place (Ocampo *et al.,* 2015a), these events have not been described according to the BBCH scale, and there is no information on growth and development under this climatic condition. Therefore, the present study was proposed to identify the main phenological stages and approximate times of duration in order to generate a guide that estimates some growth and development parameters that help achieve integrated crop management.

MATERIALS AND METHODS

The study was carried out in the municipality of Rivera (02°44’29.4’’ N, 075°77’19.5’’ W), province of Huila (Colombia), located at 595 m a.s.l., during the period of December, 2014 to December, 2015. The fruit growth curve was used on a commercial crop in the municipality of Campoalegre (2°43’26.9’’ N, 75°15’50.7’’ W), located at 788 m a.s.l., with an average temperature of 27ºC and a relative humidity of 65%. The plants, 15 d old in a 4×4 m arrangement, were evaluated with a trellis system that consisted of a 2 m high mesh, on which the different types of crop branches were conducted and fixed. The climate of the region is classified as As according to Köppen, dry tropical area with a dry summer. For the characterization of meteorological conditions during the experiment, the climatological data, relative humidity (RH, %) and temperature (°C) were obtained from datalogger weather sensors (Onset HOBO UX100-003 humidity and temperature data logger, Micro-DAQ, Contoocook, NH) every 6 h, with an accuracy of 3.5%. The precipitation variable was taken from climate databases (Wordclim).

Starting at 18 days after planting (DAP), the following variables were recorded weekly for 150 DAP in the selected plants: Length (cm) and number of nodes of the main stem and primary and secondary branches. Subsequently, for the fruit growth curve, ten flowers were marked in anthesis and weekly records were taken of the longitudinal and the transverse diameters of ten fruits per plant until ripening. the One plant was the sampling unit for the growth variables. The variables of each sample were used to model growth with the logistic function (1)

$$Y = \frac{\alpha}{1 + Exp(b - c \times X)} \tag{1}$$

Where, α, the upper asymptote, is the maximum magnitude of the variable, c is the parameter that determines the slope of the curve, b is the moment when the maximum growth rate is achieved and X is the time (Seber and Wild, 1989). Based on these coefficients, obtained with STATISTIX 9, 2008, the growth curves for the plants established in the field were obtained. The BBCH-scale was used to identify the phenological development stages of the plants.

RESULTS AND DISCUSSION

Behavior of climatic variables

For the average temperature (Fig. 1A), an irregular behavior was observed with values between 21 and 30°C and marked peaks for the months of February and March, 2015 (39.4 and 37.3°C, respectively). An oscillating trend was observed for the relative humidity throughout the evaluation; the lowest values were in the month of February, 2015 (Fig. 1B). During this period, a high rate of vegetative growth of the stem and primary branches was detected.

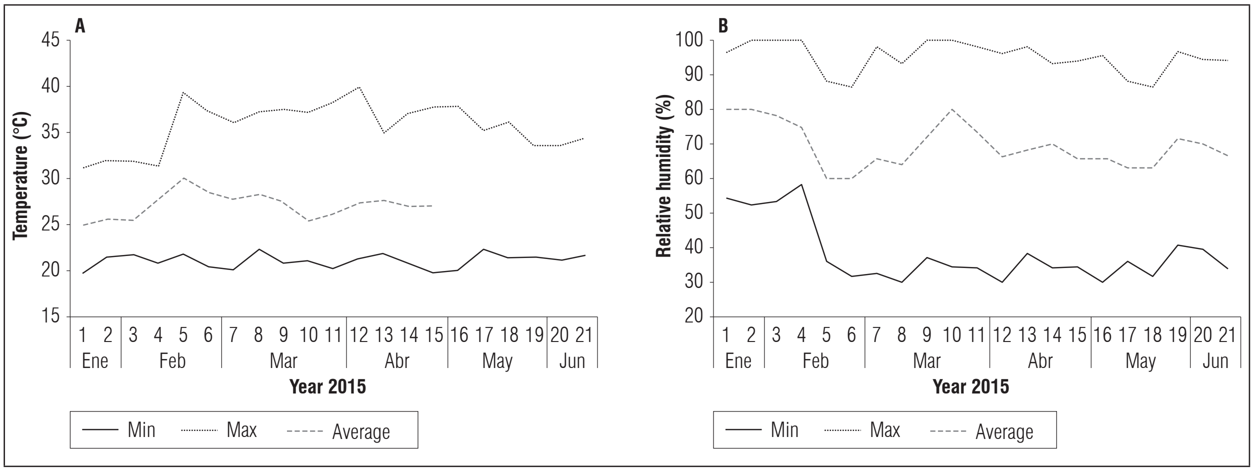

Figure 1. Behavior of (A) temperature and (B) relative humidity in the municipality of Rivera, Huila for 2015.

Differences were observed between the daytime and nighttime temperatures, with fluctuations of 19.7°C; likewise, at night, the RH reached 99.8%, which was a positive condition for the occurrence of diseases, and, during the day, it dropped to 32.2%, with a difference of 67.7% between the 2 d. It should be noted that this low RH caused dehydration of the pollen and stigmatic fluid, reducing the fertilization process and facilitating flower abortion (Ocampo, 2013; Fischer *et al*., 2009).

Vegetative phase

During the vegetative phase, the development stages 1 (leaf development) and 3 (main stem elongation) were identified according to the BBCH scale (Meier, 2001). The times are presented in days after planting (DAP), averaging the date on which 50% of the plants reached each stage of development.

In the principal growth stage 1 (Tab. 1), it was possible to identify the secondary stages corresponding to the development of the third leaf (code 13) on day 6 DAP, along with the appearance of the fifth leaf (code 15) at 10 DAP, until obtaining nine true leaves (BBCH code 19) on 17 DAP. Afterwards, the development of new leaves continued, and it is possible to observe the appearance of the first tendril accompanying leaves, which had the characteristic climber habit of these species, one of the parameters that producers take into account when transplanting to the definitive place, along with the height of the plant.

The cholupa is characterized as a liana whose growth is continuous, with lateral flowering and basitonic branching (Tovar, 2009). During its development, lateral shoots begin to appear in the basal portion, below and above the knot with the first tendril.

Growth and development of the principal stem

This plant is a climbing semi-perennial vine, with a cylindrical stem, glabrous or finely pubescent, green in color, striated, herbaceous and woody towards the base, with up to 12 cm in diameter (Ocampo *et al*., 2015a). At 23 DAP, the main stem reached an average of 37.03 cm. Starting at 30 DAP, the plants increased the growth rate, which, at 88 DAP, reached a maximum length of 455 cm, with a total of 68 nodes, presenting an emission rate of 2.8 nodes per week (Fig. 2) and an R^2 of 0.91 (Tab. 2). Schwartz (2013) stated that the phenological stages are good indicators of plant development rates, and, according to Angulo (2003), this behavior can be calculated with the period that lapses from node appearance to the appearance of the next node. At that time, pruning was carried out with cutting. The cut was made at the top of the node in order to activate lateral buds to stimulate the emission of the primary branches.

Growth and development of primary branches

The branches can reach up to 30 m in length, with knots and internodes that form a lower bud, two linear, stipulated provisions (orange), a leaf and a tendril that provide plant support for the plant (Ocampo *et al.,* 2015a). According to the typical behavior of passion flower crops, the growth of lateral shoots is successive, meaning that vegetative primary branches

Table 1. **BBCH scale established for the growth and development of cholupa (*P. maliformis*) in Colombia.**

Phenological growth stages		
BBCH	Description	DAP
10	Total developed cotyledons	n.d
11	Development of the first leaf	n.d
12	Development of the second leaf	n.d
13	Development of the third leaf	6
14	Development of the fourth leaf	8
15	Development of the fifth leaf	10
16	Development of the sixth leaf	12
17	Development of the seventh leaf	14
18	Development of the eighth leaf	15
19	Development of the ninth leaf	17
Principal growth stage 3: main stem development		
31	10% of maximum length of the main stem	25
32	20% of maximum length of the main stem	34
33	30% of maximum length of the main stem	42
34	40% of maximum length of the main stem	47
35	50% of maximum length of the main stem	52
36	60% of maximum length of the main stem	57
37	70% of maximum length of the main stem	66
38	80% of maximum length of the main stem	71
39	The main stem of the plant reached 90% - 100% of its maximum length.	80
Principal growth stage 5 and 6: inflorescence emergence and flowering		
51	Visible floral bud	90
55	Floral cartridge with 50% of the characteristic size of the species	95
58	Maximum size of the floral cartridge	99
61	Beginning of flowering: 10% of flowers are open	100
65	Full bloom. 50% of flowers in anthesis	108
69	End of flowering: fruit set	112
Principal growth stage 7: development of fruit		
70	Visible fruit	114
71	The fruit reaches 10% of the final size	117
72	The fruit reaches 20% of the final size	121
73	The fruit reaches 30% of the final size	126
74	The fruit reaches 40% of the final size	132
75	The fruit reaches 50% of the final size	140
76	The fruit reaches 60% of the final size	150
77	The fruit reaches 75% of the final size	161
78	The fruit reaches 90% of the final size	173
79	Fruit has reached typical form and size	180

DAP = days after planting.

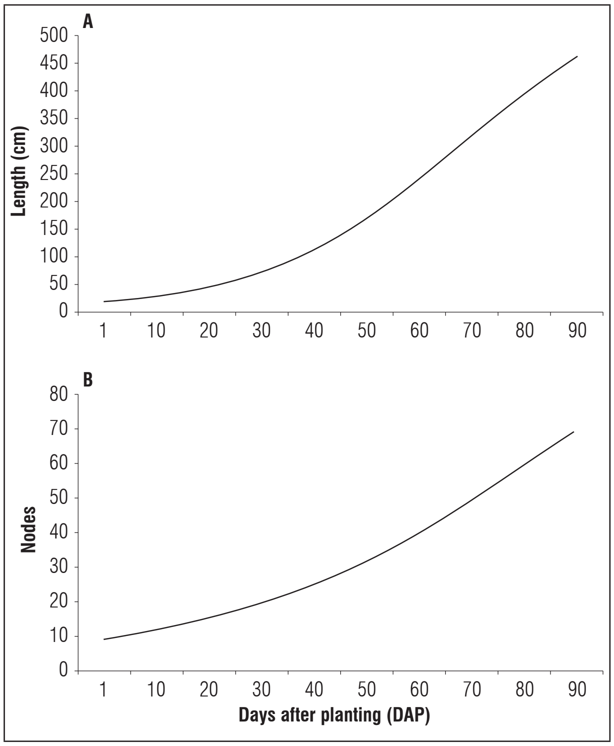

Figure 2. **Length (A) and average number of nodes (B) of the main stem of cholupa (*P. maliformis*) plants.**

are generated from the main stem; afterwards, secondary branches originate from nodes of the primary branches, which, in the first production cycle, make up the group called "loaders or producers", along with some tertiary branches, which must be thicker to support the weight of the reproductive structures that become fruit The curves for the length of the primary branching were sigmoid curves, characterized by a very rapid growth phase because of the budding response of the vegetative buds after emergence (Fig. 3).

The maximum length of the primary branches (214 cm) was achieved near day 57 after pruning, along with the greatest number of nodes (35) in the evaluated period, with an emission rate of 0.86 nodes per day. After the emergence of the primary branches, the re-sprouting of secondary and tertiary branches begins because, physiologically, there is always a branch bud and the anterior buds break their dormancy.

Growth and development of secondary branches

The length and number of nodes of the secondary branches adjusted to the logistic model of three

Table 2. Equation of growth parameters in cholupa (*P. maliformis*) plants.

Parameter	Equation	Pseudo R^2
Length of the main stem	$Y = \frac{615.66}{1 + Exp(3.5987 - 0.0525 \times DAP)}$	0.9165
Number of nodes of the main stem	$Y = \frac{153.38}{1 + Exp(2.8092 - 0.0327 \times DAP)}$	0.9181
Length of the primary branches	$Y = \frac{225.35}{1 + Exp(5.0968 - 0.1621 \times DAP)}$	0.8921
Number of nodes of the primary branches	$Y = \frac{42.45815}{1 + Exp(2.7818 - 0.0899 \times DAP)}$	0.884
Length of the secondary branches	$Y = \frac{143.8392}{1 + Exp(2.7323 - 0.1934 \times DAP)}$	0.8137
Number of nodes of the secondary branches	$Y = \frac{29.92532}{1 + Exp(1.79061 - 0.1115 \times DAP)}$	0.73
Longitudinal diameter of the fruit	$Y = \frac{54.069}{1 + Exp(2.2440 - 0.4571 \times DAP)}$	0.8752
Transverse diameter of the fruit	$Y = \frac{54.361}{1 + Exp(2.8669 - 0.4888 \times DAP)}$	0.870

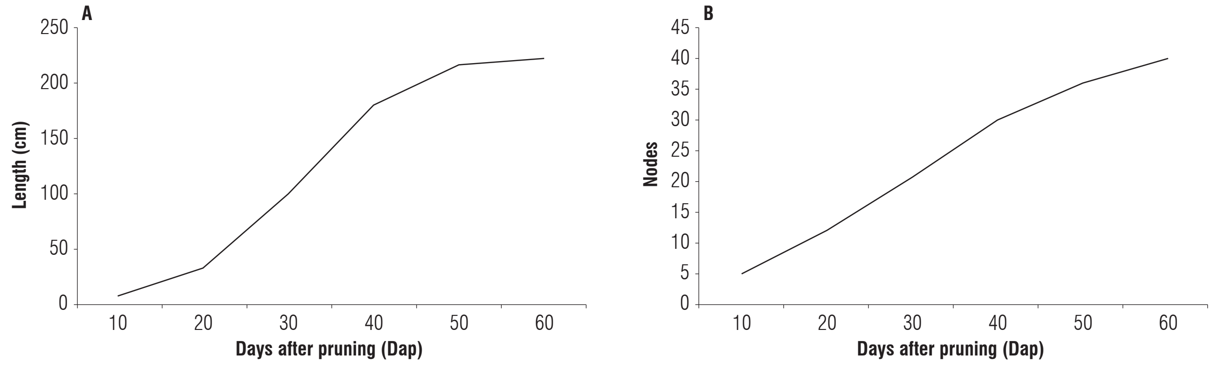

Figure 3. Length (A) and average number of nodes (B) of the primary branches of cholupa (*P. maliformis*) plants.

parameters over time; the highest average value was 138.4 cm, reaching maximum length approximately on 30 DAP (Fig. 4). The maximum number of nodes was achieved at 25-30 DAP (Fig. 4B), and the final number of nodes was 30. Thus, the rate of emission of nodes in the secondary branches in the initial phase (6-14 DAP) was 12 nodes per day, decreasing progressively, until reaching 0.9. This condition could be due to the presence of thrips (*Neohydatothrips* sp.) that caused damage to the terminal shoots and retarded branch growth but not the emission of nodes, resulting in branches with shorter internodes than healthy branches (longer internodes) (Santos *et al*., 2012).

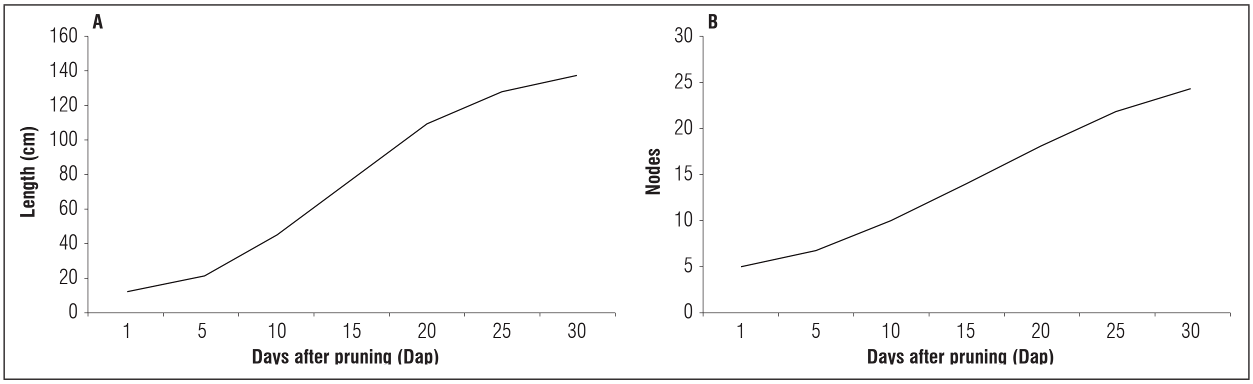

Figure 4. Length (A) and average number of nodes (B) of the secondary branches of cholupa (*Passiflora maliformis* L.) plants.

The description of stage 39 with the elongation of the main stem (Tab. 1) included the maximum length of the main stem, 4.0 m, with a tutored system with an average height of 2 m. The grower allowed growth of the stem 2 m beyond the wiring before emergence and stimulated primary branch growth. Ten percent of the main stem growth of the cholupa was reached at 25 DAP on average, 50% was reached at 52 DAP, and 100% was obtained at approximately 80 DAP. The leaves are inserted on the stem using a petiole that is 2.3 to 8.5 cm in length with two subsesile glands (eventually two pairs), located in the lower half. The peduncle can have 3 to 9.5 cm in length and three bracts (cap) at its apex that are green and resemble leaves that are 5 to 8 cm long and 2.5 to 5 cm wide, which protect the flower and fruit in the stages of development (Ocampo *et al.,* 2015a) (Fig. 5).

Reproductive phase

In the *Passiflora* species family, as in the cholupa, a stage of completely vegetative growth (juvenile phase of crop development) occurs with subsequent annual cycles of growth, where vegetative and reproductive stages occur simultaneously or slightly overlapping (Melgarejo *et al.,* 2015). This fact results from the indeterminate growth habit of the crop, the origin and the adaptation to environments where it currently grows. The apex of growth of all branches is apt to form a floral primordium at the level of each node. The reproductive phase begins with the appearance of flower buds and ends with fruit ripening, in which the fruit has developed characteristics of appearance and texture, which is related to physicochemical changes in the pulp, such as flavor and aroma. Stages 5 and 6 include the appearance and development of the floral organ and flowering (Fig. 6 and Tab. 1). This process was evaluated on the secondary branches of the crop because it was the first cycle of production. The stages were identified from the emergence of the floral bud (stage 51), which is known as a floral cartridge until it reaches its maximum size (stage 58), where all parts of the flower are fully developed

Figure 5. Principal growth stage 3: main stem development in cholupa (*P. maliformis*) plants. 5a) Stage 19, development of the ninth leaf. 5b) Stage 34, 40% of maximum length of the main stem. 5c) Stage 33, 70% of maximum length of the main stem. 5d) Stage 39, the main stem of the plant has reached 90% - 100% of its maximum length.

and the process of floral opening begins. The flower is generally alone, or rarely in pairs, pendular, pentamera, hermaphrodite, showy and with a pleasant aroma, with a length of 4.5 to 6.5 cm and a width of 4 to 5 cm, along with five petals and five sepals, lanceolate, reflexed, white and an interior mottled red-purple color (Ocampo *et al.,* 2015a) (Fig. 6).

The full flowering (stage 65) and end of flowering or fruit set (stage 69) were also established. It should be noted that, in cholupa crops, the process of floral opening, pollination and flower closure takes place in 12 h, and the fruit set is estimated at approximately 2 days after anthesis (DAA).

The beginning of flowering is not uniform because plants originate from seeds, and the species is allogamous (Tovar, 2009). Flowering begins with the appearance of flowers on the lateral and basal branches. The flower, one per leaf axilla, is ephemeral, and anthesis takes only 12 h (Tovar, 2009). The first flowers in anthesis were observed at 108-110 DAP, and, at 180 DAP, the first ripe fruits were received. However, a high abortion of flowers was observed before

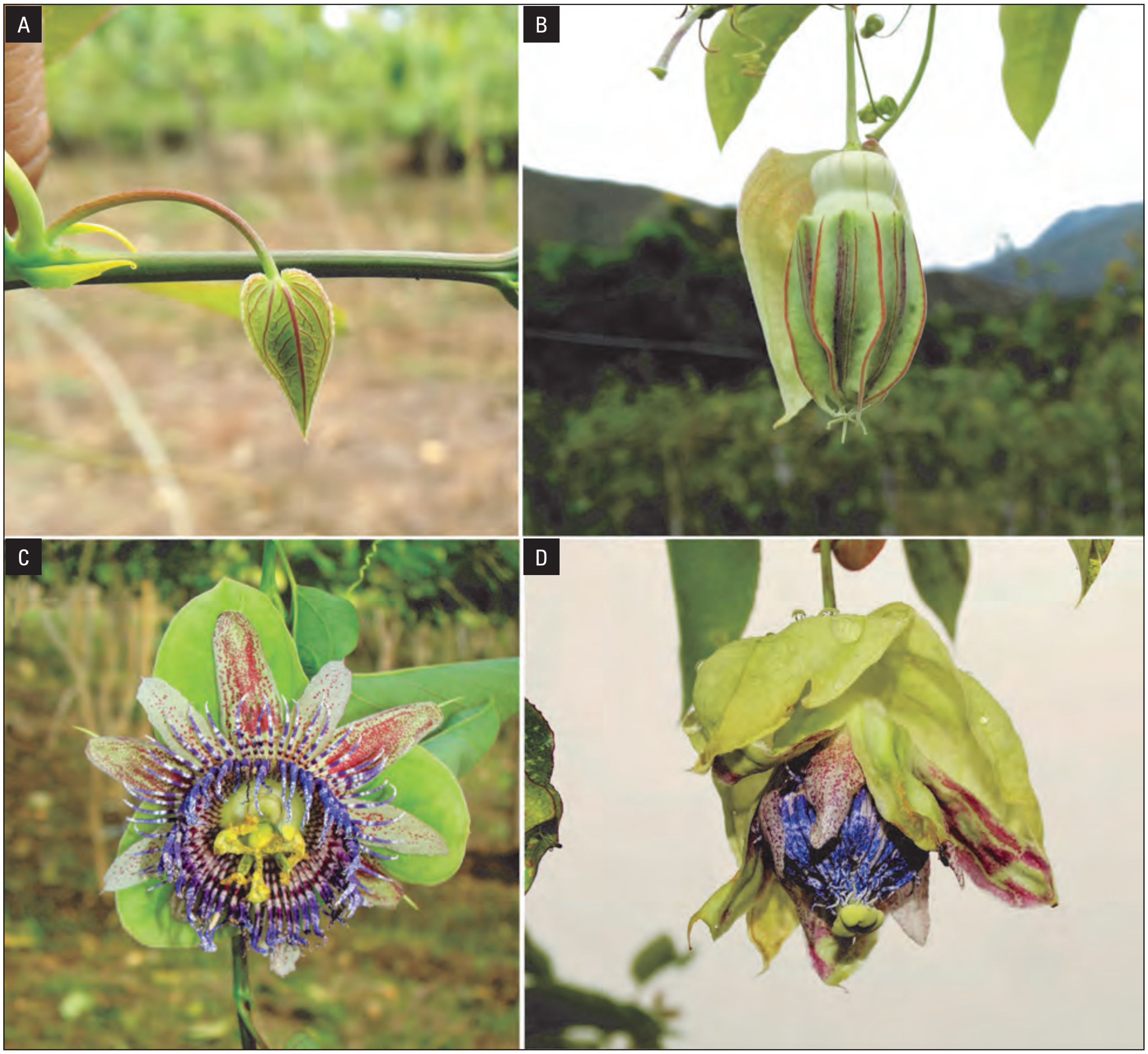

Figure 6. Principal growth stages 5 and 6: inflorescence emergence and flowering in cholupa (*P. maliformis*) plants. 5a) Stage 5. Stage 51: Visible floral bud. 5b) Stage 58, maximum size of the floral cartridge. d) Stage 6. Stage 61, Beginning of flowering: 10% of flowers are open and stage 65, full bloom. 50% of flowers in anthesis.

anthesis, which was manifested by the presence of vain structures between the apex and the flowers. After this event, a new fall of flowers can be explained by natural abortion, excess humidity and/or lack of pollination, which turns into a decrease in production since it has been indicated that 67% of production and fruit quality depend on pollinators that decrease activity at that time (Tovar, 2009).

The BBCH codes with the respective description for stage 7: fruit formation (Fig. 7 and Tab. 1) was determined at 117 DAP, which is equivalent to a fruit between 7-10% of the final size, following the BBCH stages for fruit growth (stages 71-78), where 50% of the fruit size is reached at 140 DAP, 75% of the size is achieved by 161 DAP, 90% is seen by 180 DAP, and, finally, the maximum size on average is reached at 185 DAP. The fruits, upon maturation, did not have a color change, so abscission above the bracts that support it is used as an indicator of maturity.

Growth and development of fruits

The fruit is a berry in shape, spherical or ovoid, with an extremely hard shell (pericarp) (eventually soft) that is smooth and waxy, about 3.0 to 4.5 mm thick, with a white mesocarp (Ocampo *et al.,* 2015a). Among the factors that determine development and final fruit size, Cavichioli *et al*. (2006) referenced genetic characteristics, temperature, number of flowers per plant and fruits in development; grower techniques could directly influence final size, such as irrigation, fertilization and pruning.

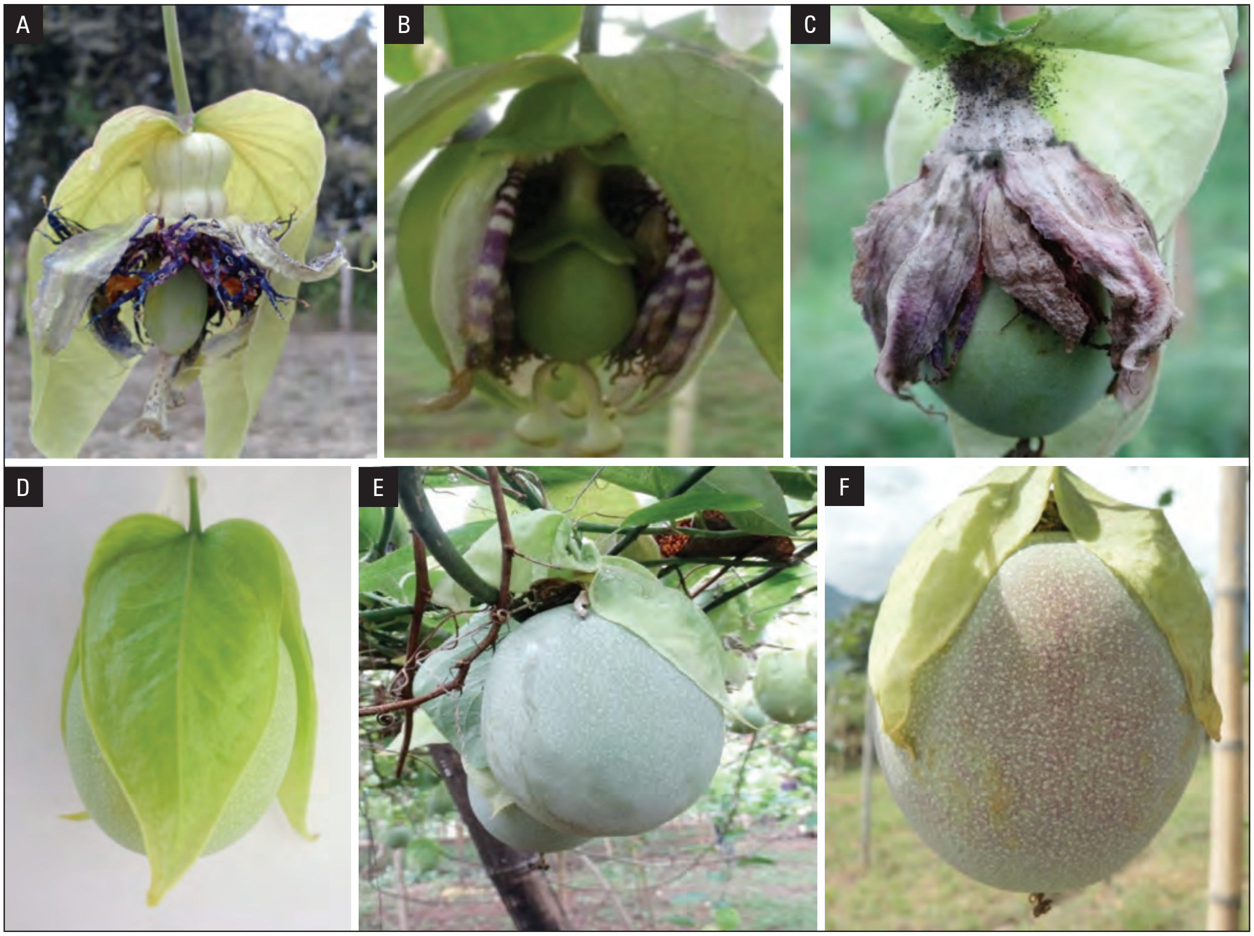

Figure 7. Principal growth stage 7: Fruit development in cholupa (*P. maliformis*) plants. 7a) Stage 70, visible fruit. 7b) Stage 71, The fruit reaches 10% of the final size. 7c) Stage 74, the fruit reaches 30% of the final size. 7d) Stage 74, the fruit reaches 40% of the final size. 7e) Stage 77, the fruit reaches 75% of the final size. 7f) Stage 79, fruit has reached typical form and size.

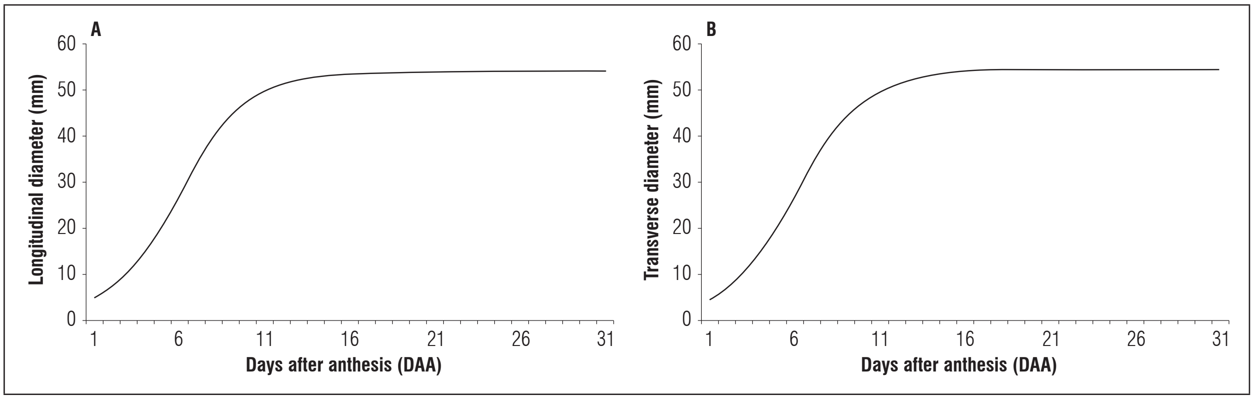

Figure 8. Growth curves adjusted to a logistic model with three parameters of A, longitudinal and B, transverse diameters of the cholupa fruits.

The analyzed variables showed a simple sigmoidal growth pattern (Fig. 8) as the result of the analysis with the fruit weight or size as a function of time (Hunt, 2003). The simple sigmoid pattern is consistent with that found in passion fruits (Arjona *et al*., 1991) and in gulupas (Lederman and Gazit, 1993). For each variable, the model with the best fit was chosen according to a more homogeneous distribution of the residuals, the highest coefficient of determination for prediction (R^2, Tab. 2), and the lowest mean square error.

The evolution of the longitudinal diameter was adjusted to the logistic model with three parameters, with an R^2 of 0.87 and a simple sigmoid growth pattern (Fig. 5A). Accelerated growth was observed until 15 DAA, where it tended to stabilize; a similar behavior was found in gulupas by Flórez *et al*. (2012), in passion fruits by Gómez *et al.* (1999), and in sweet granadillas by García (2008). The longitudinal diameter presented values very close to the transverse diameter (54 and 53 mm, respectively), which differed from that observed by García (2008) in sweet granadillas and reflected the characteristic spherical shape of cholupa fruits.

The increase in the transverse diameter of fruits was described with the logistic model with three parameters, with an R^2 of 0.91 (Fig. 5B) and presented a behavior similar to that of yellow passion fruits by Villanueva *et al*. (1999). In related species, the exponential phase occurs between 7 and 15 DAA, depending on the environment where the fruits grew; the growth rate decreased afterwards and tended to be almost constant after 20 d.

Based on the model adjusted to the fruit growth, it is possible to predict that harvest can be programmed 50 or 60 d after flowering since the longitudinal and transverse diameters tended to stabilize. The values estimated by the model explained each variable in relation to the field observations and, therefore, properly interpreted the physiological processes taking place in the fruits in each stage.

CONCLUSIONS

The vegetative and reproductive development phases of cholupa plants established in the province of Huila were described for the first time using the phenological BBCH scale.

The growth pattern of cholupas is a simple sigmoid type with three phases, which may vary slightly depending on the environmental conditions, relative humidity and precipitation.

The logistic model showed a good fit for describing the growth of the fruits under the specific conditions in the municipality of Campoalegre. These models can be used to schedule crop labor and predict the harvest time.

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Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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SCIENTIFIC NOTE

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Promissory *Passiflora* L. species (Passifloraceae) for tolerance to water-salt stress

Especies promisorias de *Passiflora* L. (Passifloraceae) por su tolerancia al estrés hídrico-salino

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Promissory Passifloraceae species under hydroponic conditions with water-salt stress.

Photo: A. Hurtado-Salazar.

ABSTRACT

The aim of this study was to determine tolerance to water and salt stress in four cultivated *Passiflora* L. species. Eleven accessions of four *Passiflora* species of commercial interest from different plantations were evaluated. The experiment design used sub-split plots, where the plot was the percentage of water saturation in relation to the requirements of the crop (33 and 100%). The subplot was established with the saturation levels of salt (sodium chloride reactive level - 99.9% purity) (EC: 1.5 and 5.5 dS m^{-1}) with the 11 accessions in a completely randomized array with five replications, where the experiment unit was one plant. The results showed that accessions m11, m13 (*P. edulis* f. *flavicarpa*) and m15 (*P. tarminiana*) were tolerant to salinity and drought; accessions m2 (*P. edulis* f. *edulis*), m12 and m14 (*P. edulis* f. *flavicarpa*) were moderately tolerant. These observations provided a basis for future studies on drought tolerance in *Passiflora*, which must be followed up with field evaluations.

Additional key words: *Passiflora* spp.; abiotic stress; dry weight; genetic resources; stress physiology.

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RESUMEN

El objetivo de este estudio fue determinar la tolerancia al estrés hídrico y salino en cuatro especies cultivadas de *Passiflora* L. Se evaluaron 11 accesiones de cuatro especies de *Passiflora* de interés comercial provenientes de diferentes plantaciones. El diseño experimental fue en parcelas subdivididas, donde la parcela principal fue el porcentaje de saturación de agua en relación con los requisitos del cultivo (33 y 100%). La subparcela se estableció por los niveles de saturación de sal (cloruro de sodio nivel reactivo – 99,9% de pureza) (CE: 1.50 y 5.5 dS m^{-1}) con 11 accesiones en una disposición completamente aleatoria, con cinco repeticiones, donde la unidad experimental fue una planta. Los resultados mostraron que las accesiones m11, m13 (*P. edulis* f. *flavicarpa*) y m15 (*P. tarminiana*) se consideraron tolerantes a la salinidad y la sequía; las accesiones m2 (*P. edulis* f. *edulis*), m12 y m14 (*P. edulis* f. *flavicarpa*) fueron moderadamente tolerantes. Estas observaciones son la base para futuros estudios de tolerancia a la sequía en *Passiflora*, que deben ser seguidos por evaluaciones de campo.

Palabras clave adicionales: *Passiflora* spp.; estrés abiótico; peso seco; recursos genéticos; fisiología del estrés.

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INTRODUCTION

Colombia is among the countries with a high number of cultivated *Passiflora* species because of its variety of ecological habitats (Ocampo *et al*., 2016). A total of nine species are cultivated from sea level to 2,800 m, and the more important ones are *P. edulis* f. *flavicarpa* Degener (yellow passionfruit), *P. ligularis* Juss. (sweet granadilla), *P. edulis* f. *edulis* Sims (purple passion fruit), *P. maliformis* L. (stone granadilla) and *P. tarminiana* Coppens (Banana passion fruit). The cultivated area in Colombia for these species covers about 10,000 ha (Agronet, 2019). Water is essential for passion fruit plants because it promotes good growth and increases productivity, allowing continuous and regular production with good fruit quality (Jehová *et al*., 2013).

The productivity and development of plants from the genus *Passiflora* are affected by solar radiation, temperature, sunshine and soil moisture loss. Factors such as water stress and extreme salinity conditions (> 2.5 dS m^{-1}) limit the productive potential of passion fruit plants (Cavalcante, 2012). A frequent and adequate water supply allows continuous flowering and fruiting so long as other environmental conditions are favorable. Therefore, to achieve good yield and good fruit quality, water must be supplied in adequate amounts.

According to Rodríguez *et al*. (2019), the term water deficit defines an essentially ecophysiological concept related to the limitation of water in tissue that is close to being synonymous with drought stress because it refers to any limitation in the ideal operation of a plant imposed a limited availability of water.

There are two types of water stress: one that is due to a total lack of rain and another caused by poor distribution of rain throughout a plant's growth period (Rodríguez *et al*., 2019); in both cases, agricultural production is seriously affected. Anthropogenic global climate changes and phenomena such as *El Niño* (Phenomenon of the Pacific) are typical examples that induce abnormal rainfall distribution in agricultural regions. When this phenomenon occurs in the Colombian regions of the Caribbean and the Andes, with the exception of Magdalena Medio, there are reductions in precipitation values for accumulated volume while the phenomenon lasts, near or above 20%. Regions such as Guajira, northern Cesar, the municipalities Atlantico, Bolivar, Sucre, Cordoba, the coffee triangle, Santander and Cundinamarca, in Colombia, show a highly marked decrease in precipitation, with values equal or higher to a 60% reduction in normal rainfall (Fan *et al*., 2017). Some of the affected regions due to drought are induced by the El Niño phenomenon, which hinders the production of the areas cultivated in passionfruits due to non-tolerance to the conditions of hydric stress. Likewise, fluctuations in the distribution of rainfall caused by global warming can increase risk from being repeatedly exposed to plant droughts (FAO, 2020).

Saline soils are defined as those adversely modified for the growth of most of these species as the result of the presence of soluble salts, exchangeable sodium or both in the root zone (Yertayeva *et al*., 2019). Soils affected by these salts are common in arid and semiarid regions because of low rainfall and high evaporation (Fageria *et al*., 2010).

Saline stress is related to two types of effects: osmotic and ionic. The first is caused by excess salts that are biophysical in nature, with osmotic effects standing out, restricting water transport (Silveira *et al*., 2010). Then, a sequence of reactions modulated by hormones is quickly triggered, leading to a restriction of stomatal opening and photosynthetic assimilation of CO_2. These effects predominate in the first phase of salt stress "osmotic phase", which occurs in the initial stages of plant exposure to salinity or in the presence of moderate levels of salts in contact with the root system. The same authors reported that the "osmotic phase" has a physiological response: acclimatization to stress in response to damage suffered caused by the salt stress. This is often diagnosed as symptoms of negative effects of salt stress but are actually normal plant physiological responses to overcome or acclimate to an adverse situation.

As excess saline ions accumulate in the cytosol of the cells, toxicity problems will arise (toxic or ionic phase) in plants exposed to salinity (Silveira *et al*., 2010). However, species differ widely in protoplasmic resistance or tissue resistance to salt stress. This ability to resist is linked mainly to the intensity of compartmentalization of saline ions in vacuoles and to the maintenance of a favorable K^+/Na^{2+} balance in the cytosol. Currently, this has been a target for the selection of resistant cultivars from some cultures. This study aimed to determine tolerance to water and salt stress in four cultivated *Passiflora* L. species.

MATERIALS AND METHODS

The present study was carried out between February and September, 2016, under hydroponic conditions in a greenhouse. The city of Manizales is in the coffee triangle, in the center of the Department of Caldas, with the coordinates 5°03'23.31" N and 75°29'41.56" W, at an altitude of 2,130 m a.s.l., with an average external temperature of 18°C, average annual rainfall of 2,000 mm and average relative humidity of 78%.

Eleven elite accessions of four *Passiflora* species of commercial interest were evaluated, which have been previously assessed, characterized and selected in several producing regions of Colombia based on the improvement program of Ocampo *et al*. (2013) and different gene banks from several institutions (Tab. 1).

Table 1. **Commercial *Passiflora* accessions from different gene banks in Colombia selected to evaluate abiotic stresses (salinity and drought).**

Species	Origin	Identification
P. edulis f. *edulis* Sims	Colombia-UNAL	m2–Cumbia2014
P. maliformis L.	Colombia-UNAL	m3–Cholupa2014
P. edulis f. *flavicarpa* D.	Colombia-UNAL	m4–Calfla04/2014
P. edulis f. *flavicarpa* D.	Colombia-UNAL	m6–Caufla01/2014
P. edulis f. *flavicarpa* D.	Colombia-UNAL	m7-Tolfla02/2012
P. edulis f. *flavicarpa* D.	Colombia-UNAL	m9-Atlafla01/2011
P. edulis f. *flavicarpa* D.	Colombia-UNAL	m11-Valfla10/2010
P. edulis f. *flavicarpa* D.	Colombia-UNAL	m12-Valluna/2014
P. edulis f. *flavicarpa* D.	Colombia-CIAT	m13-Huifla07/2014
P. edulis f. *flavicarpa* D.	Colombia-UCAL	m14-Calfla01/2015
P. tarminiana C & B	Colombia-UCAL	m15-Calmol01/2015

Source: Hurtado-Salazar *et al*. (2017).

The experiment design was completely randomized in an array of sub-split plots, where the plot was the percentage of water saturation in relation to the requirements of the crop (100 and 33%). The subplot consisted of saturation levels of salt (sodium chloride reactive level - 99.9% purity) (EC: 1.5 and 5.5 dS m^{-1}), and each sub-subplot contained the 11 *Passiflora* accessions in a completely randomized array with five replicates, where the experiment unit consisted of one plant.

240 seeds of each accession were planted, germinated in a hydroponic bed using rice husk as substrate, which was constantly kept wet until transplantation. After 15 d of germination, 54 seedlings of each accession were selected to ensure greater uniformity in the root system.

Subsequently, the plants were transferred to hydroponic beds that were 6 m long and 1.2 m wide to ensure the irrigation regime of the treatments (percentage of water saturation 100 and 33%, and salt saturation levels EC: 1.5 and 5.5 dS m^{-1}), according to the experiment design. A conduit system was used in a vertical trellis, with 12 gauge galvanized wire, 2 m above the hydroponic greenhouse bed soil. The plants were conducted on a single stem, with weekly pruning of the side branches. When the main stems surpassed the wire at 20 cm, they were bent down, forming a loop. The secondary and tertiary branches were maintained.

The Hoagland and Arnon's universal nutrient solution (1950), as modified by Niu *et al*. (2015), was used. To ensure the salinity of the treatments, each solution was brought to a value of EC: 1.5 and 5.5 dS m^{-1}, with the addition of reagent level sodium chloride (99.9% purity). To evaluate the interaction between the percentage of water saturation and salinity, electrical conductivity was added to the different treatments (1.5 and 5.5 dS m^{-1}) to the percentage of water of 100 and 33% for each experiment units of the evaluated accessions.

The production efficiency index (PEI) for dry matter was estimated to assess the effect of the water and saline stress. This index classified the tolerant genotypes. This ratio can be calculated as described below (Fageria *et al*., 2010):

$$\mathrm{PEI}=\left(\frac{\mathrm{PANS}}{\mathrm{PMANS}}\right)\times\left(\frac{\mathrm{PBNS}}{\mathrm{PMBNS}}\right)$$

where, *PEI* – efficiency index of dry matter production, *PANS* – production of dry matter with high salinity; *PMANS* – average production of the experiment with high salinity; *PBNS* – production with low salinity; *PMBNS* – average production of the experiment with low salinity.

Analysis of variance was performed, and the averages were compared using the Tukey test, with a confidence level of 95 using SAS statistical package (Statistical Analysis System, 2013).

RESULTS AND DISCUSSION

According to Fageria *et al*. (2010) and their proposed efficiency index of dry weight production in the aerial part. When the EIP is greater than 1.0, cultivars are classified as tolerant; the range of 0.5 to 1.0 indicates moderate salinity tolerance; a value less than 0.5 means the genotypes are classified as sensitive to salinity; therefore, it is worth noting that three accessions were tolerant, and three accessions were moderately tolerant (Tab. 2).

The total dry weight per accession decreased by approximately 21.2% under the water (33% humidity) and saline (5.5 dS m^{-1}) stress conditions, except in the tolerant accessions m15, m13 and m11, which showed a dry matter accumulation higher than other accessions evaluated under similar conditions of maximum stress (33% moisture and 5.5 dS m^{-1}) and optimum humidity and electrical conductivity (100%

Table 2. Influence of salinity on the dry matter of the aerial part in 11 *Passiflora* accessions and its classification in terms of tolerance to salinity according to the Production Efficiency Index (PEI).

Accessions	Level of salinity (dS m^{-1})		PEI	Classification[1]
	Control (1.5) (100% humidity) (g)	5.5 (33% humidity) (g)		
m2	5.41 c	0.70 de	0.51 e	MT
m3	4.00 d	0.60 e	0.32 f	S
m4	1.61 ef	0.42 e	0.09 g	S
m6	1.51 f	1.42 cd	0.29 f	S
m7	2.42 e	0.78 de	0.25 fg	S
m9	3.42 d	0.80 de	0.37 ef	S
m11	6.37 b	4.42 a	3.76 a	T
m12	5.42 c	0.69 de	0.50 e	MT
m13	6.42 b	2.17 c	1.86 c	T
m14	7.42 a	0.86 de	0.85 d	MT
m15	5.50 c	3.55 b	2.61 b	T
Mean	4.45	1.68 g		

[1] T= tolerant, MT= moderately tolerant, MS= moderately sensitive, and S= sensitive. Methodology according to Fageria *et al.* (2010). *The values followed by different letters differ significantly ($P<0.05$) according to the Tukey's test.

and 5.5 dS m^{-1}). The accessions m2 (*P. edulis* f. *edulis*), m12 (*P. edulis* f. *flavicarpa*), and m14 (*P. edulis* f. *flavicarpa*) obtained PEI values between 0.5 and 1.0 and were classified as moderately tolerant.

Cellular osmoregulation gives plants the ability to tolerate conditions with a lack of water and high salinity, with the expression of adaptive mechanisms that prevent the reduction of photosynthesis, changes in translocation and distribution of photoassimilates, and decreased yield. These events are of great importance in the normal growth and development of plants and, consequently, in crop productivity.

On the other hand, the sensitive accessions showed a 45% decrease in the accumulation of dry weight. A study on *P. edulis* by Gomes *et al*. (2012) showed a reduction in the dry matter content in the leaves, stem and roots when plants were exposed to water stress. Rodríguez *et al*. (2019) concluded that exposure of *P. ligularis* to droughts resulted in a significant decrease in leaf area, number of leaves per plant and length of the branches. However, the root-aerial ratio, based on the dry weight and specific weight of the leaves, increased as the water stress increased in the plants. The distribution pattern of dry leaves, branches, stems and roots was altered by the drought.

The negative effect of the water deficit was reflected in a growth reduction in the aerial part since a lot of assimilates were invested in the growth of the root system. Modifications in the root system occur because the root volume increases under water stress conditions, which favors water uptake and decreases the aerial part in order to reduce the rate of leaf transpiration (Rodríguez *et al*., 2019). Nevertheless, Hurtado-Salazar *et al*. (2018) found that the accession m15 (*P. tarminiana*) may be able to survive with salinity up to 5.5 dS m^{-1} (NaCl), which is high, with the removal of toxic ions. Possibly, the excretion of excess salt was done through epidermal glands. Sodium, in addition to other inorganic ions, is used for osmotic adjustment.

CONCLUSION

According to the PEI (Efficiency Index of dry matter production) methodology, accessions m11 (*P. edulis* f. *flavicarpa*), m13 (*P. edulis* f. *flavicarpa*) and m15 (*P. tarminiana*) were classified as salt tolerant, while accessions m2 (*P. edulis* f. *edulis*), m12 (*P. edulis* f. *flavicarpa*) and m14 (*P. edulis* f. *flavicarpa*) were classified as moderately tolerant. Studies on the tolerance mechanisms of the plants that presented potential, such as stomatal closure, expression and signaling of abscisic acid at the cellular level, osmotic adjustment, and modification of gene expression, under the levels of abiotic stress are recommended.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Relationship between soil fertility and plantain nutrition in Cundinamarca (Colombia) with the incidence of two bacterial diseases

Relación entre la fertilidad del suelo y la nutrición del plátano en Cundinamarca (Colombia) con la incidencia de dos enfermedades bacterianas

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Symptoms of bacteriosis and moko of plantain in the Department of Cundinamarca (Colombia).

Photos: L.G. Bautista-Montealegre, S.D. García-Guzmán and M.M. Bolaños-Benavides

ABSTRACT

Bacteriosis and moko of plantain are two of the more important phytosanitary problems in musaceae production in Colombia. To contribute to the management of these diseases in the Department of Cundinamarca, soils and foliar tissues were analyzed, along with symptoms of the diseases on 149 producing farms in the municipalities of Chaguani, La Palma, La Vega, and Viota (Cundinamarca). A descriptive analysis of the chemical properties of the soils and foliar concentration of nutrients was carried out; the Student-Welch *t*-test was applied ($P<0.05$); the distribution patterns of the diseases were represented cartographically. According to the results, the sampled soils were not saline and had extreme to strong acidity, low effective cation exchange capacity and low fertility; meanwhile, the plants presented a deficit of elements such as nitrogen, phosphorus, sulfur, copper, zinc, and boron. Considering the total number of farms evaluated, the incidence of bacteriosis and moko disease was reported in 63 and 14% at the departmental level, respectively. A higher concentration of sulfur and zinc was observed in farm soils free of both diseases. Finally, the plants free of bacteriosis showed a higher concentration of potassium and lower concentrations of calcium and manganese, while the plants free of moko disease showed higher concentrations of nitrogen, sodium, and copper and a lower concentration of calcium. The results can contribute to the planning of large-scale management strategies to reduce the risk of the dissemination of diseases of economic importance for plantain crops.

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Additional key words: *Dickeya* sp.*;* *Ralstonia solanacearum;* incidence*;* Dominic Hartón; soil properties; leaf tissue analysis.

RESUMEN

La bacteriosis y el moko de plátano son considerados como uno de los problemas fitosanitarios más importantes en la producción de musáceas en Colombia. Para contribuir al manejo de dichas enfermedades en el departamento de Cundinamarca, se analizaron suelos y tejidos foliares, así como los síntomas de las enfermedades en 149 fincas de productores de los municipios de Chaguaní, La Palma, La Vega y Viotá. Se llevó a cabo un análisis descriptivo de las propiedades químicas de los suelos y concentración foliar de nutrientes, se aplicó la prueba *t* de Student-Welch ($P \leq 0{,}05$), y se representaron cartográficamente los patrones de distribución de las enfermedades. Según los resultados obtenidos, los suelos muestreados no son salinos, presentan acidez extrema a fuerte, baja capacidad de intercambio catiónico efectiva, y baja fertilidad; mientras que las plantas mostraron déficit de elementos como nitrógeno, fósforo, azufre, cobre, zinc y boro. Considerando el número de fincas evaluadas, se reportó una incidencia de bacteriosis y moko de 63 y 14%, respectivamente. Se observó mayor concentración de azufre y zinc en suelos de fincas libres de ambas enfermedades. Finalmente, plantas reportadas como sanas de bacteriosis arrojaron mayor concentración de potasio y menores concentraciones de calcio y manganeso; mientras que plantas reportadas como sanas de moko, mostraron mayor concentración de nitrógeno, sodio y cobre, y menor concentración de calcio. Los resultados obtenidos contribuyen en la planificación de estrategias de manejo a gran escala, con el fin de disminuir el riesgo de diseminación de enfermedades de importancia económica para el cultivo de plátano.

Palabras clave adicionales: *Dickeya* sp.; *Ralstonia solanacearum;* incidencia; Dominic Hartón; propiedades del suelo; análisis de tejido foliar.

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INTRODUCTION

In 2019, Colombia produced 4,376,922 t of plantain and reported a cultivation area of 450,329 ha, with Arauca as the leading producer department, followed by the departments of Antioquia, Meta, and Valle del Cauca. Cundinamarca ranked 12th with a cultivated area of 12,234 ha and a production of 102,350 t (Agronet, 2020).

Phytosanitary problems in any crop cause a decrease in production, a reduction in the quality of the final product, and, in extreme cases, depending on the causal agent, a total loss of the plantation crop. Plantain production in Colombia is exposed to phytosanitary problems as the result of the attack of fungi, bacteria, viruses, and phytoparasitic nematodes, among others. In this sense, bacteriosis or wet rot and moko are caused by the bacterial species *Dickeya* sp. and *Ralstonia solanacearum* Race 2, respectively.

Dickeya sp. degrades tissues and releases cellular fluids that attract secondary bacteria, which proliferate in the cells and often release unpleasant odors. Pathogens that cause soft rot secrete extracellular enzymes, such as pectate lyases, cellulases, nucleases, and proteases, that degrade pectin, cellulose, nucleic acid, and cellular proteins, respectively (Kado, 2016a). This species survives mainly in the tissue of diseased plants, crop residues, soil and compost (Kado, 2016a); it generally appears during the summer season, generating losses of up to 100% on plantations where adequate management measures are not implemented (Aránzazu *et al*., 2002). Initially, plants affected by bacteriosis show necrosis at the edge of the oldest leaves, which progresses to the entire leaf blade, causing total chlorosis (Alarcón and Jiménez, 2012). If the bacterium spreads rapidly, the plant dies with its brown, dead leaves remaining erect, and the older leaves often fall while green, with the younger center leaves turning yellow (Jones, 2018). However, in Colombia, the primary symptomatology is observed in the pseudostem, where aqueous rot develops, accompanied by translucent spots with a yellowish color at the beginning that later turn reddish to dark brown. This weakens the middle part of the plant, accompanied by a strong foul odor that attracts pests (weevils), aggravating the problem by disseminating the bacterium on the plantation (Aránzazu *et al*., 2002; Alarcón and Jiménez, 2012).

On the other hand, *R. solanacearum* Race 2 is one of the more destructive bacteria in the world, with a negative impact on banana and plantain production systems in the tropics and warm temperate zones (Kado, 2016b). In Colombia, losses generated by attack of this bacterium in musaceas can range up to 100% since the fruits are severely affected; moreover, higher management costs are related to the application of eradication measures and quarantine times (Yabuuchi *et al*., 1992; Castañeda and Espinosa, 2005; Bejarano, 2010). In plantain cultivation, this bacterium attacks in any physiological state of the plant, moving downwards through mechanical damage caused by an infected tool or by insect attack on the branch or upwards when penetrating the root system or pseudostem through wounds (Hurtado, 2012). Once inside the tissue, the vascular parenchyma and the xylem get infected, and exopolysaccharides (acidic extracellular polysaccharide - EPS I) and β-1,4-endoglucanase are produced when exceeding 10^7 cells/mL, promoting bacterial colonization of the plant (Kado, 2016b), vascular bundle blockage, weakening through water and nutrient deficiency, wilting, and, finally, plant death.

According to Álvarez *et al*. (2015), in newly planted seedlings affected by moko disease, widespread yellowing appears with subsequent necrosis and when the pseudostem is cut, and a few reddish spots or brown lines appear, corresponding to the vascular bundles where the pathogenic bacterium has degraded tissues. In young plants, dry leaves appear in the middle of asymptomatic leaves or the flag leaf appears completely necrosed. When cutting the rachis, fruits, pseudostem, or corms, internal damage can be observed as lesions that are, at first, pale yellow, becoming reddish brown, and then black. When attack is late or transmission is by vector insects to the bunch, reddish or black coloration appears in the affected plantain fingers; upon cutting across the rachis, spots can be seen, corresponding to infected vascular bundles through which the bacterium has moved throughout the plant.

The integrated management of these two diseases involves prevention measures, such as disinfection of tools and footwear, weed control, and use of healthy seeds. Furthermore, the implementation of cultural practices, such as removing dry leaf sheaths, management of the weevils-worm screws complex, and, after identifying symptoms on plantations, zoning the plots and implementing the management schemes recommended by Corporación Colombiana de Investigación Agopecuaria - AGROSAVIA (Formerly CORPOICA), CIAT and ICA (Aránzazu *et al*., 2002; Álvarez *et al*., 2007, 2013; Alarcón and Jiménez, 2012). Additionally, managing phytosanitary problems as reported by other authors in Colombia (Aránzazu *et al*., 2002; Alarcón and Jiménez, 2012; Ramírez *et al*., 2014; Bautista-Montealegre *et al*., 2016) requires analyzing plant nutrition and soil fertility in pathogen-plant interactions.

In accordance with the latter, adequate crop nutrition contributes to a reduction of disease attacks (Méndez and Viteri, 2007; Viteri *et al*., 2012; Bautista-Montealegre *et al*., 2017) and is directly related to the occurrence of plant resistance and predisposition factors according to nutritional imbalances (Chaboussou, 1967). Deficiencies in crop nutrition can increase susceptibility to various diseases and, therefore, increase aggressiveness seen in weak infections (Huber, 1997; Velasco, 2000; McMahon, 2012).

Accordingly, the aim of this study was to analyze the incidence of bacteriosis and moko diseases in the plantain cultivar Dominico Harton (*Musa* AAB Simmonds) in four producing municipalities in the Department of Cundinamarca and its relationship with chemical soil properties and foliar nutrient concentrations.

MATERIALS AND METHODS

This study was carried out in the municipalities of Chaguani, La Palma, La Vega and Viota, located in the Department of Cundinamarca. The latter is located in the Andean region, on the eastern mountain range (Cordillera) of the Colombian central area, between 3°42’ - 5°51’ N and 73°3’ - 74°54’ W (IGAC, 1999). One hundred forty-nine (149) previously selected plantain farms were visited and georeferenced with a Global Positioning System (GPS) (Garmin® brand). These farms are in areas that register average temperatures of 16 to 26°C, precipitation of 900 to 2750 mm year^{-1}, relative humidity of 75 to 85% (AGROSAVIA, 2020) and altitudes of 1,121 to 1,770 m a.s.l. (Tab. 1).

Table 1. Biophysical characteristics of the territory and number of plantain farms visited by municipality in the Department of Cundinamarca.

Municipality	Temperature (°C)	Precipitation (mm year^{-1})	Humidity (%)	Altitude (m a.s.l.)	Farms visited
Viotá	16 - 26	900 - 1750	75 - 85	1202 - 1617	53
La Vega	16 - 22	2000 - 2750	80 - 85	1500 - 1770	43
Chaguani	16 - 24	1300 - 2000	75 - 85	1121 - 1848	29
La Palma	18 - 24	1750 - 2500	80 - 85	1305 - 1640	24
Total					149

The incidence of bacteriosis and moko diseases in the department was calculated from visual observation in the field of internal and external symptoms of randomly selected plants, qualifying the farms as healthy or sick according to the presence of characteristic symptoms described by Aránzazu *et al*. (2002), Alarcón and Jiménez (2012) and Álvarez *et al*. (2015).

Moreover, soil samples were collected from each farm according to the methodology described by Bolaños (2006) for chemical analysis. Plant tissue samples were obtained by taking 10 cm at the middle of the third leaf from the flag leaf on each farm for nutrient concentration analysis. The collected samples were processed according to the protocols of the analytical chemistry laboratory of AGROSAVIA at the Tibaitata Research Center in Mosquera (Cundinamarca), following norm NTC ISO/IEC 17025.

Once the laboratory results were obtained, a descriptive analysis of the chemical properties of the soil and the foliar concentration of nutrients was carried out by analyzing the range frequency according to the classification published by ICA (1992), along with the macro and micronutrient approximations in foliar tissues by Munson (1998). Additionally, the data were subjected to comparison of means for independent groups using the *t*-Student-Welch test with a level of significance of $P<0.05$. Finally, ArcGIS 10.3 (ESRI ®) was used according to the sampling carried out to cartographically represent the distribution patterns of the diseases in the field.

RESULTS AND DISCUSSION

Incidence of *Dickeya* sp. and *R. solanacearum*

Of the total number of farms evaluated in four municipalities, 63 and 14% of these farms were affected by bacteriosis and moko, respectively. In the case of bacteriosis, a higher number of sick plants were observed on farms in the municipalities of La Vega and Viota, followed by the municipalities of Chaguani and La Palma, with reports of 36, 25, 18 and 16 farms, respectively. However, the incidence of the disease was higher in the municipalities of Viota and La Palma, with 68 and 67%, followed by the municipalities of Chaguani and La Vega with 62 and 50%, respectively (Fig. 1).

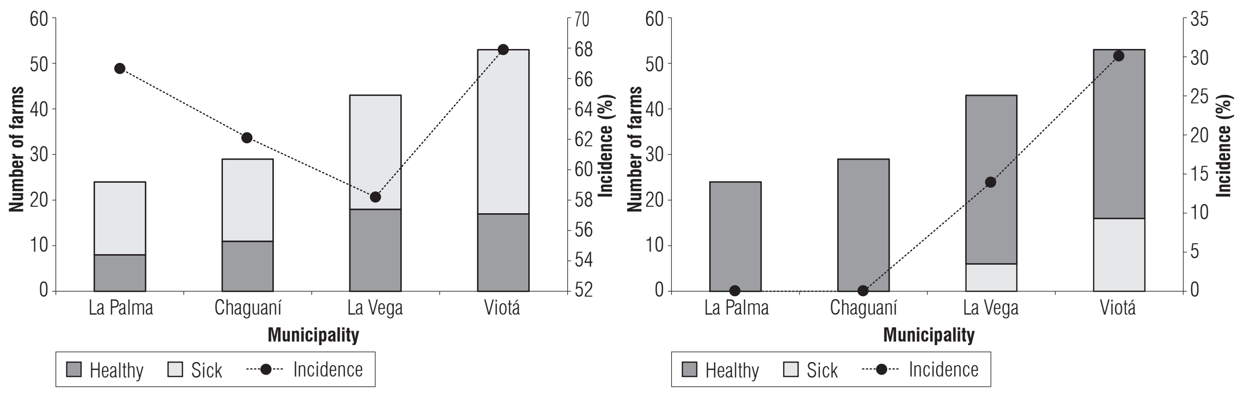

Figure 1. Number of plantain farms per municipality affected by bacteriosis (left) and moko (right).

On the other hand, more plants were observed on farms affected by moko in the municipalities of Viota and La Vega, with 16 and 6, respectively, while the municipalities of La Palma and Chaguani did not report the presence of this disease. Hence, the incidence was higher in the municipality of Viota with 30%, followed by the municipality of La Vega with 14% (Fig. 1).

In the municipality of Chaguani, the presence of bacteriosis was confirmed on the eastern edge of the municipality in the veredas (settlements) Llanadas, Montefrio, and El Rincon, while, in the municipality of La Palma, the majority of the farms reported bacteriosis in the southern part of the municipality (Fig. 2).

The plantations affected by bacteriosis in the municipality of La Vega were concentrated in settlement El Chupal, with isolated cases in settlement Hoya Grande, while the presence of moko on the plantations was on the northern edge of settlement Naguy, and only one case was found in the southwest of settlement El Chupal (Fig. 2).

Finally, in the municipality of Viota, settlement Bajo Ceilan reported a higher number of plantations affected by both diseases; additionally, in this settlement, there were several farms with the presence of only one of the diseases. Both in Bajo Palmar and Mogambo, the most prevalent phytosanitary problem was bacteriosis, which was concentrated in the southern part (Fig. 2).

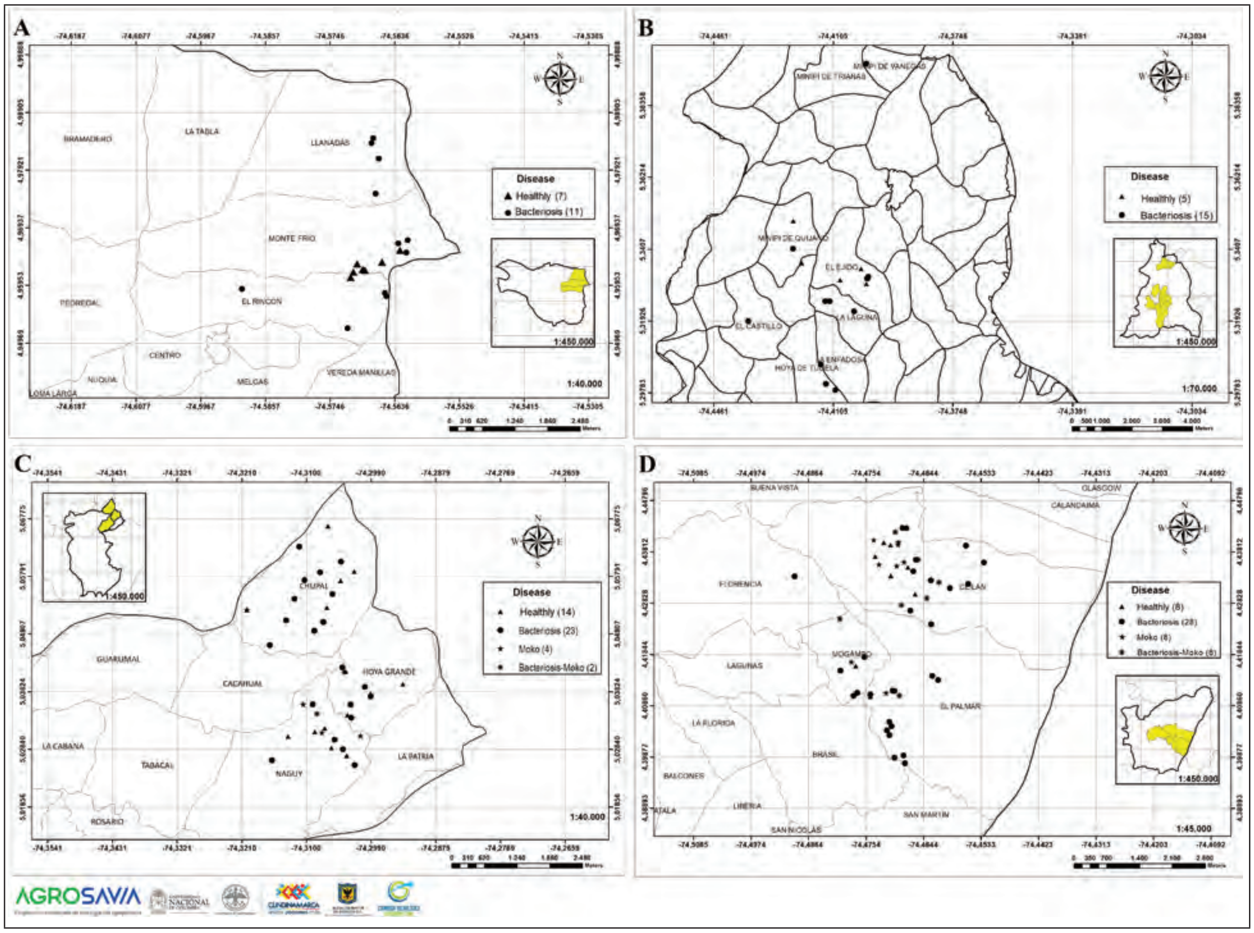

Figure 2. **Spatial distribution of the bacterial diseases bacteriosis and moko on the Dominico-Harton plantain farms in the municipalities of Chaguani (A), La Palma (B), La Vega (C) and Viota (D), in the Department of Cundinamarca. Source: The authors.**

The diseases evaluated in the municipalities did not show a defined distribution pattern (Fig. 2), and their dispersion was possibly influenced by specific epidemiological aspects of each pathogen, as well as by biophysical and sociocultural conditions and crop management techniques. The epidemiology of the diseases, in the case of bacteriosis, was focused on the endemic nature of the bacterium and its ability to develop and establish in a wide range of temperatures (i.e., 5, 22 and 37°C for minimum, optimum and maximum temperatures, respectively), as well as long periods of drought alternated with heavy rains (Alarcón and Jiménez, 2012), particularly if the ground is poorly drained, cool, and plants are waterlogged (Jones, 2018). Furthermore, other factors that stand out are the survival of the pathogen in alternate hosts and plant debris that remain attached to the pseudostem as the result of defoliation (Martínez and García, 2003), high incidence of insects such as *Metamasius hemipterus* L. (Coleoptera: Curculionidae) and nutritional imbalances, especially considering elements such as potassium and boron (Belalcázar, 1991; Bolaños-Benavides and Benalcázar-Carvajal, 2000; Aránzazu *et al*., 2002).

For moko in plantain, although its incidence in the municipalities was low (14%), its prevalence and spread to new areas or farms is possible because of transmission by insect vectors and movement of infected material from other affected areas (Álvarez *et al*., 2015). According to a study carried out by Cardona *et al*. (2017) in which technical and socioeconomic particularities of the productive system were established in the same municipalities studied in the instant research, non-disinfestation of tools, highly variable disease management strategies and use of seeds from the same farm are related to the presence of bacteriosis and moko in plantain. This translates into a constant threat to plantain production in producing areas, particularly since management decisions are made based on traditional knowledge or the recommendation of other farmers.

Chemical soil properties and foliar nutrient concentrations

According to the descriptive analysis of the chemical characteristics of all soils sampled (Tab. 2), the results were: a higher frequency of extreme to strong acidity (44-46%, respectively); low effective cation exchange capacity (63%); non-saline soils for electrical conductivity of less than two (100%); very low sodium content (63%); low levels of phosphorus (46%), magnesium (54%), copper (54%), boron (59%), calcium (42%), manganese (49%), potassium (39%) and the magnesium potassium ratio (68%); sufficient zinc content (49%) as well as high sulfur content (61%), and high values for calcium/magnesium, calcium/boron and calcium/magnesium + potassium ratios (71, 98 and 62%, respectively); moreover, there were very high contents of organic matter (46%) and iron (67%) and low to very high aluminum concentration frequencies (37%).

The nutrient concentration in foliar tissue collected to evaluate bacteriosis and moko on the plantain farms (Tab. 3 and Tab. 4, respectively) showed frequent deficits of nitrogen (61 and 62%, respectively), phosphorus (60 and 63%, respectively), sulfur (82 and 84%, respectively), copper (92 and 95%, respectively), zinc (95 and 97%, respectively) and boron (90 and 69%, respectively). The plantain foliar tissue showed normal concentrations of calcium (94 and 86%, respectively), potassium (91 and 88%, respectively), iron (96 and 86%, respectively), magnesium (62 and 63%, respectively) and manganese (65 and 60%, respectively).

However, the student's t-test, used to compare the health of the crops on the farms and the chemical properties of the soil, showed statistically significant differences ($P<0.05$) in the content of sulfur affected by bacteriosis (8.28) and in the content of zinc (4.17) affected by moko; these values were low when compared with the concentration in the farm soils without the diseases (S: 10.36 and Zn: 6.37) (Tab. 5).

Table 2. Chemical characteristics of the soils sampled on 149 plantain farms.

pH			ECEC			EC			OM		
Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic
>5.0	44	Extreme acidity	<8.0	63	Low	<2	100	Not saline	<2	1	Very low
5.0 - 5.5	46	Strong acidity	8.0 - 15.0	33	Medium	>2	0	Saline	2.0 - 3.0	5	Low
5.5 - 6.0	9	Moderate acidity	>15.0	4	high		0		3.0 - 5.0	13	Sufficient
6.0 - 6.5	1	Light acidity							5.0 - 10.0	35	High
									>10.0	46	Very high
Phosphorous (P)			Sulfur (S)			Iron (Fe)			Manganese (Mn)		
Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic
<5.0	7	Very low	<3.0	0	Very low	<10.0	0	Very low	<2.5	28	Very low
5.0 - 15.0	46	Low	3.0 - 6.0	3	Low	10.0 - 25.0	0	Low	2.5 - 5.0	54	Low
15.0 - 30.0	32	Sufficient	6.0 -12.0	24	Sufficient	25.0 - 50.0	2	Sufficient	5.0 - 10.0	16	Sufficient
30.0 - 45.0	7	High	12.0 - 15.0	61	High	50.0 - 100.0	31	High	10.0 - 20.0	1	High
>45.0	8	Very high	>15.0	12	Very high	>100.0	67	Very high	>20.0	1	Very high
Copper (Cu)			Zinc (Zn)			Boron (B)			Calcium (Ca)		
Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic
<0.5	0	Very low	<0.5	0	Very low	<0.2	39	Very low	<1.0	3	Very low
0.5 - 1.5	54	Low	0.5 - 1.5	16	Low	0.2 - 0.5	59	Low	1.0 - 3.0	42	Low
1.0 - 3.0	25	Sufficient	1.5 - 5.0	49	Sufficient	0.5 - 1.0	1	Sufficient	3.0 - 6.0	27	Sufficient
3.0 - 5.0	13	High	5.0 -10.0	24	High	1.0 - 1.5	1	High	6.0 - 9.0	18	High
>5.0	8	Very high	>10.0	11	Very high	>1.5	0	Very high	>9.0	10	Very high
Magnesium (Mg)			Potassium (K)			Sodium (Na)			Aluminium (Al)		
Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic
<0.5	30	Very low	<0.05	0	Very low	<0.5	63	Very low	<0.5	26	Very low
0.5 - 1.5	49	Low	0.05 - 0.15	39	Low	0.5 - 1.0	25	Low	0.5 - 2.0	37	Low
1.5 - 2.5	19	Sufficient	0.15 - 0.30	37	Sufficient	>1.0	12	Very high	>2.0	37	Very high
2.5 - 3.0	1	High	0.30 - 0.50	14	High						
>3.0	1	Very high	>0.50	10	Very high						
Ca/Mg relation			Mg/K relation			Ca/B relation			Ca/Mg + K relation		
Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic
<2.0	1	Low	<6.0	68	Low	<60	1	Low	<12.0	17	Low
2.0 - 4.0	28	Medium	6.0 - 8.0	17	Medium	60- 400	1	Medium	12.0 - 20.0	21	Medium
>2.0	71	High	>8.0	15	High	>400	98	High	>20.0	62	High

EC: dS m^{-1}; ECEC: $cmol_{(+)}$ kg^{-1}; OM: g/100 g; P, S, Fe, Mn, Zn, Cu, B: mg kg^{-1}, K: $cmol_{(+)}$ kg^{-1}; Ca, Mg, Na, Al: %.

Table 3. Approximate concentration ranges of macro and micronutrients in foliar tissues of plants evaluated for bacteriosis symptoms on 149 plantain farms.

Nitrogen (N)			Phosphorous (P)			Potassium (K)		
Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic
<2.5	61	Deficit	<0.15	60	Deficit	<1.0	8	Deficit
2.5 - 4.5	39	Normal	0.20 - 0.75	40	Normal	1.5 - 5.5	91	Normal
>6.0	0	Excess	>1.0	0	Excess	>6.0	1	Excess
Calcium (Ca)			Magnesium (Mg)			Sulfur (S)		
Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic
<0.5	16	Deficit	<0.2	38	Deficit	<0.2	82	Deficit
1.0 - 4.0	84	Normal	0.25 - 1.0	62	Normal	0.25 - 1.0	18	Normal
>5.0	0	Excess	>1.50	0	Excess	>3.0	0	Excess
Iron (Fe)			Copper (Cu)			Manganese (Mn)		
Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic
<5	4	Deficit	2.0 - 5.0	92	Deficit	15 - 25	4	Deficit
100 - 500	96	Normal	5.0 - 30	8	Normal	20 - 300	65	Normal
>500	0	Excess	30 - 100	0	Excess	300 - 500	31	Excess
Zinc (Zn)			Boron (B)					
Range	Percentage	Characteristic	Range	Percentage	Characteristic			
1.0 - 20	95	Deficit	5.0 - 30	90	Deficit			
27 - 100	5	Normal	30 - 200	10	Normal			
100 - 400	0	Excess	50 - 200	0	Excess			

N, P, K, Ca, Mg, Na, and S: %. Fe, Cu, Mn, Zn and B: mg kg^{-1}.

Table 4. Approximate concentration ranges of macro and micronutrients in foliar tissues of total plants evaluated for moko symptoms on 149 plantain farms.

Nitrogen (N)			Phosphorous (P)			Potassium (K)		
Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic
<2.5	62	Deficit	<0.15	63	Deficit	<1.00	12	Deficit
2.5 - 4.5	37	Normal	0.20 - 0.75	37	Normal	1.5 - 5.5	88	Normal
>6.0	1	Excess	>1.0	0	Excess	>6.0	0	Excess
Calcium (Ca)			Magnesium (Mg)			Sulfur (S)		
Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic
<0.5	14	Deficit	<0.2	37	Deficit	<0.2	84	Deficit
1,0 - 4.0	86	Normal	0.25 - 1.0	63	Normal	0.25 - 1.0	16	Normal
>5.0	0	Excess	>1.5	0	Excess	>3.0	0	Excess
Iron (Fe)			Copper (Cu)			Manganese (Mn)		
Range	Percentage	Characteristic	Range	Percentage	Characteristic	Range	Percentage	Characteristic
<50.0	14	Deficit	2.0 - 5.0	95	Deficit	15 - 25	3	Deficit
100 - 500	86	Normal	5.0 - 30.0	4	Normal	20 - 300	60	Normal
>500	0	Excess	30.0 - 100.0	1	Excess	300 - 500	37	Excess
Zinc (Zn)			Boron (B)					
Range	Percentage	Characteristic	Range	Percentage	Characteristic			
1.0 - 20.0	97	Deficit	5.0 - 30.0	69	Deficit			
27.0 - 100.0	3	Normal	30.0 - 200.0	31	Normal			
100.0 - 400.0	0	Excess	50.0 - 200.0	0	Excess			

N, P, K, Ca, Mg, Na, and S: %. Fe, Cu, Mn, Zn and B: mg kg^{-1}.

Table 5. Chemical soil properties from 149 plantain farms according to the presence and absence of bacteriosis and moko.

Bacteriosis										
State	pH	EC	ECEC	OM	P	S*	K	Fe	Mn	Zn
Sick	5.0	0.33	7.55	8.99	19.40	8.28	0.22	307.01	3.95	5.63
Healthy	5.2	0.34	8.74	9.50	30.87	10.36	0.32	330.93	4.42	6.74
State	Cu	B	Sat_Ca	Sat_Mg	Sat_Na	Sat_Al	Ca/Mg	Ca+Mg/K	Mg/K	Ca/B
Sick	2.63	0.23	52.02	11.53	6.11	29.26	4.63	28.97	5.21	4,718
Healthy	2.56	0.25	52.02	11.53	6.11	29.26	4.77	29.22	5.43	5,103
Moko disease										
State	pH	EC	ECEC	OM	P	S	K	Fe	Mn	Zn*
Sick	5.2	0.31	8.54	9.61	20.26	9.27	0.27	299.14	3.78	4.17
Healthy	5.0	0.33	7.88	9.09	24.16	8.99	0.25	318.70	4.18	6.37
State	Cu	B	Sat_Ca	Sat_Mg	Sat_Na	Sat_Al	Ca/Mg	Ca+Mg/K	Mg/K	Ca/B
Sick	2.73	0.29	60.73	13.30	5.29	18.91	4.70	28.67	5.36	4,756
Healthy	2.58	0.23	60.73	13.30	5.29	18.91	4.68	29.14	5.27	4,876

EC: dS m^{-1}; CICE: $cmol_{(+)}$ kg^{-1}; OM: g/100 g; P, S, Fe, Mn, Zn, Cu, B: mg kg^{-1}, K: cmol(+) kg^{-1}; Sat_Ca, Sat_Mg, Sat_Na, Sat_Al: %.
*: Significant at $P<0.05$.

As seen in the soil results and the importance of its availability for plants, S is a constituent of proteins and amino acids, such as cysteine and methionine; these amino acids are precursors of other compounds that contain this element, such as coenzymes and plant subproducts, which are directly involved in metabolic reactions (Hawkesford *et al*., 2012). This element has been recognized in the control of diseases through its use as a fungicide, which was initially implemented in the agricultural sector in 1847 for the control of *Oidium* in vineyards (Paul, 1978). Further, for vascular diseases, researchers have demonstrated that levels of S accumulation in vascular tissue were fungitoxic for *Verticillium dahliae* in tomato plants, inhibiting spore germination and growth of fungal hyphae (Williams *et al*., 2002), whilst, in tobacco and French bean susceptible to *Fusarium oxysporum* and tomato susceptible to *R. solanacearum*, sulphur was detected in xylem excised from infected plants but not in controlled plants (Williams and Cooper, 2003).

Zn is the second most abundant transition metal in living organisms, after Fe; the metabolic functions of Zn are based on its strong tendency to form tetrahedral complexes with N, O, and, mainly, S ligands, playing a functional and structural role in enzymatic reactions. Additionally, it is related to protein molecules involved in DNA replication and the regulation of gene expression. Further, the metabolic changes induced by a Zn deficiency are complex. However, some can be explained by the functions of this element in enzymatic and metabolic reactions, so when they affect the expression and regulation of defense mechanisms of genes and proteins, Zn contributes to the tolerance of plants to environmental stressors (Broadley *et al*., 2012).

In the foliar tissue, the concentration of nutrients showed significant differences ($P<0.05$) for K, Ca and Mn in the plants with bacteriosis, while the plants with symptoms of moko had differences in the concentrations of N, Ca, Na and Cu (Tab. 6). The plants free of bacteriosis showed a higher concentration of K and lower concentrations of Ca and Zn, while the plants free of moko showed higher concentrations of N, Na and Cu and a lower concentration of Ca (Tab. 6).

Bacteria such as *Dickeya* sp. that produce soft rot release polygalacturonase and pectolytic enzymes as they spread within the tissues of the host, while vascular diseases such as moko caused by *R. solanacearum* spread within plants through the xylem and lead to the formation of "slime" that clogs the vessels (Huber *et al*., 2012). Calcium and manganese are related to the resistance of plants to bacterial diseases through stabilization of the medium lamella, inhibition the formation of pectolytic enzymes and decrease in accumulation of sugars and amino acids in the apoplast in the case of Ca, along with hypersensitivity

Table 6. Foliar nutrient concentration from 149 plantain farms according to the presence and absence of bacteriosis and moko.

Bacteriosis												
State	N	P	K*	Ca*	Mg	Na	S	Fe	Cu	Mn*	Zn	B
Sick	2.37	0.13	1.66	1.23	0.231	0.007	0.16	82.69	3.53	450	14.88	17.93
Healthy	2.46	0.14	2.02	0.88	0.243	0.008	0.16	88.48	4.75	192	15.23	18.84
Moko disease												
State	N*	P	K	Ca*	Mg	Na*	S	Fe	Cu*	Mn	Zn	B
Sick	2.14	0.140	2.05	1.32	0.197	0.003	0.15	75.96	3.19	281	14.61	20.22
Healthy	2.46	0.143	2,03	0.88	0.256	0.009	0.16	82.38	3.74	255	15.52	17.33

N, P, K, Ca, Mg, Na, and S: %. Fe, Cu, Mn, Zn, and B: mg kg^{-1}. *: significant at $P<0.05$.

responses to infections by activation of enzymes in the case of Zn (Humphries *et al.*, 2006; Huber *et al.*, 2012; Gupta *et al.*, 2017; Schumann *et al.*, 2017).

However, the response of plants to these elements is affected to some extent by how they are applied, as well as by changes in pH and use of chemical molecules as herbicides (Huber *et al*., 2012; Rahman and Punja, 2007). For *Dickeya* sp. in plantain cultivation, authors have reported that a K deficiency predisposes plants to attacks by this pathogen (Bolaños-Benavides and Belalcázar-Carvajal, 2000; Aránzazu *et al*., 2002; Alarcón and Jiménez, 2012). Consequently, the results coincide with the results reported by Bolaños-Benavides and Benalcázar-Carvajal (2000). These authors studied the relationship between the attack of bacteriosis and the concentration of nutrients in tissue and soils, finding that, in addition to K, plantain plants affected by disease have low contents of N, P, Mn, B, and Zn. Moreover, no correlation between the concentration of nutrients in soil and plant tissues was found.

Physiologically, K is essential to metabolic processes and influences membrane potential, cell turgor, carbohydrate translocation, protein synthesis, osmoregulatory activities and enzyme catalysis (Mengel, 2007; Pyo *et al*., 2010). Its importance has been demonstrated because its deficiency facilitates the entry and development of pathogens through physical and metabolic changes that counteract the natural defense mechanisms of plants (Amtmann *et al*., 2008).

The influence of N on susceptibility to diseases is related to the metabolic aspects of plants, the effect on the virulence of phytopathogenic microorganisms, and abiotic and biotic factors (Huber and Thompson, 2007). However, results may be inconsistent because it has not been clearly established whether the effect presents when the nutrient supply is low, optimal or excessive, if the effect depends on the form of N supplied (ammonium or nitrate that are metabolized differently), or if there are differences between infection patterns of obligate and facultative parasites (Huber *et al*., 2012). Although excess N is related to attacks by pathogens, mainly foliar, the susceptibility of plants to bacterial spots caused by a facultative parasite decreases with an increase in N supply, as reported for *Xanthomonas vesicatoria* in tomato plants (Huber *et al*., 2012).

On the other hand, the results observed in the current research for the concentration of foliar Cu in the plants without moko symptoms coincide with the results reported by Bautista-Montealegre *et al*. (2016), who found a low probability of occurrence of the disease in plants with a high concentration of this element at the leaf level. This response might be due to the effect of Cu on the lignification of the xylem in photosynthetic processes, in the transfer of electrons and oxidative proteins, as well as in the synthesis of DNA-RNA (Evans *et al*., 2006; Broadley *et al.*, 2012). There is some uncertainty about the effect of Na as a nutrient and as a disease suppressant, partly because of the semantics of “essentiality” (Reid *et al*., 2001; Gorham, 2006), and it is only considered a beneficial nutrient when it influences the stimulation of growth and interaction with other nutrients (Gorham, 2006). However, excess Na in soil and plants causes restricted growth of the root, marginal burning, or at the tip, of leaves, inhibition of flowering and reduction of crop yield (Greenway and Munns, 1980). Conversely, positive effects are attributed to the control of diseases caused by vascular-type soil pathogens by decreasing damage and increasing dry matter production (Reid *et al*., 2001; Okada and Matsubara, 2012; Matsubara *et al*. 2015).

CONCLUSION

All evaluated farms in the Department of Cundinamarca registered incidences of 63 and 14% for bacteriosis and moko, respectively, with the highest presence in the municipalities of Viota and La Vega.

The information collected on the diseases evaluated in the municipalities did not show a defined distribution pattern, and their dispersion was possibly influenced by the soil fertility and the nutritional status of the plants, as well as by the epidemiological aspects and management practices carried out by the plantain producers.

The farm soils cultivated with plantain in the four municipalities of Cundinamarca had an extreme to strong acidity and low effective cation exchange capacity; moreover, the non-saline soils had low concentrations of most elements. The plantain plants had a deficit of elements such as nitrogen, phosphorus, sulfur, copper, zinc, and boron.

Soils from the plantain farms with low concentrations of sulfur and zinc reported the presence of bacteriosis and moko, respectively. The plants free of bacteriosis showed a higher concentration of potassium and lower concentrations of calcium and manganese; while the plants free of moko showed higher concentrations of nitrogen, sodium, and copper and a low concentration of calcium.

The spatial distribution analysis of the bacterial diseases can contribute to the planning of management strategies on a large scale to reduce the risk of dissemination of phytosanitary problems of economic importance in plantains, such as bacteriosis and moko.

Although the incidence of moko in plantain in the Department of Cundinamarca was low (14%), as compared to bacteriosis (64%), attention should be paid to this disease because, if adequate control measures are not implemented, Musaceae productivity in the studied municipalities may be compromised.

Nonetheless, it is recommended that the exploratory scale or number of evaluated farms be expanded to more precisely determine the distribution of bacteriosis and moko, as well as the current phytosanitary situation of plantain production systems in the main producing municipalities of the Department of Cundinamarca.

The soil and nutritional requirements of plantain must be analyzed to implement nutritional management of this productive system in the Department of Cundinamarca since a nutritional imbalance in the soil matrix and a deficiency of some nutrients in the plants can influence tolerance to bacteriosis and moko.

Local entities in the municipalities of La Vega, Viota, Chaguani, and La Palma should work jointly with producers in the adoption of recommended integrated diseases management schemes to reduce the impact on plantations.

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Pitaya fruit quality (*Hylocereus undatus* [Haworth] Britton & Rose) according to physiological maturity. A review

Calidad de los frutos de pitaya (*Hylocereus undatus* [Haworth] Britton & Rose) en función de la madurez fisiológica. Una revisión

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Physiological maturity of pitaya fruits

Photo: T.A. Ortiz

ABSTRACT

The pitaya fruit has occupied a growing niche in the fruit market because its organoleptic characteristics and rusticity have attracted the attention of consumers and producers, respectively. The organoleptic and nutritional quality of fruits are due to the maturity stage. Therefore, determining the period in which the fruits reach physiological maturity is important since it aids the planning of the harvest and fruit quality control. In this sense, the aim of this review was to establish the ideal period for harvesting pitaya fruits (*Hylocereus undatus*) by determining the physiological maturity point. For this species, the number of days from anthesis to full development of the fruit has been the most reliable variable to determine harvest timing. With this designation, it is possible to indicate the stage (physiological maturity) in which the fruits present characteristics that are favorable to the species, for example, color, soluble solids content, ratio, and others. The place of production and edaphoclimatic conditions can interfere and cause variation in the period in which fruits reach physiological maturity. Some studies in Mexico have confirmed that the physiological maturation of pitaya fruits occurs between 25 and 31 days after anthesis (DAA). Other research, including in Brazil, has confirmed that this point occurred from 28 to 32 DAA. Thus, based on the scientific studies in the literature, it is recommended to harvest pitaya fruits between 25 and 32 DAA to avoid losing the commercial value of fruits.

Additional key words: dragon fruits; maturity index; physiological quality; fruit quality.

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RESUMEN

Los frutos de pitaya han ocupado un creciente nicho en el mercado de frutas ya que sus características organolépticas y rusticidad han atraído la atención de los consumidores y productores, respectivamente. La calidad organoléptica y nutricional de los frutos son consecuencia de la fase de maduración. Por lo tanto, determinar el período en que los frutos alcanzan la madurez fisiológica es importante, pues apoya la planificación de la cosecha y el control de calidad de los frutos. En este sentido, esta revisión tiene como objetivo establecer el período ideal para realizar la cosecha de los frutos de la pitaya (*Hylocereus undatus*), a través de la determinación del punto de madurez fisiológica. Para esta especie, el número de días de la antesis hasta el desarrollo pleno del fruto se ha mostrado como la variable con mayor confiabilidad para determinar el momento de la cosecha. A partir de esta designación, es posible indicar la fase (madurez fisiológica) en que los frutos presentan características favorables a la especie, a ejemplo del color, contenido de sólidos solubles, ratio, entre otras. Se sabe que el lugar de producción y las condiciones edafoclimáticas son capaces de interferir y causar variación en cuanto al período en que los frutos alcanzan la madurez fisiológica. Algunos estudios en México confirmaron que la maduración fisiológica de los frutos de pitaya ocurrió entre los 25 y 31 días después de la antesis (DAA). Otras investigaciones, incluso en Brasil, confirmaron que este punto ocurrió en el período de 28 a 32 DAA. Por lo tanto, a partir de los estudios científicos llevados a cabo y publicados en la literatura, para evitar perder su valor comercial de los frutos de pitaya se recomienda cosecharlos entre 25 y 32 DAA.

Palabras clave adicionales: fruta de dragón; índice de madurez; calidad fisiológica; calidad de la fruta.

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INTRODUCTION

The fruit cultivation is constantly evolving in terms of new varieties and species for commercialization, which have been boosted by consumer awareness of healthy diets. In this sense, pitaya or dragon fruits (*Hylocereus undatus* [Haworth] Britton & Rose), a fruit plant belonging to the Cactaceae family, has occupied a growing niche in the fruit market, with wide demand in the global market (Rodríguez, 2000; Le Bellec *et al*., 2006; Cordeiro *et al*., 2015).

Thus, with the increase in acceptance of exotic fruits worldwide, pitaya's market has been favored, increasing the economic and agronomic potential of its cultivation (Rodríguez, 2000). The organoleptic characteristics, together with the nutraceutical properties, make pitaya products desirable in consumer markets (Silva *et al*., 2006; Andrade *et al*., 2007; Perween *et al*., 2018). In addition, the plant's rusticity and the aggregate commercial value of the fruits have contributed to expansion of the cultivation of this species, which makes it attractive to fruit growers (Ortíz-Hernández *et al*., 1999; Bastos *et al*., 2006; Santos *et al*. 2010; Xu *et al*., 2019).

Fruit production depends on intrinsic factors, extrinsic factors and a combination of both, and the organoleptic and nutritional quality depend on the maturation stage (Centurión Yah *et al*., 2000). The maturation stage of fruits at harvest influences final quality, interfering in the post-harvest process. When fruits are harvested immaturely, the maturing process is impaired, which is reflected in the quality since they become susceptible to physiological disorders, for example, cell disorganization and cell wall disruption. However, fruits harvested at the supermature stage will favor senescence, reducing shelf-life and hampering handling, storage and transport as the result of low physical resistance and sensitivity to injuries and diseases, causing quantitative and qualitative losses (Kays, 1991; Chitarra and Chitarra, 2005).

Fruit maturation is not a clearly event in plant phenology models (Chuine *et al*., 2003). Thus, in order to obtain a product with commercial value, it is necessary to precisely determine when the fruit reaches physiological maturity, which can be identified by physical parameters (longitudinal length, equatorial diameter, thickness, weight, and color) and/or chemicals parameters (soluble solids content and titratable acidity ratio and pH).

Because studies on pitaya are still restricted, little is known about harvest rates and post-harvest behavior of the fruits of this species. In addition, a lack of knowledge on pre- and post-harvest techniques for the management of pitaya fruit quality has made it difficult to market them globally (Centurión Yah *et al*., 2008). Thus, this review aims to establish the ideal period for harvesting pitaya fruits (*Hylocereus undatus*) by determining the physiological maturity point.

ORIGIN AND MORPHOLOGY OF PITAYA

Pitaya, part of the Cactaceae family, originated from the tropical and subtropical Americas, whose adaptability to different environmental conditions has favored introduction in countries with edafoclimatic differences (Mizrahi *et al*., 2002; Tel-Zur *et al*., 2004). It is grouped into four genera: *Stenocereus* Briton & Rose and *Cereus* Mill. (which they are columnar cactaceae), and *Selenicereus* (A. Berger) Riccob and *Hylocereus* Britton & Rose (both are epiphyte cacti) (Zee *et al*., 2004; Le Bellec *et al*., 2006).

The Antilles words 'Pitahaya' or 'Pitaya', meaning fruit covered by scales, are used for both the plant and the fruit (Rodríguez, 1993; Zee *et al*., 2004). The names for these species include 'Moonflower' and 'Queen of the night' because the flowers open only at night and close in the early hours of the following day, along with the beauty of its flowers, one of the more beautiful in the world (Mizrahi and Nerd, 1999).

In eastern countries, they are known as 'Dragon fruit' because the external bracts resemble dragon scales (Mizrahi and Nerd, 1999; Meráz *et al*., 2003). However, use of the term 'Pitahaya' has become widespread, not only in Colombia and Nicaragua, where the word 'Pitaya' has been used repeatedly, but also in European and Asian countries where it is traded and where the name 'Pitahaya' is often added to the local designation to better identify it in the market (Rodríguez, 2000; Xu *et al*., 2019).

Pitaya is an epiphytic, rupiculate or terrestrial branched, perennial and succulent plant. The taxonomic group '*Hylocereus*' is diploid, as in most cacti, characterized by plants with a scandal or climbing habit that grow in both trees and rocks (Ortíz-Hernández, 2000). Commercial cultivation commonly uses the staking system in production.

The stem is classified morphologically as cladodium, segmented with trigone or trialled branches, reaching up to 35 m in length and 5 to 7 cm in diameter, with wings that have an approximately 2.3 cm height (Rodríguez, 1993). The cladodium is succulent, with sharp, creased and horny edges; it is greenish with a photosynthesizing function or greyish with aging because of the wax that covers it (Donadio, 2009). It is devoid of true leaves; however, there are modified leaves, i.e. thorns (Paula and Ribeiro, 2004; Socha, 2007). The stem has a sub-winged areolas with a dilated base in a bulb; they are of 2 to 3 cm in diameter, 3 to 5 cm from each other, and have 3 to 6 thorns each that are 1 to 4 mm long (Donadio, 2009).

Numerous adventitious roots originate from the cladodes in the intercostal spaces that contribute to the absorption of nutrients and anchoring the plant (Rodríguez, 1993; Ortíz-Hernández, 2000). The root system is fasciculate and superficial; however, it has the capacity of extracting nutrients from the soil even when they are at low concentrations (Le Bellec *et al*., 2006).

The flower is complete, androgynous, solitary, aromatic and lateral, with white or pink coloring, measuring from 20 to 35 cm in diameter. It contains numerous stamens, with over 800 in a single flower. They are arranged in two rows around the pistil, formed by 14 to 28 cream colored stilettos. The sepals are light green in color. and the pollen is abundant and yellow in color (Crane and Balerdi, 2005; Le Bellec *et al*., 2006; Donadio, 2009).

In *H. undatus*, flower buds are formed shortly before anthesis, exhibiting rapid development, about three weeks (Nerd *et al*., 2002b). The anthesis is nocturnal, lasting approximately 15 h. The maximum flower opening occurs between 23.00 and 01.00 h. The emission of floral buds occurs in the areolas, appearing as a bud in each one only once, and other reproductive or vegetative organs are not developed in this region (Marques, 2010). The areolas are protruding or depressed points, where the axillary buds are located. In order to avoid self-pollination, *H. undatus* flowers have higher stigma than anthers (Marques, 2008).

The fruit is a berry, globose or subglobous, and measures from 10 to 20 cm in diameter, with mass varying from 200 to 1.000 g, but the average mass of the fruit varies from 350 to 450 g (Nerd and Mizrahi, 1997). Its formation takes place from the development of the ovary (Mizrahi and Nerd, 1999).

According to the species, the fruits may present diversified characteristics, such as shape, presence of thorns and pericarp and pulp color, reflecting high genetic variability (Junqueira *et al*., 2010). The pericarp may have a pink, red or yellow tonality, with foliar scales (bracts) varying in number and length, which are formed from the receptacle surrounding the ovary (Mizrahi and Nerd, 1999). The pulp is juicy, white, red or magenta color; according to the species, with approximately 18 °Brix (Le Bellec *et al*., 2006; Lorenzi *et al*., 2006; Livera-Muñoz *et al*., 2010).

The fruits have climacteric or non-climacteric respiration. In this respect, some studies on pitaya have concluded that it is part of the group of climacterics (Chávez and Stevenson, 1992; Garnica and Quintero, 1994; Camargo and Moya, 1995; Rudas, 1995). However, other authors, based on the low ethylene concentration obtained in their experiments, classified it as non-climacteric (Nerd and Mizrahi, 1997, 1999; Zee *et al*., 2004; Arévalo-Galarza and Ortíz-Hernández, 2004).

The seeds are distributed numerically throughout the pulp, approximately 3 mm in diameter, dark in color, obovate in shape, smooth, shiny and with a high germination capacity (Crane and Balerdi, 2005; Le Bellec *et al*., 2006). Weiss *et al*. (1994) and Nerd and Mizrahi (1997) observed a positive correlation between fruit mass and number of seeds.

PRODUCTION AND ECONOMIC IMPORTANCE OF PITAYA

The most widespread pitaya species are the red pericarp (*Hylocereus* spp.), whose fruits may have white pulp (*Hylocereus undatus* [Haworth] Britton & Rose) or red pulp (*H. polyrhizus* [Weber] Britton & Rose), and the yellow pericarp (*Selenicereus megalanthus* [Schum] Britton & Rose) (Ortíz-Hernández, 2000; Nerd *et al*., 2002b; Le Bellec *et al*., 2006).

Among them, the species *Hylocereus undatus*, red pitaya with white pulp, is the world's most widely cultivated (Nerd *et al*. 2002a) in Oceania (Australia and New Zealand), Asia (Malaysia, Indonesia, Philippines, Vietnam, Cambodia, Thailand, China, Korea, Taiwan, Japan and Israel), of Europe (Spain), North America (United States and Mexico), Central America (Guatemala, El Salvador, Nicaragua, Costa Rica and Panama), and South America (Colombia, Venezuela, Ecuador, Peru, Uruguay and Brazil) (Rodríguez, 1993; Mizrahi and Nerd, 1999; Xu *et al*., 2019).

A few decades ago, pitaya was little known but in the 90s received more attention, occupying a growing niche in the exotic fruit market. The number of producing countries of this species has grown, being widely consumed in Asia (Le Bellec *et al*., 2006, Fernandes *et al*., 2018; Xu *et al*., 2019). Significant production is occurring and expanding in many countries, including Colombia, Mexico, Nicaragua, Ecuador, the United States, Thailand, Malaysia, Indonesia, Vietnam, China and Australia (Junqueira *et al*., 2010; Cavalcante *et al*. 2011; Paull and Chen, 2019). Vietnam, the leading exporter of pitaya fruits in the world, has almost 40,000 ha devoted to cultivation of this species, with a production volume reaching about 1 million metric tons (ADAWR, 2017).

The interest in this fruit is increasing in consumers because of its organoleptic characteristics and nutraceutical properties (Silva *et al*., 2006; Andrade *et al*., 2007). It is a nutritious fruit and can be consumed both in natura or as raw material in a range of industrialized products, such as beverages, cosmetics and medical products (Esquivel, 2004). Some species are rich in flavonoids, vitamins and fibers, besides being a source of vitamin A and B (B_1, B_2 and B_3), phosphorus, calcium, potassium and sodium, with a caloric value of 38 kcal/100 g pulp (Crane and Balerdi, 2005; Wu *et al*., 2006; Esquivel *et al*., 2007a; Gunasena *et al*., 2007; Perween *et al*., 2018).

On the other hand, fruit growers are attracted by the aggregate commercial value of the pitaya, an alternative for cultivation. In addition, because of its rusticity, it has become an option with agronomic, economic and nutritional potential in shallow, sandy and stony soils (Ortíz-Hernández *et al*., 1999; Bastos *et al*., 2006; Santos *et al*. 2010; Xu *et al*., 2019).

Pitaya propagation can be with seeds or vegetative structures, notably cutting, grafting and micropropagation. The average productivity of this species is variable according to the soil and climatic conditions, cultivation techniques and orchard age, ranging from 10 to 30 t ha^{-1} (Le Bellec *et al*., 2006). Vaillant *et al*. (2005) confirmed that, in Nicaragua, well-managed crops can produce up to 26 t ha^{-1}. Bastos *et al*. (2006) reported, in Brazil, an average yield of 14 t ha^{-1}, and, according to Nguyen *et al*. (2015), yield averages 22-35 t ha^{-1} in Vietnam.

PHYSIOLOGICAL MATURITY OF PITAYA FRUITS

The fruits go through a series of transformations during the development process. Fruit ripening is a complex and genetically programmed process, resulting in changes in color, aroma, texture and taste, which are important to consumer acceptance. However, it is necessary to determine the point of harvest because its absence causes post-harvest losses in fruits and vegetables. Reducing post-harvest losses and quality deterioration are essential to increasing food availability from existing production. Minimizing this loss is important to food security, economic growth and social welfare (Ayub *et al*., 1996; Kasso and Bekele, 2018).

In the pitaya crop, the annual flowering period is related to the cultivation region because it is dependent on the photoperiod, characterized here as long days (Nerd *et al*., 2002b; Luders and McMahon, 2006). However, it is important to determine the period in which the fruits reach physiological maturity to optimize harvest and obtain quality product since edaphoclimatic conditions may exert influence during the development of the fruits.

The importance of determining the appropriate phase for fruit harvest means it is necessary to evaluate the pattern of growth and development after flowering, helping to establish maturation indices based on both environmental and varietal variations that are practical for efficient use by producers (Cavalini, 2004).

A properly timed harvest requires a determination of the physiological maturity stage of the fruit, which be identified by physical parameters, such as longitudinal length, equatorial diameter, mass, shape, color and firmness, and/or with chemicals using the soluble solids content, titratable acidity, and pH, among others. However, one of the criteria used in determining the harvest point is number of days of anthesis until full development of the fruit (Warrington *et al*., 1999; Lemos *et al*., 2018).

The determination of the physiological maturity phase based solely on fruit appearance is empirical since it is a subjective measure subject to variations and errors. In this sense, the maturation stages of some species are defined by the pericarp color, which is used by consumers to judge maturity (Kays, 1991; Cavalini, 2004).

According to Thé *et al*. (2001), pericarp color is closely related to fruit maturation and climatic conditions during the growth period. Color is an important parameter for producers and consumers because it indicates whether the fruit has the ideal conditions for commercialization and consumption. However, color in most cases does not contribute to an effective increase in the nutritional value or quality of the product (Chitarra and Chitarra, 2005), but consumers prefer fruits with a bright and strong color.

The literature reports that the first change in the pericarp color in *H. undatus* occurs between 24 and 29 days after anthesis (DAA), becoming completely red between 2 and 5 d after the first color change (Castillo-Martínez and Ortíz-Hernández, 1994; Nerd *et al*., 1999; Centurión Yah *et al*., 2008; Ortiz and Takahashi, 2015). Centurión Yah *et al*. (2008) and Ortiz and Takahashi (2015) obtained completely red fruits at 31 and 30 DAA, respectively.

Previously unpublished images obtained from studies by Ortiz and Takahashi (2015) are in this literature review to make this study more instructive and illustrative (Fig. 1).

For the maturation of pitaya fruits, studies have observed a reduction in the hue color angle (*h*°), that is, fruits changed from green to red when they reached physiological maturity (Centurión Yah *et al*., 2008; Ortiz and Takahashi, 2015). Van To *et al*. (2002) confirmed that the range of *h*° in pitaya fruits suitable for marketing must be equal to or less than 30°. Osuna Enciso *et al*. (2011) obtained completely mature fruits with values below 30°.

According to Wybraniec and Mizrahi (2002) and Le Bellec *et al*. (2006), the red coloration of *Hylocereus* spp. fruit pericarp is due to betacyanin pigments, whose synthesis is activated by a high availability of sugars and light, among other factors (Castellar *et al*., 2003). Phebe *et al*. (2009) found a significant negative correlation between hue color angle values and total content of betacyanins in *H. polyrhizus* pericarp, with a drastic increase of 90% in betacyanins between 25 and 30 DAA.

Another important characteristic to take into consideration in pitaya fruits is luminosity since contrasts in luminosity make fruits more attractive. Thus, the lightness index (L^*) has been evaluated in studies carried out to determine the time at which pitaya fruits reach physiological maturity (Ortiz and Takahashi, 2015).

Centurión Yah *et al*. (2008) did not obtain significant differences when analyzing the lightness index during the development of *H. undatus* fruits. However, Ortiz and Takahashi (2015) observed that L^*

decreased in fruits of this same species, which is not to say that the fruits became less attractive; however, they verified that chroma (*C**) increased with maturation, indicating that the pericarp color became more intense, which would possibly increase its acceptance by consumers.

According to Tucker (1993), Chitarra and Chitarra (2005) and Silva *et al*. (2019), the reduction of green in fruit pericarp is a process of color change that, in addition to allowing the appearance of the typical color of the analyzed species, is indicative of the maturation stage in fruits and the harvest point. Therefore, the evolution of coloring in pitaya fruits from 21° to 32° DAA through the external and internal fruit pericarp (Fig. 1 and 2) shows the reduction of green in the pericarp and the beginning of the typical coloration of this species starting at the 28th DAA, becoming completely red at 30 DAA.

In addition to color, fruit size variability is also used to define fruit maturation stages (Ortiz and Takahashi, 2015; Almanza-Merchan *et al*., 2016; Chacón-Padilla and Monge-Pérez, 2016). Ortiz and Takahashi (2015) did not find a significant difference in the longitudinal length during the evolution of the maturation of pitaya fruits from 21st to 32nd DAA, which ranged from 10.6 to 11.7 cm. However, Centurión Yah *et al*. (2008) obtained an increase in this parameter with proximity to physiological maturity, obtaining fruits

Figure 1. Transition in the external coloring of the pericarp of pitaya fruits. DAA, days after anthesis.

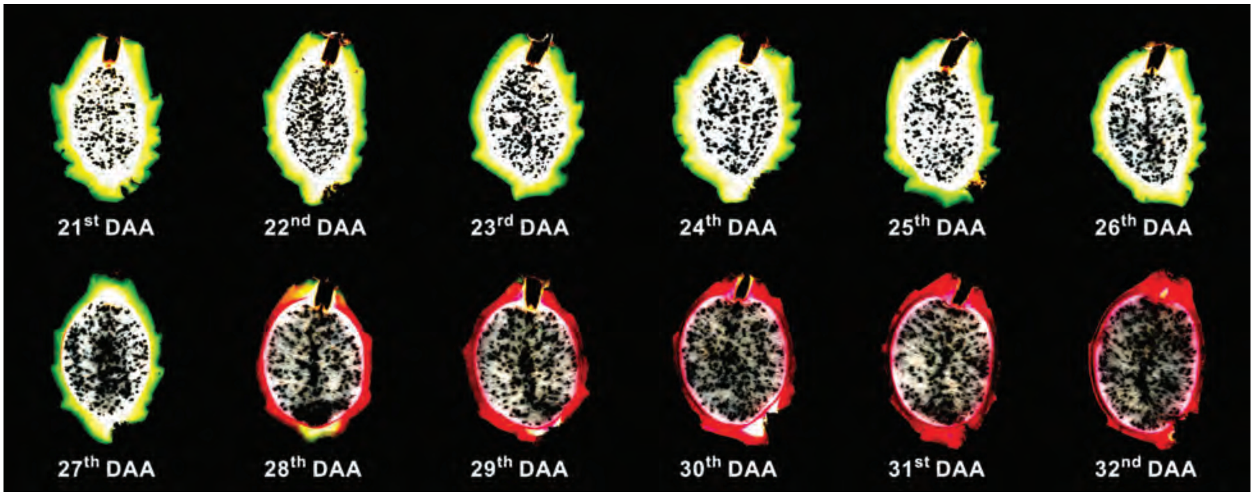

Figure 2. Transition in the internal coloring of the pericarp of pitaya fruits. DAA, days after anthesis.

with a longitudinal length of 8.9 cm at 31 DAA; Osuna Enciso *et al*. (2007) reported fruits of this species with a mean longitudinal length of 14.3 cm.

The equatorial diameter of pitaya fruits has also been reported in the literature with a continuous increase as the physiological maturity advances, obtaining mature fruits between 7.9 and 8.2 cm (Osuna Enciso *et al*., 2007; Ortiz and Takahashi, 2015; Centurión Yah *et al*., 2008). Martínez (2011) observed *Hylocereus* spp. fruits with an equatorial diameter varying from 5.2 to 7.8 cm.

Other characteristics were studied and reported in the literature during the evolution of the maturation of pitaya fruits, including pericarp and pulp thickness. According to Ortiz and Takahashi (2015), the pericarp and pulp thickness presented a decreasing and increasing tendency, respectively, from the 21st to the 32nd DAA. They observed that the pericarp thickness reduced from 1.06 to 0.17 cm and the pulp thickness increased from 4.60 to 7.17 cm (Fig. 2). Martínez (2011), when evaluating six pitaya genotypes, obtained materials with a pericarp thickness from 0.22 to 0.42 cm, and Castillo-Martínez *et al*. (2005) reported a thicknesses from 0.26 to 0.37 cm when studying five *H. undatus* genotypes.

Fruit, pulp and pericarp mass have been considered when determining the physiological maturity point of pitaya fruits. Ortiz and Takahashi (2015) observed that the fruit and pulp mass showed an increasing tendency from 21° to 32° DAA, varying from 293.1 to 416.2 g and from 87.6 to 253.3 g, respectively. Centurión Yah *et al*. (2008) observed the same trend, reporting a fruit and pulp mass of 469.2 and 368.9 g at 31 DAA, respectively.

Osuna Enciso *et al*. (2007) reported an average fruit mass of 442.0 g, and Nerd *et al*. (1999) recorded 437.5 g. Castillo-Martínez *et al*. (2003); Osuna Enciso *et al*. (2007) and Castillo-Martínez *et al*. (2005) observed pitaya fruits with a pulp mass of 188.4 to 297.8 g and from 139.6 to 320.1 g, when working with five *H. undatus* genotypes, respectively. For pericarp mass, Centurión Yah *et al*. (2008); Ortiz and Takahashi (2015) reported decreasing trends following anthesis. The authors concluded that, with as maturation of pitaya fruits advanced, the pericarp mass tends to decrease, and the pulp mass tends to increase, which is desired by consumers. Castillo-Martínez *et al*. (2005) and Martínez (2011), working with different *H. undatus* genotypes, obtained fruits with a pericarp mass from 72.4 to 120.5 g and from 57.9 to 140.6 g, respectively, reflecting variability within the species.

The progression of pericarp and pulp percentage of pitaya fruits during maturation has also been studied, and Ortiz and Takahashi (2015) noted that from the 21st to the 32nd DAA the pericarp percentage of the fruits decreased from 71.3 to 34.0%, and there was an increase in the pulp percentage from 28.7 to 66.0%, which is favorable and expected since the pulp is the edible portion of the fruit.

Castillo-Martínez *et al*. (2005) reported *H. undatus* fruits with 73.0% pulp, and Osuna Enciso *et al*. (2007), at 31 DAA, reported 79.0%. Martínez (2011), working with six pitaya genotypes, evaluated materials that had 40.5 to 80.6%, and it is possible to observe that there is intraspecific variation in this variable. As for the pericarp, Centurión Yah *et al*. (2008) obtained ripe pitaya fruits with 20.4% pericarp, and Nerd *et al*. (1999) reported 32.5%.

Thus, Ortiz and Takahashi (2015) observed that the pulp/pericarp ratio showed a tendency to increase throughout harvest from 0.40 at 21 DAA to 1.98 at 32 DAA because of the increase in pulp mass and the decline of pericarp mass. Centurión Yah *et al*. (2008) observed an increased pulp/pericarp ratio, from 1.5 to 3.9 from 25th to 31st DAA.

In addition to the physical transformations during fruit maturation, several biochemical transformations also occur, including the increase in soluble solids and the reduction of titratable acidity. According to Gross *et al*. (2016), there is a relationship between these variables and the maturation stage of the fruit.

The soluble solids content is dependent on the maturation stage and generally increases during maturation evolution through biosynthesis or degradation of polysaccharides (Chitarra and Chitarra, 2005). Some authors evaluated the soluble solids content in pitaya fruits and observed an increase in this variable with the advancement of maturation, obtaining ripe fruits with 12.2 °Brix (Ortiz and Takahashi, 2015) and 13.6 °Brix (Osuna Enciso *et al*., 2011). Other authors, when evaluating different pitaya genetic materials, reported values from 10.9 to 14.1 °Brix (Castillo-Martínez *et al*., 2005), 14.5 to 17.6 °Brix (Martínez, 2011), 12.0 to 16.0 °Brix (Livera-Muñoz *et al*., 2010) and 16.0 to 17.0 °Brix (Nerd *et al*., 1999). Centurión Yah *et al*. (2008), working with *H. undatus*, observed a close relationship between color development and soluble solids increases, where fruits with 20 d of development recorded 4.6 °Brix, while at 31 d they reached 12.6 °Brix.

An important aspect that has an effect on the concentration of soluble solids in the pulp is the exposure of the fruit to light during growth because it activates the formation of pigments responsible for the red coloring of the pericarp, betacyanin compounds and sugars in the pulp (Castellar *et al*., 2003; Esquivel *et al*., 2007b).

For Centurión Yah *et al*. (2008), the taste of the pitaya fruit ranged from bittersweet to sweet between 27 and 31 DAA, and the highest fruit acceptance was between 29 and 31 DAA. According to Nerd *et al*. (1999), the accumulation of sugars during the maturation of pitaya fruits is related to a decrease in the content of starch and mucilages in the pulp; there is no contribution to metabolism from the pericarp, as occurs in *Opuntia ficus-indica* (L.) Miller fruits (De La Barrera and Nobel, 2004).

Titratable acidity is another chemical variable used in the evaluation of the physiological maturity of pitaya fruits; Osuna Enciso *et al*. (2011) and Ortiz and Takahashi (2015) reported ripe fruits with an acidity of 0.63 and 0.27%, respectively. According to Centurión Yah *et al*. (1999), fruits with a titratable acidity of 0.24% present an acceptable flavor for consumers.

Several other authors, evaluating the fruit acidity of *Hylocereus* spp., reported different values, such as 0.24% (Centurión Yah *et al*., 1999), 0.30% (Sornyatha and Anprung, 2009), 0.36% Arévalo-Galarza and Ortíz-Hernández, 2004), 0.40% (Centurión Yah *et al*., 2008) and between 0.30 and 0.60%, when studying six genotypes (Martínez, 2011).

In fruits of *H. undatus* and *H. polyrhizus*, Nerd *et al*. (1999) found that the acidity was higher in color-changing fruits than in fruits with advanced coloring, as did Ortiz and Takahashi (2015) in *H. undatus*. According to Arévalo-Galarza and Ortíz-Hernández (2004), the increase of acidity before a color change shows the beginning of the maturation process. In addition, a reduction of acidity is a problem in pitaya pulp since this indicator does not detect the sweetness of the fruit.

Thé *et al*. (2001) and Cavalini (2004) indicated that the ratio provides an indication of fruit flavor since it relates the amount of sugars and acids present. This relationship tends to increase during maturation because of the increase of sugars and the decrease of acids, evidencing the conditions of harvest, storage and immediate consumption.

However, Chitarra and Chitarra (2005) recommend caution in establishing this relationship because insipid fruits, containing low levels of soluble solids and acids, present high ratios, which can lead to erroneous interpretations of quality. As reported by Osuna Enciso *et al*. (2011), the increase in the ratio of pitaya fruits is due to the drastic reduction in titratable acidity, not an indicator of quality since soluble solids do not increase. Martínez (2011) and Centurión Yah *et al*. (2008) also observed that the increase in the ratio was due to the decrease in acidity. However, Ortiz and Takahashi (2015) found that in addition to the titratable acidity reduction, the increase in soluble solids content also contributed to the increase in the ratio in *H. undatus* fruits.

For Van To *et al*. (2002), the best indicator of flavor in pitaya is the ratio, whose ideal value is around 40. Ratios higher than recommended were reported by Ortiz and Takahashi (2015), who obtained ripe fruits with a ratio of 55.5 at 32 DAA. However, Centurión Yah *et al*. (2008) obtained fruits with a ratio of 35.5 at 31 DAA. Martínez (2011), when evaluating six pitaya genotypes, obtained materials with a ratio ranging from 33.1 to 48.6.

For pH, Esquivel *et al*. (2007b) reported pitaya fruits (*Hylocereus* spp.) with a pH between 4.2 and 4.9, and Stintzing and Carle (2006) reported a value between 4.3 and 4.7. Similar values were cited by Ortiz and Takahashi (2015) in fruits considered mature (32 DAA), with an average pH of 4.6. However, Cálix de Dios and Castillo-Martínez (2008) reported a pH of 1.7 in the *H. undatus* subspecies *luteocarpus*.

Some aspects are important to timing the harvest of pitaya fruits, such as the pericarp color, which is considered determinant, in addition to soluble solids content and ratio. However, the number of days of anthesis to full fruit development has been considered one of the criteria that are commonly used in determining the harvest point (Nerd *et al*., 1999; Warrington *et al*., 1999).

Centurión Yah *et al*. (2008) observed that the physiological maturity of pitaya fruits (*H. undatus*) in Yucatán-Mexico occurred between 25th and 31st DAA because, in this period, the color of the pericarp varied from light green mixed with incipient red tones to purple-red on the entire surface. These authors also observed that, at 31 DAA, growth was still active although this age was considered the harvest limit in order to avoid cracking of the pericarp. During the evolution of the maturation, there was accumulation of the edible portion of the fruit and increases in the content of reducing sugars, soluble solids and ratio; however, firmness and acidity were reduced. The authors concluded that there was variation in flavor, from bittersweet to sweet in fruits harvested between the 29th and 31st DAA, the most accepted by consumers.

Castillo-Martínez and Ortíz-Hernández (1994) observed that the development period of *H. undatus* fruits in Oaxaca-Mexico occurs from May to September, with physiological maturity from 31st to 41st DAA. The onset of maturation in pitaya fruits is characterized by a color change in the pericarp between the 24th and 25th DAA; maturation entails the first manifestation of red coloration in the pericarp until the appearance of a brindle brown color. However, because of the subsequent loss of the commercial value of the fruit, these authors stated that the useful harvest stage occurs from the 25th to the 31st DAA. Similar periods for the stage in which fruits of this species reach physiological maturity were obtained by other authors, such as from 28th to 30th DAA (Van To *et al*., 2002) and from 25th to 31st DAA (Martínez, 2011).

Nerd *et al*. (1999) reported that, in *H. undatus* fruits, the onset of color change also occurs from 24th to 25th DAA, and, after 4 to 5 d, they become completely red, confirming that the development of the pericarp color is related to the soluble solids content. Van To *et al*. (2002) stated that *H. undatus* fruits must be harvested when they acquire the red color, suggesting that the best indicator of pitaya flavor is the ratio, whose ideal value is close to 40.

Ortiz and Takahashi (2015) verified that the physical and chemical characteristics evaluated were influenced by the maturation of pitaya fruits, with the exception of the longitudinal length. Thus, they concluded that *H. undatus* fruits in Paraná-Brazil reached physiological maturity between the 30th and 32nd DAA, proving this as the ideal period for harvesting the fruits since, in this period, the fruits have become completely red and have reached the soluble solids content, titratable acidity, pH and ratio recommended and characteristic for this species.

Many characteristics are important and essential in the designation of the ideal period for harvesting pitaya fruits. Therefore, determining the physiological maturity of the fruits is necessary to optimize the harvest and quality of the final product. According to Cavalini (2004), using more than one variable to characterize a maturity stage allows greater precision when classifying fruits in their stages.

CONCLUSIONS

For the pitaya, the number of days of the anthesis until the full development of the fruit is the most reliable variable to determine the timing of harvest. With this designation, it is possible to indicate the phase (physiological maturity) in which the fruits present characteristics that are favorable to this species, such as color, soluble solids content, and ratio, among others, making it possible to obtain a product with quality and consumer acceptability.

The place of production and edaphoclimatic conditions can interfere and cause variation in the period in which the fruits reach physiological maturity. Based on studies carried out in this area, the recommendation is to harvest fruits between the 25th and 32nd days after anthesis to avoid loss of the commercial value.

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The ecophysiology of cape gooseberry (*Physalis peruviana* L.) - an Andean fruit crop. A review

La ecofisiología de uchuva (*Physalis peruviana* L.) - un frutal andino. Una revisión

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Cape gooseberry with flower buds, flowers and developing fruits in calyx.

Photo: G. Fischer

ABSTRACT

In a literature review of the ecophysiology of cape gooseberry (*Physalis peruviana* L.) it was found that in Colombia this typical Andean plant adapts to a wide altitudinal range of the tropical cold climate, between 1,800 and 2,800 m a.s.l., with optimal medium temperatures between 13 and 16°C and base (minimum) temperatures for stem and fruit growth of 6.3 and 1.9°C, respectively. However, this fruit does not withstand temperatures $<0°C$. The Andean conditions of the tropics such as high solar radiation and rather short day lengths <12 hours favor flower initiation. A duration of 1,500-2,000 hours year^{-1} of direct sunshine are the most favorable for the size, quality and ripening of the fruit. Under field conditions in Bogota we measured a photosynthesis rate of $A = 10.545$ μmol CO_2 m^{-2} s^{-1} and light compensation point $Ic = 13.645$ μmol photons m^{-2} s^{-1}. As this species with an indeterminate growth habit requires a constant supply of water, while high amounts or heavy rains after a dry season cause cracking of the fruits, the plant does not tolerate waterlogging for more than 4 days. Cape gooseberry is classified as moderately tolerant to salinity and 30 mM NaCl curiously promotes growth, since the plant has mechanisms such as increased antioxidant activity to protect against saline conditions.

Additional key words: temperature; altitude; photosynthetic rate; water; salinity.

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RESUMEN

Con el fin de revisar la literatura sobre la ecofisiología de la uchuva se encontró que esta típica planta andina en Colombia se adapta a un rango amplio de altitud de clima frío tropical, es decir entre 1.800 y 2.800 metros sobre el nivel del mar (msnm), con temperaturas medias óptimas entre 13 y 16°C y temperaturas bases (mínimas) para el crecimiento del tallo y del fruto relativamente bajas, 6,3 y 1,9°C, respectivamente, sin embargo no tolera temperaturas <0°C. Igualmente, las condiciones andinas del trópico como es la radiación solar alta y las longitudes del día <12 horas, más bien cortas, favorecen el inicio de la floración. Entre 1.500 y 2.000 horas año^{-1} de brillo solar (luz solar directa) son los más favorables para el tamaño, calidad y maduración del fruto. Bajo condiciones de campo en Bogotá, se midió una tasa de fotosíntesis A = 10,545 μmol CO_2 m^{-2} s^{-1} y punto de compensación por luz Ic = 13,645 μmol fotones m^{-2} s^{-1}. Como planta con un hábito de crecimiento indeterminado, la provisión constante de agua es indispensable, mientras altas cantidades o lluvias fuertes, después de una época seca, causan el rajado de los frutos, igualmente como la planta no tolera más que 4 días el anegamiento. La uchuva es calificada como una planta moderadamente tolerante a la salinidad y 30 mM NaCl curiosamente fomentan el crecimiento, teniendo mecanismos, como el aumento de la actividad antioxidante, para protegerse contra condiciones salinas.

Palabras clave adicionales: temperatura; altitud; tasa fotosintética; agua; salinidad.

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INTRODUCTION

Ecophysiology studies the environmental effects on plant physiology, describing the physiological mechanisms during plant growth and development and their interactions with biotic and physicochemical environmental factors (Lambers *et al*., 2008). Climatic factors influence crops at the same time, and when they become stressful as occurs during excessive heat, drought, strong ultraviolet light and wind, and other environmental stressors, their action can be very damaging to the plant (Mittler, 2006). No factor alone impacts physiology and performance (Fischer *et al*., 2016).

Site growth conditions, like climate, soil and crop management, affect the size of the plant, the duration of its phenological stages, and the period and volume of the harvest (Fischer *et al*., 2016; 2018) This means that planting a crop in an unsuitable ecophysiological site increases the production costs by reducing economic success (Fischer and Orduz-Rodríguez, 2012).

In recent decades, many environmental imbalances caused by deforestation and increased greenhouse gas emissions have caused significant changes in worldwide climatic dynamics (Menezes-Silva *et al*., 2019). These have major implications for global food security affecting, among other factors, the growth and productivity of plants (Dhankher and Foyer, 2018). For these climate changes, UNEP (2019) predicted that near the end of the 21st century (2100) the global average temperature will increase by 3.2°C, if governments do not take more stringent measures to control the greenhouse effect. Shukla *et al*. (2019), in their IPCC technical summary, stated clearly that fruits and vegetables, as key elements of healthy diets, are susceptible to climate change and that production, quality, and crop suitability will decline as temperatures increase, particularly in the tropics and semi-tropical regions.

Especially for the tropical Andes, the average precipitation will increase by 20-25% (Marengo *et al.*, 2011). Likewise, they affirm that in the high Andean zones - where the greatest production of cape gooseberry is concentrated - the magnitude of the warming will tend to be greater than in the low zones. Baldwin *et al.* (2019) reports that extreme weather events, including heat waves and droughts, are expected to increase in occurrence and severity as atmospheric CO_2 rises.

The cape gooseberry (*Physalis peruviana* L., Solanaceae) is native to the South American Andes (Fischer and Melgarejo, 2014), in Peru, Brazil, Chile, Ecuador, and Colombia. Commercial production has spread far more widely than these previously named countries, including now Kenya, South Africa, Egypt, Zimbabwe, New Zealand, Australia, India, China and Hawaii, and now also in Caribbean countries (Fischer *et al*., 2014; Ramadan and Mörsel, 2019). The CABI Invasive Species Compendium (2019) reports the appearance of *P. peruviana* in most countries of the world, classifying it as a plant of the high tropics that also grows well in the subtropics and in the not excessivley cold conditions of the temperate climate, where it behaves like an annual plant.

In Colombia, according to Agronet (2019) data for 2017 the annual production of cape gooseberry fruits was 18,889 t over a harvested area of 1,259 ha, located mainly in the departments of Boyaca (616 ha), Cundinamarca (465 ha), Antioquia (149 ha) and Nariño (130 ha). Colombia is not only the largest producer, but also an exporter of this fruit (with USD 32.4 million in 2018), especially to European countries, primarily the Netherlands, Belgium, the United Kingdom and Germany (Agronet, 2019).

The plant develops as a semi-shrub, semi-perennial with an indeterminate growth habit (Ramírez *et al*., 2013), and attains commercial fruit production during the first 18 months of the crop. It reaches a height of 1-1.5 m, generally forming four erect productive branches (Fischer *et al*., 2014). If not controlled, the plant can become invasive displacing other crops and be considered a weed (CABI Invasive Species Compendium, 2019).

On the aerial part of the plant and particularly on the leaves - which are simple, heart-shaped and alternate – the cape gooseberry develops full pubescence (Fischer and Miranda, 2012) that protects against sudden changes between day/night temperatures and high UV radiation from the tropical highlands (Fischer, 1995).

The flowers are hermaphroditic with a yellow tubular corolla and are solitary, while the calyx with a cupuliform structure (Nocetti *et al*., 2020), consists of five persistent sepals (or modified leaves). These form a husk 4-5 cm long that encloses and protects the fruit (against pests, UV light, rain, hail and cold) until maturity. It then changes into a translucent and parchment husk (Fischer and Miranda, 2012), due to the degradation of its chlorophyll and the translocation of almost all its carbohydrates, especially during the first 20 d of development of the fruit, (Fischer *et al.,* 2015). The development of the fruit, according to the agro-ecological condition, takes 60 - 80 d (Fischer and Miranda, 2012).

The almost round fruits of cape gooseberry are yellow-orange berries with a diameter of 1.25 - 2.5 cm, weighing 4 - 10 g (Dostert *et al*., 2012); while the ‘Kenya’ ecotype is up to twice this size, also showing a polyploid chromosomal number of twice (2n=48) that of the Colombian wild ecotypes (2n=24). The commercial ecotype ‘Colombia’ has a chromosomal endowment of 2n=32 (Rodríguez and Bueno, 2006). The fruit contains up to 350 seeds, which are small (about 1.1 mg in weight), flattened and lenticular (Fischer, 2000a; Fischer *et al*., 2007).

The fruits of the cape gooseberry contain multiple health properties due to the elevated content of antioxidants, minerals and vitamins (Ramadan and Mörsel, 2019). It is an antioxidant and anti-inflammatory (Puente *et al*., 2019) and stands out for its contents of provitamin A (648-5,000 IU), ascorbic acid 11-43 mg/100 g fresh weight and iron (1.1-1.7 mg), 13-15°Brix (Fischer *et al*., 2011). Akbaba (2019) emphasizes the important medical use of this fruit in the control of hepatitis, malaria, rheumatism, dermatitis, diabetes and weight loss. Furthermore, the sap of the plant synthesizes withanolides that serve as repellents against coleopteran pests (Fischer *et al*., 2011).

Ecofisiological studies and related topics are of special importance for finding adaptation strategies for the crops to changing environmental conditions (Sánchez-Reinoso *et al*., 2019; Cardona *et al*., 2016; Cleves-Leguízamo *et al*., 2017), therefore the objective of this review is to report on the current state of knowledge of the ecophysiological factors in the growth and physiology of the cape gooseberry plant and thus facilitate significant decisions for research and production of this important Andean fruit species.

ENVIRONMENTAL FACTORS

Temperature

Temperature is the most complex factor in ecophysiology (Gariglio *et al*., 2007). Like all plants, the cape gooseberry needs a base (or minimum) temperature to begin growth (Parra *et al*., 2015). This was established by Salazar *et al*. (2008) at 6.3°C by the appearance of nodes on the stem. This temperature confirms the species’ good adaptation to cold climatic zones in Colombia (Fischer and Miranda, 2012).

The cape gooseberry base temperature for the different phenological stages in its reproductive phase (investigated so far in a few species) is shown in Table 1. Salazar *et al*. (2008) calculates a light extinction coefficient (k) of 0.469, a light use efficiency (LUE) of 0.46 g MJ^{-1} for the vegetative state and 2.62 g MJ^{-1} for the reproductive phase, registering 69% of the plant´s total dry matter (DM) for the distribution in the fruit (Tab. 1).

In a cape gooseberry experiment, Aguilar-Carpio *et al*. (2018) applied the base temperature of 6.3°C for stem growth in order to calculate the heat units (HU = [(Tmax - Tmin)/2] - 6.3). Comparing different concentrations of Steiner's nutrient solution Salazar *et al*. (2008) found that the solution of 150% required only 1,370 HU, compared with 1,435 and 1,527 HU at 100 and 50% of the Steiner's solution, respectively. Therefore, the plant had 15 d of precocity in fruit production compared to the 50% solution.

The calculated base temperature of 1.9°C for the development of the fruit is real, taking into account that they resist temperatures as low as 1.6°C during storage (Alvarado *et al*., 2004) without physiological damage.

The average temperature range guaranteeing good adaptation and production of cape gooseberry at a Colombian site is 13 to 16°C (Fischer and Miranda, 2012). However, Carillo-Perdomo *et al*. (2015) reported an optimal average temperature of 18°C. Possibly, the most favorable temperatures for the production and quality of cape gooseberry fruits also depend on the ecotype or genotype, because in Cundinamarca at 17.5°C medium temperature in Subia (1,900 m a.s.l.) the fruits of the Colombia ecotype were of a smaller size and weight (5.12 g), compared to the site with more elevation, San Raimundo (2,100 m a.s.l., 16.0°C), with a weight of 6.95 g (Mazorra *et al*., 2003). From 30°C an inhibition of flowering has been observed (Wolff, 1991). However, optimal temperatures for seed germination in cape gooseberries collected in Sertão (Rio Grande do Sul, Brazil) and Capelinha (Minas Gerais, Brazil), were 27 and 32°C, with 98 and 96% germination respectively (Nunes *et al*., 2018). Diniz *et al*. (2020) observed that cape gooseberry seeds germinated within a wide range of temperatures between 15 and 30°C, with the best results at 25°C of constant temperature or 20-30°C alternating temperatures, under a lighting of 8 h d^{-1} light.

Constant night temperatures <10°C impair plant development (National Research Council, 1989). Likewise, in a growth chamber at a constant temperature of 12° C, the cape gooseberry did not prosper because of a reduction of the amount of fruit set and the formation of very flattened fruits that are not marketable (Pacheco and Sáenz, 1991).

In a study of agricultural zoning of climatic risk in the Southeast of Brazil, 10% of land suitable for the cultivation of *P. peruviana* was identified when taking into account air temperature between 13 and 18°C and an annual precipitation of 1,000-2,000 mm. This ruled out regions superior to 30°C and inferior to 13°C (Aparecido *et al*., 2019).

The fruit of the cape gooseberry is protected by a calyx against large variations in temperature and that encloses the fruit throughout its development. Fischer (1995) found that in Villa de Leyva (Boyaca, Colombia), at 2,300 m a.s.l., the temperature at noon was up to 5°C lower inside this organ than that outside (Fig. 1), but this protective effect did not appear at a higher and colder site in Tunja at 2,690 m a.s.l. Apart from the extreme temperatures, the calyx protects the fruit against sunburn, hail and mechanical damages such as air-distributed diseases, insects, and birds (Fischer *et al*., 2011).

Table 1. Base temperature and dry matter distribution for the different phenological stages of the cape gooseberry (Salazar *et al*., 2006).

Phenological state	Base temperature (°C)	Distribution of DM in the different aerial organs		
Appearance of knots on stem	6.3	Vegetative state	Leaves	72%
First flower bud appearance	10.8		Stem	28%
Time between first flower bud until first flower	5.6	Reproductive state	Leaves	9%
Time between first flower until first fruit	10.5		Stem	22%
Time between first fruit and ripe fruit	1.9		Fruits	69%

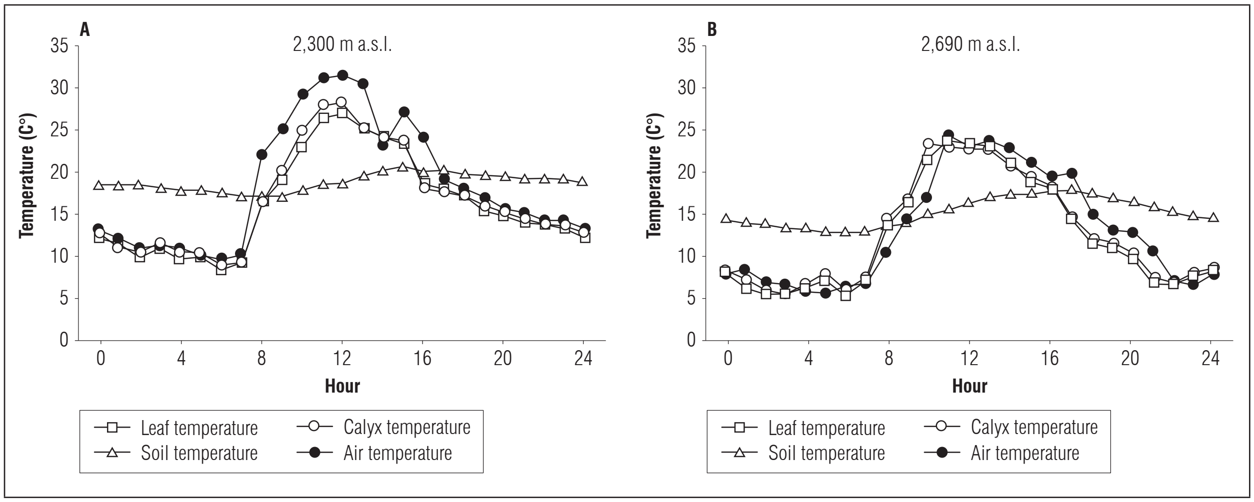

Figure 1. **Changes in air temperature in the leaf shoot, inside the calyx, and at 10 cm soil depth for 24 hours in cape gooseberry (*Physalis peruviana* L.) in a lot in (A) Villa de Leyva (2,300 m a.s.l., 17.4°C, 66.6% RH) and (B) Tunja (2,690 m a.s.l., 12.5°C and 79.0% RH) in Boyaca Colombia (Fischer, 2019).**

The temperatures of the root surroundings exert a great influence on the metabolism and the functions of the roots, for which Fischer and Melgarejo (2014) reported that temperatures between 15 and 22°C of the cape gooseberry roots best favor leaf growth, while at 8°C the development of the plant is very poor (related to the longitudinal growth of branches, insertions and leaf surface). Possibly this is induced by the higher viscosity of the water and the lower permeability of the membranes at this low temperature. The membrane lipids become less fluid at these low temperatures and their protein components no longer function properly (Taiz and Zeiger, 2010), resulting in less absorption of water and nutrients and a reduction in growth and photosynthesis of the plant (Fischer *et al*., 2000a).

A herbaceous plant such as cape gooseberry suffers from frost that causes burns and dark colorations, especially in young plants, flowers, new shoots, leaves and calyces at 2°C (Fischer and Melgarejo, 2014). Irreparable burns occur at temperatures below 0°C (Carillo-Perdomo *et al*., 2015). In the plantations of the Colombian Cundinamarca-Boyacense zone from 2,400 m a.s.l., there are drops in temperature, especially in the early morning, that coincide with dry weather and a clear sky and that are often without wind and these temperature drops induce radiation frost (Torres *et al*., 2016). The cape gooseberry is capable of a resprouting of the basal shoots if the frost is short (up to -6°C) (Fischer and Melgarejo, 2014).

Light

Solar radiation plays a crucial role as an energy source for the production of DM and fruits of the cape gooseberry (Fischer and Orduz-Rodríguez, 2012). The plant depends on visible light to maintain a positive carbon balance through photosynthesis (Taiz and Zeiger, 2010). Consequently, the DM of the plant depends on the incidence and absorbed radiation, in addition to the ability to convert the radiation to carbohydrates through photosynthesis (Gariglio *et al*., 2007).

The amount of light (light intensity or irradiance), the quality of light (wavelength) and the photoperiod (light hours/day) are some of the light factors perceived by the cape gooseberry (Fischer and Melgarejo, 2014), taking into account that the solar radiation that falls on the green fruit calyx and the two adjacent leaves are decisive for fruit maturation and quality (Fischer *et al*., 2015). In general, Mora *et al*. (2006) estimate that for cape gooseberries 1,500 to 2,000 h of direct sunlight per year are the most favorable for the size, quality and ripening of the fruit. This characterizes the cape gooseberry as a light demanding plant (Carrillo-Perdomo *et al*., 2015).

Under conditions of restricted luminosity - as in the case of a dense plantation or in a greenhouse - the cape gooseberry reacts with an elongation of the branches compared to plants in the open field that are well-distanced and where a higher incidence of UV light exists (Fischer, 2000b).

In the case of direct solar radiation onto the cape gooseberry fruit in which the calyx has been removed, the first author of this review found that in Villa de Leyva (Boyaca, 2,300 m a.s.l.) the fruit epidermis was burned after one week and mined by beetle larvae. This underlines the importance of the calyx against direct sunstroke and the production of a withanolide repellent at the base of the calyx against insects. Furthermore, as previously mentioned, the calyx protects against an extreme increase in the temperature of the fruit, thus, avoiding its cracking (Peet, 2009).

In some studies related to foliar photosynthesis (gas exchange) in plants grown under field conditions in Bogota, the authors of this document found that by constructing light response curves (range from 0 to 1,400 μmol photons m^{-2} s^{-1}; Cref constant 400 ppm, constant 18° C) that the plants have a photosynthesis rate (A = 10.545 μmol CO$_2$ m^{-2} s^{-1}; light compensation point Ic = 13.645 μmol CO$_2$ m^{-2} s^{-1}); light saturation constant (defined as ½ of the saturating photon flux density equal to 207.91 μmol photons m^{-2} s^{-1}; dark respiration Rd = 0.6496 μmol CO$_2$ m^{-2} s^{-1}); and apparent quantum yield (Φ = 0.03011 μmol CO$_2$ μmol^{-1} photons) (Fig. 2). Additionally, through the chlorophyll *a* fluorescence measurement methodology a maximum photochemical efficiency of photosystem II (Fv/Fm) of 0.82 was found, which is an indicator of a good state of operation of photosystems (Fischer and Melgarejo, 2014).

When net photosynthesis response curves were constructed at different CO$_2$ concentrations (Ci: 0 to 600 ppm; Cref 400 ppm, constant 18°C, constant PAR 600 μmol photons m^{-2} s^{-1}), in the same plants and leaves where light curves were constructed, and using adjustments by the Farquhar FvCB model, the authors recorded a maximum carboxylation rate of RuBisCO Vc_{max} = 75.70 μmol CO$_2$ m^{-2} s^{-1} and a maximum rate of regeneration of ribulose-1,5-bisphosphate controlled by electron transport J_{max} = 288.09 μmol CO$_2$ m^{-2} s^{-1}.

The cape gooseberry behaves like a facultative (or quantitative) short day plant. Heinze and Midash (1991) found that an 8-h photoperiod shortens the juvenile phase producing faster flower induction than a plant under 16 h d^{-1} light. This shorter photoperiod also develops more elongated internodes. This behavior as a short-day plant coincides with other species that originate in low latitudes such as corn, rice, soybean varieties and coffee, and other species (Dwivedi and Dwivedi, 2012).

P. peruviana seeds show low germination percentages in the absence of light. Nunes *et al.* (2018) observed germination percentages of 97% in a light regime of 8 h or more daily, indicating that the seed is photoblastically positive.

Ultraviolet (UV) light

Due to its growth in high-altitude tropical areas, the cape gooseberry receives large amounts of UV light for which it is adapted through its pubescent green epidermis, includaing a calyx that protects the fruit against this type of radiation (Fischer *et al*., 2016). In a study at two altitudes in Boyaca, Fischer (1995) found that these plants develop a shorter stem with

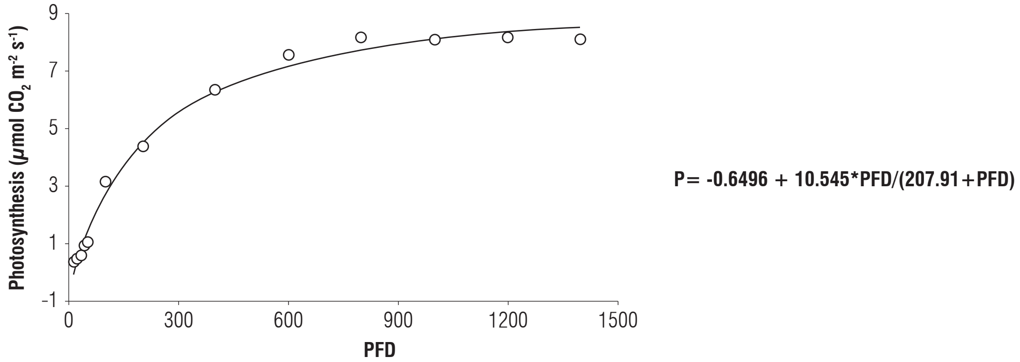

Figure 2. Response of net photosynthesis to light in leaves of cape gooseberry plants. Fitting a hyperbolic Michaelis-Menten model. Photon flux density (μmol photons m^{-2} s^{-1}).

1,399 mW m^{-2} UV-B (at 2,690 m a.s.l.) than with 1,294 mW m^{-2} UV-B (at 2,300 m a.s.l.). Fischer and Melgarejo (2014) proposed three possible causes for this phenomenon: (1) the increase in UV-B radiation (280-320 nm) can reduce the longitudinal growth of the stem due to the reduction of auxins (Kulandaivelu *et al*., 1989); (2) the lower night temperature at 2,690 m a.s.l. markedly decreases stem growth; and (3) the reduced atmospheric pressure at the higher altitude with 736 mb compared to 776 mb at the lower altitude could have negative effects on the elongation of the internodes.

Altitude

The increase in tropical altitude is characterized by a decrease in the average temperature (6°C/100 m), increased radiation (mainly UV-B) and wind incidence, and a reduction in precipitation that reduces the growth rate and leaf area producing thicker leaves compared to lower elevation sites (Fischer, 2000b). Thus, the leaves increase the number of layers of the parenchyma, forming a thicker cuticle with greater pubescence, which better filters the mutagenic effects of high UV radiation and improves the phytosanitary status of the cape gooseberry (Fischer and Orduz-Rodríguez, 2012).

Cape gooseberry is a crop adapted to a wide range of altitudes that can reach up to 3,300 m a.s.l. in Ecuador (Carrillo-Perdomo *et al*., 2015). Fischer and Miranda (2012) characterize between 1,800 and 2,800 m a.s.l. for Colombia altitudes that are the most commercially suitable for this plant. They specify that altitudes between 2,200 and 2,400 m a.s.l. can have the best production, if the microclimate and plantation management are adequate.

Possibly due to the reduced partial pressure of gases such as CO_2 and O_2 at a higher altitude, cape gooseberries develop a greater number of stomata per leaf area at these elevations. Fischer (1995) found in African ecotypes in Kenya and South Africa that 678 and 719 stomata/mm^2 leaf area at 2,690 m a.s.l. were typical. But they found only 564 and 507 stomata at 2,300 m a.s.l., respectively. However, this behavior did not show up in the Colombian ecotype that had 534 and 547 stomata at the two respective altitudes. Also, the same author recorded that the roots grew smaller and more superficial in the highest areas (2,690 m a.s.l.), because the soil cools down so much at night at this altitude (Fig. 1), that it must take better advantage of solar heating during the day.

In the higher and colder zone (2,690 m a.s.l., 12.5°C) the contents of sucrose and soluble solids, and also the number and weight of the seeds of the gooseberry fruit decreased significantly. Thus, the first production peak is postponed, although, the production cycle can be longer, about 2 years, compared to a lower site (2,300 m a.s.l., 17.4°C) that takes only 1.5 years (Fischer *et al*., 2007). For that, latter authors suppose that at the lower altitude the higher sucrose content, the most common sugar in this fruit (Fischer *et al*., 2015), was fostered by the higher seed number and weight which probably increased the sink strength and the greater influx of sucrose in the fruit, But also the higher temperature at the lower site probably promoted the hydrolysis of starch to soluble sugars. This was found by Mayorga *et al*. (2020) in curuba fruits that were grown at 2,006 m a.s.l. (19.4°C day time temperature) and 2,498 m a.s.l. (14.9°C) in Pasca (Cundinamarca, Colombia).

In the lower and warmer zone the fruits contained higher concentrations of provitamin A carotenoids (alpha- and beta-carotene) than at the higher site. This was a result that Fischer *et al*. (2000b) did not observe for ascorbic, citric and malic acids that were not influenced by altitude.

Water

Since in a plant of indeterminate growth, as in many nightshades, vegetative and reproductive development occur at the same time (Ramírez *et al*., 2013), the cape gooseberry needs a constant supply of water. The most demanding phases between bud sprouting, flowering and fruit filling (Fischer and Miranda, 2012), require uniformly distributed rainfall of between 1,000 and 1,800 mm $year^{-1}$ of precipitation per year.

During the initial growth of a plantation, the cape gooseberry is water demanding (Carillo-Perdomo *et al*., 2015). This contrasts with fruit harvesting when the crop needs water levels below field capacity so as not to harm the quality of the fruit (Torres *et al*., 2004) and stagnate the vegetative growth of the plant (Fischer, 2000a).

The cape gooseberry fruit contains 80% water, and this amount is increased or reduced according to the water available to the plant (Fischer and Melgarejo, 2014). The size of the fruit depends highly on the soil humidity provided by irrigation and rain (Fischer, 2005). The fruit constantly accumulates water and sucrose until its organoleptic maturity (yellow-orange color), during which it would take advantage of irrigation until harvest (Fischer and Martínez, 1999). However, this is at the cost of postharvest quality and durability.

In a study of irrigation frequencies and levels with calcium applications on cape gooseberry plants, Álvarez-Herrera *et al*. (2015) found that one irrigation every 4 d produced fruits of greater fresh mass, while irrigation every 14 d caused fruits of smaller size. However, the percentage of small fruits was reduced when applying Ca (50 or 100 kg ha^{-1}). The cape gooseberry responds favorably to irrigation, also in amounts that exceed the crop evapotranspiration value. With an irrigation coefficient of 1.3, the quantity of fruits increased increasing production per plant (Álvarez-Herrera *et al*., 2015), whereas the irrigation coefficient of 1.1 exhibited maximum values of ψ_{leaf} and ψ_{stem} (Álvarez-Herrera *et al*., 2019). The irrigation coefficient of 1.1 every 4 d had the second highest irrigation water-use efficiency (WUEi) and represented the most appropriate water level for cape gooseberry growing because this generated the highest amount of large and marketable fruits and the smallest number of cracked fruits (Álvarez-Herrera *et al*., 2019).

The cracking of the cape gooseberry fruit is the physiological disorder most related to excess water due to overly abundant rains or irrigation. This is especially true when interrupting a dry season and, especially during the rainy seasons. The fruits rejected by exporters because of cracking can reach 50% (Fischer, 2005). This physiological damage occurs particularly in large fruits, with high volume and weight (Gordillo *et al*., 2004). Plant breeding programs must take this situation into account and orient breeding programs to the size of this organ, an attribute for which the expression of genes is highly dependent on environmental conditions (Trevisani *et al*., 2017).

Supposedly, elevated water content and the high concentration of solutes exert high pressure on the epidermis of the fruits (Peet, 2009), and because they cannot resist the pressure, they crack (Fischer, 2005). This situation is accentuated if there are only a few fruits on the plant, as for example in the first production cycle (Gordillo *et al*., 2004). The relationship between the leaf area and the number of fruits during the formation of the first harvest is high, increasing the influx of carbohydrates to the fruit (Torres *et al*., 2016). Gordillo *et al*. (2004) observed two types of cracking, deep (splitting) and superficial (cracking).

Therefore, fruit growers prefer to eliminate the first flowers of the plant, and to also maintain an adequate level of nutrients that promotes firmness and an extension of the epidermis, such as calcium, boron and magnesium (Garzón-Acosta *et al*., 2014; Cooman *et al*., 2005). Álvarez-Herrera *et al*. (2012) reported 38% cracking in cape gooseberry fruits without adding Ca to the substrate, but this dropped to 27% after applying 100 kg ha^{-1} of calcium.

Relative humidity (RH)

A RH between 70 and 80% is optimal for growth and production of the cape gooseberry (Fischer and Miranda, 2012). Higher RH favors the incidence of diseases such as *Botryti*s sp., *Phom*a sp. and *Xanthomonas* sp. Serious leaf and calyx damage during high RH causes *Cercospora* sp., preventing fruit export with calyx (Fischer *et al*., 2011). Prolonged periods of high RH inhibit the plant's potential to form a thick cuticle or it can modify its composition, reducing cuticular protection (Opara *et al*., 1997).

Fischer (2005) reported that high RH (>95%) occurring for 6 consecutive hours in the field, with or without rain suppresses transpiration of the fruit causing high pressure on the epidermis that consequently can crack.

Waterlogging

As a solanaceous plant the cape gooseberry does not tolerate waterlogging or flooding conditions for longer than 4 d in plants submitted for 0, 2, 4, 6 and 8 d to waterlogging (5 cm above the substrate of the pot) (Aldana *et al*., 2014). After 6 d of waterlogging the plants showed significant reductions in vegetative growth (plant height, number of leaves, leaf area, diameter of the basal stem), including the number of nodes on the branches. Consequently, the number of flower buds, flowers and fruits decrease, and taking into account that at each node of the fruiting branches the reproductive organ forms (Ramírez *et al.*, 2013). The chlorophyll index is highly reduced especially from the 29th day after the beginning of the experiment, so that after 50 d the plants that were waterlogged for 8 d register a SPAD index of 17.48, compared to the control (35.85), in addition to severe leaf wilt (Aldana *et al*., 2014). Sánchez-Reinoso *et al*.

(2019) observe with increasing waterlogging periods (0, 3, 6 and 9 d) great chlorophyll content reductions, especially at 6, 12 and 18 d after the beginning of the waterlogging period. The effect of waterlogging on the development of the cape gooseberry DM is clear (Fig. 3).

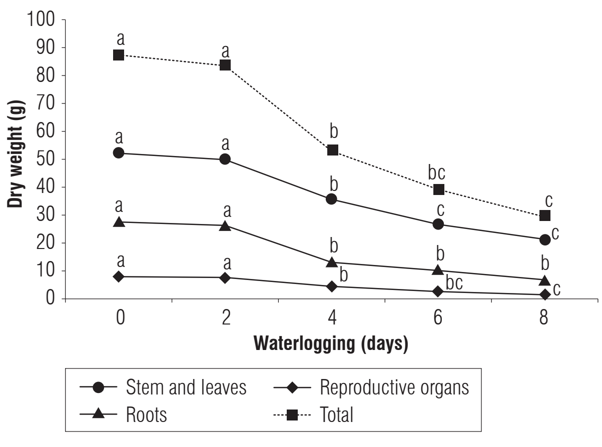

Figure 3. **The dry weight of organs (stem and leaves, reproductive organs [flower buds, flowers and fruits] and roots) of cape gooseberry plants in 10 L plastic pots after 50 d of the beginning of waterlogging of 0, 2, 4, 6 and 8 d, modified from data of Aldana *et al*. (2014). Means with different letters of the same organ indicate significant differences after the Tukey test ($P\leq0.05$).**

Due to the high incidence of *Fusarium* spp. in cape gooseberry plantations in the country caused by torrential rains, Villareal-Navarrete *et al*. (2017) flooded cape gooseberry plants in 2 L plastic pots for 6 d, where the substrate was or was not inoculated with *Fusarium oxysporum*. Each of the two factors, waterlogging and *Fusaium*, alone did not show significant negative effects on plant growth for 30 d, but the combination of the two stresses reduced root related variables (root dry weight, length, and neck diameter), and leaf area. In addition, the ratio of root dry weight to aerial parts of the plant decreased dramatically, from 0.45 to 0.14. In this experiment, a heavy reduction of stomatal conductance and transpiration rate is seen in addition to a strong decrease in the rate of photosynthesis in plants flooded and affected by *Fusarium* (1.75 μmol CO_2 m^{-2} s^{-1}), compared to control plants (5.06 μmol CO_2 m^{-2} s^{-1}). This means a 35% reduction when plants are affected by a combination of abiotic and biotic stress (Villareal, 2013). The author concludes that the obstruction of the vessels by the fungus and the impediment of the absorption of water and nutrients by these stressful conditions cause the closure of stomata and a decrease of photosynthesis.

Drought

Water deficit stress in the cape gooseberry reduces the longitudinal growth of the reproductive organs, damaging the amount of fruit produced (due to the lower number of "productive knots"), and, also, affecting the filling of the fruit by reducing the leaf area (Fischer and Melgarejo, 2014). When this stress occurs at the beginning of production it produces smaller fruits leading to reduced productivity and a greater sensitivity to fruit cracking. This indicates that these organs are more susceptible to a lack of water when they are in the cell division (Torres *et al*., 2004).

Moreno (2013) subjected cape gooseberry plants to 90 and 17% of the field capacity (in plastic pot), finding that the water deficit markedly reduces vegetative growth, chlorophyll content, gas exchange parameters, as well as the fluorescence of chlorophyll *a*. This author also records the effect of water deficit stress on plants from 14 d and is much greater after 21 d with increased leaf temperature, loss of electrolytes, and increased concentration of antioxidant enzymes such as catalase and peroxidase and also of the proline osmolyte

Knowing the cape gooseberry's reaction to water deficit stress that occurs in many cases under higher than optimal temperatures, it is very likely that stomatal conductance and transpiration are reduced, and this increases leaf temperature and photorespiration and, consequently, photosynthesis and the growth and production of the plant are reduced (Restrepo-Díaz *et al*., 2010). This scenario becomes more likely with increasing global warming (Menezes-Silva *et al*., 2019; Ngasoh *et al*., 2019).

Salinity

Like other nightshades, cape gooseberry not only shows a moderate tolerance to salinity, but also concentrations of 30 mM NaCl in the nutrient solution increases growth rates such as CGR (crop growth rate), RGR (relative growth rate), NAR (net assimilation rate) and LAI (leaf area index) (Miranda *et al*., 2010).

The cape gooseberry is protected from saline conditions through mechanisms such as increased total antioxidant activity in plants subjected to concentrations of 120 mM NaCl that significantly increased the activity of oxygen free radical control, compared to plants subjected to 60 mM (Miranda *et al*., 2014). Also, these authors observe an increasing tendency for the osmoprotectant proline when the salt concentration increased. Nimbolkar *et al*. (2020) state that the understanding of a salt tolerance mechanism in plant tissues is important for the integration of physiological and biochemical comprehension for increasing the salinity tolerance of fruit species.

This moderate tolerance of cape gooseberry is very similar to many tomato varieties (Chakma *et al*., 2019) and is important in the scenario of increased saline areas generated by anthropogenic effects or by factors related to climate change (Ngasoh *et al*., 2019).

Table 2. Effect of NaCl salinity on total antioxidant activity (μM Fremy's salt/g fresh weight) in leaf tissue of cape gooseberry plants, 45 and 75 d after plantating (Miranda *et al*., 2014).

NaCl (mM)	Days after planting			
	45	55	65	75
0	2.72 bC	3.16 bB	3.15 bB	3.46 bA
60	2.74 bC	3.17 bB	3.29 bB	3.78 abA
120	3.28 aC	3.84 aB	4.18 aA	4.14 aA

Means in columns followed by the same letter are not significantly different according to Tukey's test ($P \leq 0.05$). Lowercase letters are for comparing the NaCl concentration and uppercase between the days of the sample.

Wind

Cape gooseberry plantations suffer from strong winds (Carrillo-Perdomo *et al*., 2015), causing water loss due to evapotranspiration, deformation of the plant structure, and stagnation of growth that can also cause premature reproductive organ abscission (Fischer and Melgarejo, 2014). This is why in windy places plant barriers that cut the wind are necessary. Winds with speeds >30 km h^{-1} prevent the flight of bees, which are important due to entomophilic pollination for cape gooseberry (National Research Council, 1989). Likewise, wind dries out the stigma of the flowers, disabling pollination. In general, hot winds accelerate the drying of the plant tissues, replacing the humid air in the intercellular spaces with dry air (Das, 2012).

The benefits of soft winds to the physiology of the cape gooseberry are great, not only because of the transfer of mass and heat (Gariglio *et al*., 2007), but also because they dry the plants after a rain and ensure the opening of stomata (Fischer and Melgarejo, 2014). At the same time soft winds renew the air in the canopy maintaining the required CO_2 concentration for stable photosynthesis (Fischer and Orduz-Rodríguez, 2012) and they favor gas exchange in the low and dense strata of the plant (Das, 2012). Friedrich and Fischer (2000) observe in fruit trees that winds as slow as 1.7 m s^{-1} are the most optimal for producing DM.

CONCLUSIONS

As a typical Andean plant, the cape gooseberry adapts to a wide range of cold altitudinal climate, with base temperatures (minimum) for the stems (6.3°C) and fruit growth (1.9°C) being relatively low. The plant cannot stand temperatures <0°C because of the burning of leaves, young shoots, flowers, calices and young fruits.

The Andean conditions of the tropics that include high solar radiation and rather short day lengths favor the beginning of flowering. Important for fruit filling and quality is solar radiation that stimulates the green calyx and the two adjacent leaves. The cape gooseberry can be classified as a light-demanding plant, requiring from 1,500 to 2,000 h direct sunlight/year.

As a plant with an indeterminate growth habit, a constant supply of water is essential, while high amounts of water cause cracking of the fruits, and the plant does not tolerate waterlogging for more than 4 d. The cape gooseberry is classified as a moderately tolerant plant to salinity that has mechanisms such as increased antioxidant activity and proline accumulation for its protection. Increasing wind speeds with altitude can affect plantations so that plant barriers are necessary.

To our knowledge, no studies have been reported on the effects of elevated atmospheric concentrations of CO_2 on the physiology and growth of the Andean solanaceous fruit plants except tomato.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Seed-fruit relationships in fleshy fruit species: Role of hormones. A review

Relación semilla-fruto en frutos carnosos: Rol de las hormonas. Una revisión

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Fruit and seeds in apple var. Anna.

Photo: A. Cepeda

ABSTRACT

Seeds are known to have significant biological importance in nature, and they have high economic value in agriculture. This review discusses the physiological, biochemical, and hormonal aspects involved in the seed-fruit relationship, highlighting the main implications that seeds have on fruit set and growth, development, and abscission of some fleshy fruits. Fleshy fruits, with the exception of some parthenocarpic species, require pollination and double fertilization for seed formation. This contributes to the stimulation of hormone synthesis for auxins, gibberellins, cytokinins, and brassinosteroids. These hormones are required for seed formation and, in turn, for fruit development; and they determine fruit set, final fruit size, fruit shape and quality characteristics in some fruits. This knowledge is necessary for successful management of the cultivation of species producing fleshy fruits.

Additional key words: double fertilization; hormones; cellular division; cellular elongation; fruit growth.

RESUMEN

Se sabe que la semilla tiene gran importancia biológica en la naturaleza y un determinante valor económico en la agricultura. Esta revisión tiene como objetivo retomar aspectos fisiológicos, bioquímicos y hormonales necesarios para el entendimiento de la relación semilla-fruto, destacando las principales implicaciones que tienen las semillas en el cuajado, crecimiento, desarrollo y abscisión de algunos frutos carnosos. Los frutos carnosos,

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con excepción de algunos partenocárpicos, requieren de la polinización y fecundación doble para la formación de las semillas. Esto conlleva a la estimulación de la síntesis de hormonas, tales como auxinas, giberelinas, citoquininas y brasinoesteroides. Estas hormonas se requieren para el desarrollo de la semilla y a su vez para el desarrollo del fruto, determinando aspectos como el cuajado, el tamaño final del fruto, la forma y las características de calidad en varios frutos. Este conocimiento es necesario para un correcto manejo del cultivo de especies que producen frutos carnosos.

Palabras clave adicionales: fertilización doble; hormonas; división celular; elongación celular; crecimiento del fruto.

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INTRODUCTION

Seeds play a fundamental role in nature as a means of dispersal and propagation of plants, but they are also important for their nutrient contents and as an important source of food for humans and other living beings (Bewley *et al*., 2013). From the agronomic point of view, seeds have high economic value because sexual reproduction is one of the most efficient and widely used methods in crop cultivation. Additionally, their economic importance depends on their being produced and marketed for food, industrial purposes, and medicine (Hartmann *et al*., 2014).

However, the functions of seeds at biochemical and physiological levels that determine fruit development and quality have been underlined to a lesser extent. Fruit set (an early stage of fruit development) is a consequence of fertilization of the ovum or ovules within the ovary; each fertilized ovum produces a seed, varying from one seed up to several hundred seeds per fruit in different species (Peña *et al*., 2010; Fischer, 2012; Yang *et al*., 2020). Fertilization first leads to the formation of seeds (with an exception of many parthenocarpic species); but it is, in turn, hormonal activity that triggers the ovary and, in some cases, other flower parts, to transform into a fruit (Agustí, 2004; Seymour *et al*., 2013). Double fertilization causes a local increase in auxin concentration resulting in the activation of gibberellin biosynthesis. The synchronized action of the two hormones controls the differentiation of the ovary into a fruit (Alabadí *et al*., 2009; Taiz *et al*., 2017).

Hormones synthesized in seeds (auxins, gibberellins, brassinosteroids, cytokinins, polyamines, ethylene, and others) regulate seed development (Sun *et al*., 2010; Kang *et al*., 2013) and increase the activity and strength of fruits as sink organs, a factor that determines fruit size (Fischer *et al*., 2012a). In this regard, in apples, increasing numbers of seeds result in an increased size of fruits (Sapir *et al*., 2017). Additionally, hormones influence fruit shape (Matsumoto *et al*., 2012; Sheffield, 2014) and, in some aspects, the final fruit quality (Hershkovitz *et al*., 2009, 2010, 2011). Seeds can also determine the abscission of fruits because, in case of normal seed development, fruits are more likely to remain attached to the plant until harvest (Agustí, 2013).

Knowledge and understanding of the functions that seeds perform for fruits are necessary to optimize agricultural tasks like fertilization, irrigation, pollination, pruning, application of phytoregulators, etc., that lead to improving the production and quality of fruit species. So, the objective of this review was to discuss the state of the art of the main functions of seeds during initiation, growth, development, and abscission of fleshy fruits, and to report the physiological and biochemical aspects that determine the seed-fruit relationships.

SEEDS

The seed is an organ of dispersion and propagation of angiosperms and represents the final trait of the reproductive evolution of plants (Azcón-Bieto and Talón, 2013; Taiz *et al*., 2017), although frequently the ovary wall or even additional flower parts could remain in close association with seeds, forming a more complex dispersal in grasses. In this way, seeds play a very important role in the life cycle of higher plants. Likewise, seeds are the basic food for humans and domestic animals and their value depends on the storage reserves of proteins, starch and lipids synthesized during seed development (Bewley *et al*., 2013). At some point of formation, the angiosperm seed is composed of embryo, endosperm, and seed coats (Agrawal and Rakwal, 2012). In order to initiate seed development, double fertilization must take place in which one sperm cell fuses with the egg cell to form a zygote, and the other one unites with the polar nuclei to give rise to the endosperm (Bewley *et al*., 2013).

After fertilization, the fruits and seeds undergo a concomitant development; however, in contrast to the fruit that can develop in the absence of pollination (Azcón-Bieto and Talón, 2013; An *et al*., 2020), seed development is more strictly dependent on double fertilization (Taiz *et al*., 2017; Ge *et al*., 2019). Seed development includes endosperm development and embryo growth, and both processes are regulated by various hormones such as auxins, cytokinins, gibberellins (GAs) and brassinosteroids (Sun *et al*., 2010; Hartmann *et al*., 2014), etc. In *Citrus maxima* (Burm.), during the early stages of seed growth, auxins, gibberellins and cytokinins are detected in complete seeds in higher quantities than in the small empty seeds (Yang *et al*., 2020), indicating the role of seeds as endogenous hormone centers maintaining fruit development.

FRUITS

Fruits are structures derived from an ovary (true fruit) or they can also include a variety of other tissues (false fruit). For example, the receptacle contains seeds and determines their dispersion (Seymour *et al*., 2013; Taiz *et al*., 2017). Fruits can be either dry or fleshy; fleshy fruits are the subject of interest in this review. They possess a juicy pericarp with abundant pulp and water and occasionally fibrous material. Fleshy fruits include drupes, berries, pomes, pepos and hesperidia (Cerri and Reale, 2020). After double fertilization the ovary undergoes cell division followed by cell expansion, a phase responsible for the final size of the fruit. In the expansion phase fruits undergo metabolic changes responsible for biosynthesis of many classes of metabolites including plant hormones (Paliyath *et al*., 2019). The final phase is fruit ripening, generally initiated once the fruit has acquired physiological maturity, at which stage it has attained the maximum size and capability for ripening, even after detachment from the mother plant (Paliyath *et al*., 2019). Many fruits show a single sigmoid growth phase such as lulo fruit (Almanza-Merchán *et al*., 2016), while others exhibit double or multiphasic growth patterns (Paliyath *et al*., 2019), like grapevine fruit (Almanza-Merchán *et al*., 2012).

The formation and development of fruits have a close relationship to seed characters, such as the presence, number, size and distribution of seeds within the fruit. These are responsible for fruit set establishment, fruit retention on the plant, and the growth and quality of fruits (Fig. 1). These aspects, although considered essential, are poorly elucidated in fleshy fruit species, so that the objective of the present review is to address some of these issues in more detail.

Hormones, such as auxins (AIA), gibberellins (GAs), cytokinins (CYT), strigolactones (STG) and brassinosteroids (BR), are synthesized in the seeds developed within the fruit. They link to protein receptors in membranes, and trigger the signaling process that leads to changes in gene expression. As a result, the proteins being synthesized (structural and enzymatic) and that are involved in cell division, expansion,

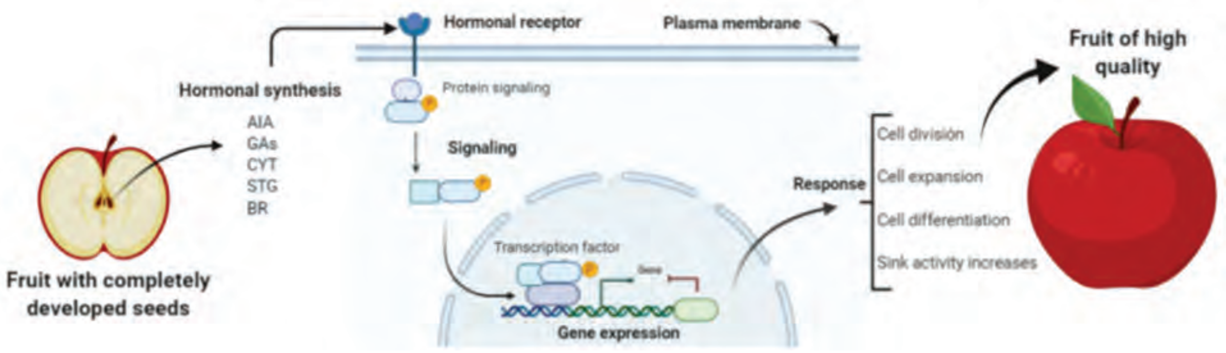

Figure 1. General role of hormones synthetized in seeds in fruit quality.

Table 1. Number of seeds per fruit in different species.

Species	Number of seeds per fruit	Author
Physalis peruviana L.	237 (ecotype Colombia) 242 (ecotype Kenia) 264 (ecotype South Africa)	Peña *et al*. (2010)
Persea americana Mill.	1	Fischer (2012)
Malus domestica Borkh.	4-9	Sapir *et al*. (2017)
Solanum quitoense Lamarck	600-1200	Ramírez and Davenport (2014)
Solanum lycopersicum L.	46-148	Salim *et al.* (2020)
Vitis vinifera L.	0-4	Almanza *et al*. (2012)
Carica papaya L.	Up to 100 seeds	Fischer (2012)
Solanum betaceum Cav.	50-180	Fischer (2012)
Passiflora edulis Sims	150	Fischer (2012)
Acca sellowiana (Berg) Burret	20-40	Fischer (2003)
Rubus glaucus Bentham	Multidrupe having 150-170 drupes with one seed per each drupe	Fischer (2012)

differentiation and increase in source capacity, physiological processes affect the final quality of the fruits.

Although the number of seeds per fruit is variable and depends mainly on the species (Tab. 1). In some cases (such as for drupes and some berries) seed number may be sufficient to guarantee fruit growth of high quality, whereas, in other species, it is necessary that the fruit contain a high number of seeds, as in Solanaceae trees or in passion fruit (Fischer, 2012). However, there are certain exceptions to this trend, and one of these is the formation of parthenocarpic fruits, described in the following section.

Parthenocarpic fruits

Parthenocarpy is an alternative way for fruit set and growth. It consists of ovary development without seed formation (Agustí, 2013; An *et al*., 2020). Some examples of these species include cucumbers, bananas, grapes, citrus, pears, and apples (Agustí, 2013). Parthenocarpy can occur without fertilization or with pollination but without fertilization, or when both processes occur but the seeds abort (Gillaspy *et al*., 1993). It has been proposed that auxins serve as an alternative signal to replace pollination and fertilization to initiate fruit growth. Also, auxins interact with gibberellins (GAs) and both hormones stimulate cell division and expansion during fruit formation (Varoquaux *et al*., 2000; An *et al*., 2020). Furthermore, with a reduced activity of DELLA proteins the growth of parthenocarpic fruits can be promoted (Marti *et al*., 2007; Dorcey *et al*., 2009).

Obtaining seedless fruits is a desirable condition for consumers, like citrus without seeds, highly valued fruits for their ease of consumption (Iglesias *et al*., 2007). Likewise, fruit development without fertilization is advantageous in agronomy when the rate of fruit set is low. Pollen maturation and fertilization are affected by environmental factors such as temperature, light, and relative humidity. Unfavorable environmental conditions can dramatically decrease fertilization and, consequently, fruit development (An *et al*., 2020). Parthenocarpic plants can avoid this problem, allowing the development of seedless fruits under adverse environmental conditions for pollination and/or fertilization. In horticulture, parthenocarpy can be implemented to accelerate fruit production (Acciarri *et al*., 2002; Pandolfini, 2009). For research purposes, parthenocarpic fruits can be model fruits to understand the complex function of seeds in fruits. The application of 0.05% naphthalene acetic acid to the stigma of chili peppers under temperatures above 18°C during anthesis generated formation of seedless fruits, although these were smaller (Heuvelink and Körner, 2001). In tomatoes, eggplant, strawberry and raspberry, expression of auxin biosynthesis genes in ovary and ovules caused parthenocarpy (Mezzetti *et al*., 2004; Kataoka *et al*., 2009). The application of GAs can also generate parthenocarpy, as frequently occurs in apple and pear varieties that present difficulties in pollination (Agustí, 2013). This is because the exogenous GAs (for example, GA_3) can generate changes in gene expression similar to those that occur with pollination (Vriezen *et al*., 2008).

In citric crops many varieties used for fresh consumption do not contain seeds, but fruit development is typical for seed-bearing varieties (Agustí *et al*., 2003). In these varieties, the stimulus of fruit growth cannot be attributed to seeds, but this process continues to be regulated by hormonal stimuli from the ovary walls (Agustí, 2013). In agreement with this, Mesejo *et al*. (2016) find that GAs are responsible for cell division during the set of parthenocarpic citrus fruits, and they conclude that in species with autonomous parthenocarpy, GAs are responsible for reactivation of cell division in ovary during anthesis. The anthesis itself improves GA biosynthesis for maintaining cell division and probably acts as a feedback mechanism responsible for fruit formation.

Seedless fruits can possess better quality than fruits with seeds, for example when the latter have hard seeds or seeds of unpleasant taste, such as in *Campomanesia lineatifolia*, a berry whose seeds represent about 30% of the fruit weight (Balaguera-López *et al*., 2012). The seeds are not edible and hinder industrialization of the fruits. In eggplant, the absence of seeds prevents browning and reduction of pulp texture (Maestrelli *et al*., 2003). Additionally, seeds can produce substances that accelerate fruit deterioration, such as in watermelon and eggplant. So, the absence of seeds can increase the shelf life of fruits, allowing better conservation (Pandolfini, 2009). However, in tomato the fruits with seeds possess a higher beta-carotene content compared to parthenocarpic fruits, an important aspect from the nutritional point of view (Rotino *et al*., 2005).

ROLE OF HORMONES IN THE SEED-FRUIT RELATIONSHIP

Seeds and fruit set

Fruit set is the initial phase of fruit development during the sexual reproduction of flowering plants in which the flower parts transform into a fruit (An *et al*., 2020). Once the ovules that convert into developing seeds are fertilized, the ovary develops and transforms into the fruit (Taiz *et al*., 2017). The fruits and seeds undergo simultaneous development; however, some fruits can develop in the absence of pollination, a situation that cannot occur in seeds, with the exception of some cases of apomictic seed formation (Hojsgaard and Hörandl, 2019). Seed development is a process that has multihormonal regulation by auxins, cytokinins, GAs, brassinosteroids, and other hormones (Sun *et al*., 2010; van der Knaap and Østergaard, 2018). Likewise, the transition from ovary to developing fruit corresponds to fruit initiation, a process that is based on cell division and requires a large amount of energy. It is through hormonal synthesis that the developing fruit requires this energy in the form of carbohydrates (Azcón-Bieto and Talón, 2013). Therefore, the formation and development of seeds and fruits are hormonally regulated (van der Knaap and Østergaard, 2018).

Auxins, cytokinins and GAs immediately begin to regulate fruit set and development after fertilization. There is strong evidence to indicate that the role of auxin is facilitated by synergistic activity with GAs (Serrani *et al*., 2008; de Jong *et al*., 2009a). After fertilization, a seed produces an auxin signal (Mezzetti *et al*., 2004; Dorcey *et al*., 2009) that is believed to regulate the increment by biosynthesis of another hormone, the GA. This leads to the activation of GA signaling to the ovule to stimulate fruit growth (Dorcey *et al*., 2009), apparently through the degradation of DELLA proteins that repress the action of GA and, consequently, of growth (Kumar *et al*., 2013; van der Knaap and Østergaard, 2018). However, it appears also that the existence of independent DELLA protein pathways mediated by seeds contributes to fruit growth (Seymour *et al*., 2013).

It is important to mention that GA application to unpollinated ovaries triggers the initiation of fruit growth without affecting the expression of auxin signaling genes (Vriezen *et al*., 2008), while the development of fruits induced by auxin is significantly reduced by the simultaneous application of GA biosynthesis inhibitors (Serrani *et al*., 2008). This data suggest that auxins may act before GAs and that the effect of auxins is mediated at least in part by GAs. According to this hypothesis, the genes for GA biosynthesis are overexpressed after pollination and by auxin treatment of emasculated flowers (Serrani *et al*., 2008). However, in tomato fruits, each hormone seems to play a specific role; for example, the application of auxin generates a large number of cells in the pericarp, while the GA treatment reduces the number of cells in this part of the fruit, but causes cell development of a larger size (Serrani *et al*., 2007). Interestingly, a simultaneous treatment with both hormones results in the formation of fruits similar to the seed-bearing fruits obtained by pollination (Serrani *et al*., 2007), suggesting that GAs and auxins are necessary for normal fruit development (Crane, 1964; Agustí, 2013).

ARFs and Aux/IAA proteins also act during early fruit development so that in *Arabidopsis* and tomatoes, the expression of aberrant forms of ARF8 result in the formation of parthenocarpic fruits (Goetz *et al*., 2007) as well as the silencing of IAA9 in tomato (Wang *et al*., 2009). It is unclear by what mechanism ARF8 regulation occurs, but based on the model for auxin signal transduction, ARF8 and the IAA9 Aux/IAA protein may form a transcriptional repressor complex that is destabilized by aberrant forms of ARF8 to allow transcription of auxin response genes (Goetz *et al*., 2007). In the tomato, pollination causes ARF9 upregulation and ARF7 repression, while the silencing of the *SlARF7* gene in transgenic plants generates parthenocarpic fruits, which suggests that *SlARF7* is a negative regulator of fruit set, and the silencing of this gene also results in a strong upregulation of *SlGH3* encoding for an IAA-amido synthetase that can be induced to compensate for the excess of auxins (de Jong *et al*., 2009b; 2011).

Accordingly, most of the hormones related to fruit set are found in seeds (Crane, 1964). In fruits of sweet oranges, pollination increases GA levels in the ovaries of seed varieties (Ben-Cheikh *et al*., 1997). But the fact that varieties with similar fruit formation capacity, with and without seeds, have similar contents of gibberellins (Talón *et al*., 1990) indicates that these hormones must be responsible for the initiation of citrus fruit formation. The presence of auxins in pollen, their production in the style and the ovary due to fertilization and pollen tube growth, and the stimulation resulting from the ovary growth together with production of parthenocarpic fruits by exogenous application of auxins, indicate the function of this hormone during fruit set (Agustí, 2013).

In pears and tangerines, when seed formation is prevented by emasculation, the content of GAs in fruits is lower than in normally developing fruits, so that Agustí (2004) stated that this hormone contributes to initial fruit development. GA content in seeds is high, so that when seeds are formed after fertilization, they produce GA, which stimulates the synthesis of indole acetic acid (IAA) (Taiz *et al*., 2017). Imanishi and Hiura (1975) indicate that fruit set and fruit development are also promoted by cytokinins produced, along with auxins, in pollen, style, and seeds during pollination, fertilization, and seed formation. However, the effects of seeds on fruit set are probably associated with other factors that affect fruit set and growth, such as the position of fruits on the bunch, position of bunches on the plant, and environmental conditions (Kinet and Peet, 1997), etc.

Role of seeds in fruit growth and development

Pollination becomes an important process for yield (Tamburini *et al*., 2019), fruit growth and development, since it is one of the most decisive factors that determines seed formation. This is reported in custard apple (*Annona cherimola*), where a higher pollen load (twice that of the normal pollination) generated significantly greater pollen adhesion to the stigma, a higher number of seeds per fruit and, consequently, the fruits become larger (González *et al*., 2006). Pollination requirements in apple are lower than for some stone fruits; a commercial apple crop requires between 2 and 5% of blossom set compared with 80% for cherry. However, growers seek higher pollination rates to maximize fruit quality and market value (Pardo and Borges, 2020). The market favors to few large and well-shaped fruits rather than many small fruits (Park *et al*., 2018). This shows that pollination is an important process because it favors the formation of seeds, which in turn determines the quality of the fruits. So, in apple fruits cv. Anna, under conditions of the high tropics, a positive correlation was established between the number of seeds, fresh and dry weight of seeds, and fruit equatorial diameter (Cepeda, 2018).

Pollen grains have very high levels of brassinosteroids (Kanwar *et al*., 2017), hormones that in certain cases duplicate auxin functions and act on the expression of genes related to growth (Sun *et al*., 2020). But the possible action of brassinosteroids of pollen on fruit growth is unclear. In apples, a higher number of pollinated stigmas positively correlate with a higher number of seeds per fruit and a lower number of deformed fruits (Matsumoto *et al*., 2012). Likewise, the asymmetric fruits observed in low-set trees might be due to inadequate pollination (Fischer *et al*., 2012b). Increased pollen load improves the production and size of Japanese pear fruits (*Pyrus pyrifolia*), where concentrations of endogenous GA_3 and GA_4 increases in pollen tubes soon after germination and positively correlates with the final fruit size (Zhang *et al*., 2010).

Seed formation may be required for the synthesis and signaling of strigolactone hormones in the fleshy receptacles (commercially known as fruits) of wild strawberry (*Fragaria vesca*) (Wu *et al*., 2019).

In particular, the expression of *D27*, *MAX1* and *LBO* genes for the synthesis of strigolactones increases in receptacles after pollination, than decreases during the development of receptacles, and a low or null expression of these genes is detected in maturing fruits, suggesting involvement of strigolactones in early fruit development (Wu *et al*., 2019).

Thanopoulos *et al*. (2013) reports, for seedless chili fruits, considerably lesser weight of fruit and pericarp compared to fruits with seeds; the growth parameters such as length, diameter, fruit volume, and volume of fruit cavity are also lower in seedless fruits. Other results in the same species indicate that the seedless chili fruits are smaller, irregular in shape and show the carpeloid structures within the pericarp that generally makes them unacceptable for the market (Tiwari *et al*., 2007). In grapes, Friend *et al*. (2009) finds that the growth of seedless fruit is a result of the expansion of single cells, while that of a normal berries with seeds had both cell division and cell expansion.

Fruit size and shape

After fruit set, fruits grow due to the increase in cell size promoted by hormones synthesized by the developing seeds (Agustí, 2004). This suggests that adequate seed formation produces a large amount of auxins, GAs and cytokinins that leads to normal fruit development with optimal cell growth that in turn, determines the final fruit size (Kang *et al*., 2013). In grapes, endogenous hormones, such as auxins and GAs synthesized by the seeds, are transported to the pericarp and regulate its growth (Van-Huizen *et al*., 1996). Endogenous GAs in growing seeds highly correlate with fruit growth, so the larger the number of seeds, the greater the sink strength of the fruits for nutrients in the tree (Buccheri and Di Vaio, 2004) In kiwi, the metabolic pathways related to the synthesis and conjugation of IAA in developing seeds can influence high levels of auxin in the internal fruit tissues as well as on the amount of auxins exported from the fruits (Sorce *et al*., 2017).

According to Agustí (2004), the hormones synthesized in seeds play an important role in fruit growth, since hormonal activity influences the expression of genes, enzymatic activity, and membrane functionality. Most of the hormones produced in fruits are synthesized in seeds and exert their action through seed development, but they are also essential for the normal development of fruits, for this reason, the size and shape of many fruits are determined by the number and distribution of seeds (Srivastava and Handa, 2005). According to Sheffield (2014), the diameter and firmness of apple fruits do not depend so much on successful pollination *per se*, but on the resulting distribution of seeds within the fruit and the hormones that the seeds produce, that has an effect on fruit symmetry. Hormones produced by developing seeds in each carpel influence the growth of adjacent empty carpels; only when two or more adjacent carpels are empty does this reduce the fruit growth in this area, generating an asymmetric fruit (Sheffield, 2014). Some authors point out that the presence of cytokinins in fruits is a consequence of their transport from the roots, rather than their synthesis in developing seeds. Despite this, cytokinins are isolated from kiwi and lemon seeds (Agustí, 2013).

Brassinosteroids from immature seeds apparently stimulate fruit growth (Montoya *et al*., 2005). According to these authors, in the tomato much higher levels of brassinolide are detected in the area of immature seed and coincide with the high activity of cytochrome P450 monooxygenase (CYP or P450) in this region of the fruit. This enzyme catalyzes the C-6 oxidation of 6-desoxocastasterone to castasterone, the immediate precursor to brassinolide, which is the most bioactive brassinosteroid (Montoya *et al*., 2005).

Despite evidence that the control of fruit shape is mainly exercised by plant hormones from seeds that stimulate growth to varying degrees, this is not true for all fruits. In bananas, for example, fertile seeds suppress the pulp development and, in this anomalous case, the failure of the fertilization allows the ovary to grow (Atwell *et al*., 2003). In tomatoes, the shape of the ovary regulates the spatial distribution of the seeds, which in turn, influences pericarp growth so that the size and shape of fruits become a function of the initial ovary shape plus subsequent fertilization and seed development (Razdan and Mattoo, 2006). Kojima (2005) affirms that the form that the fruit acquires at early stages of development is the effect of growth hormones produced by the immature seeds, of which, abscisic acid is found in high amounts in the pericarp, axils, and locular tissue at the early stages of fruit growth. Later on, large amounts of IAA appear, whose function is to determine the growth rate and shape of the fruit. However, expansion of the pericarp is not caused directly by the IAA but by the sink strength of the fruit caused by the developing seeds (Varga and Bruinsma, 1986).

In other Solanaceae, like the tomato, Agustí (2013) confirms that the IAA did not directly influence fruit development, but its action would be restricted to the seeds and involved in the growth of the embryo, where it creates a high sink strength, from which the pericarp grows. As the number of seeds increases, seed production of auxins per fruit also increases, and a strong basipetal flow of auxins results in developing high fruit capacity to attract mineral elements and photosynthates, and, thus, such fruits can grow more than fruits with few seeds (Friedrich and Fischer, 2000). The developing seed is a strong sink for carbohydrates that are supplied as sucrose via phloem (Lu *et al*., 2020), favoring the final fruit weight (Peña *et al*., 2010). This confirms that seed formation is the integral component of fruit development (Gillaspy *et al*., 1993).

In grape cv. Merlot, an increase in fruit weight is based on the number of seeds per fruit, even if the fruits came from different parts of the bunch (Prudent *et al*., 2014). In cape gooseberry, this determines the content of seeds in fruits of three ecotypes, Colombia, South Africa and Kenya (Fischer *et al*., 2007); the fruits with the highest seed weight per fruit has a larger size, being the South African ecotype that generates the largest fruits (Peña *et al*., 2010). In tomato, Varga and Bruinsma (1986) observe that fruits with fewer seeds take longer to develop than normal fruits. In this species, a higher number of seeds per fruit results in increased fruit size (Bashir *et al*., 2018; Prudent *et al*., 2014). In agreement Crane (1964) proposes that the seeds serve as a focal point for mobilization of hormones and photosynthesis from the fruits.

In *Capsicum flexuosum*, the largest fruits have the highest number of seeds, with this number being greater when manual pollination is used (Carrizo, 2011). In the 'Hass' avocado, a strong correlation is found between seed size and fruit size (Garner and Lovatt, 2016). Seedless avocados are always smaller than seed-bearing fruits (Lovatt, 1990). In the 'Arad' avocado, there is a relatively large number of seedless fruits, together with those with seeds, making this fruit an excellent experimental model to study the implication of seeds for avocado seed quality (Hershkovitz *et al*., 2011). However, studies exist reporting little or no relationship (Garmendia *et al*., 2019) between fruit size and number of seeds (Chao, 2005; Gravina *et al*., 2011). And, although the seeds can improve the fruit diameter through a direct hormonal effect, an opposite side effect must be considered for the total yield per tree (Garmendia *et al*., 2019).

Seeds are centers of metabolic activity, and once their development has begun, they appear to have a predominant effect on fruit growth in many species (Grange, 1993; Kang *et al*., 2013). Picken (1984) concludes that the precise relationship between the fruit weight and the number of seeds in tomatoes can vary due to several factors affecting fruit weight, for example, through competition between the fruits, without affecting the number of seeds. In *Cucurbita pepo*, Stephenson *et al*. (1988) show that the seeds of the first fruit can affect the growth of the second fruit, apparently, this is because the auxins produced by the seeds of the first fruit inhibit the export of auxins from the second fruit and, therefore, decrease their sink strength. This explains the differential size of the fruits of the same bunch.

The number and distribution of seeds within a developing apple affects not only the weight but also the shape of the fruit (Brault and de Oliveira, 1995; Keulemans *et al*., 1996). Apples with shape defects are classified in lower priced categories (Musacchi and Serra, 2018), and fruits with a smaller number of seeds can grow deformed. One way to measure this abnormality is to use the asymmetry index; high values indicate increased fruit deformity. The asymmetry index increases in a curvilinear manner with an increasing number of well-formed seeds and fertilized ovules in the 'McIntosh' apples (Brault and de Oliveira, 1995). Similar results are observed in the 'Granny Smith' apple (Drazeta *et al*., 2004). The unequal forms of the fruits (asymmetry) can be determined by the amount of hormones (e.g. IAA and GA) derived from the seeds, generating an uneven distribution of nutrients and water within the pulp of the fruits (Matsumoto *et al*., 2012).

Seeds and fruit maturation and quality

Seeds are not only involved in the determination of the size and shape of fruits, but can have an effect on fruit ripening and quality. In this regard, in the atemoya, the absence of seeds contributes to the smaller size of the parthenocarpic fruits and less calcium accumulation in the pericarp, making the fruits less firm (dos Santos *et al*., 2019). Hershkovitz *et al*. (2011) found that seedless 'Arad' avocado fruits have less firmness, confirming the importance of seeds in fruit development and quality. Hershkovitz *et al*.

(2010) mentions that seeds in avocado fruits are involved in delay of the ripening processes. Seedless avocado fruits ripen more quickly both in refrigeration and at room temperature, due to a faster start of the climacteric production of ethylene, significantly higher respiratory rate, and a greater softening of fruits (Hershkovitz *et al*., 2009). In the 'Arad' avocado the seeds increase the sensitivity to ethylene of mesocarp that surrounds the seed during the late ripening process (Hershkovitz *et al*., 2009). Hershkovitz *et al*. (2011) states that seedless avocado fruits have a higher relative expression of genes that code for CTR1 throughout development and ETR during ripening. This suggests the important role that seeds play in inhibiting the ethylene-induced gene response. Previous studies in this species suggest that the seeds delay the onset of climacteric stage in mature fruits (Hershkovitz *et al*., 2009). Thanopoulos *et al*. (2013) reports that seedless pepper fruits have a lower respiratory rate but a higher reddish coloration.

Seed development is necessary for cell division within the grapefruit mesocarp (Friend *et al*., 2009). In apples, the best pollinated fruits contain more seeds, are larger, and generally have better external quality characteristics, but this only occurs if the number of fruits per tree are optimal (Blažek and Hlušičková, 2006). The high number of seeds seems to accelerate maturation but decreases susceptibility to fungal rot, apparently, due to the increase in the contents of phenolic compounds (Blažek and Hlušičková, 2006). High seed numbers were also associated with higher calcium content, increased fruit firmness and acidity (Tomala, 1999; Buccheri and Di Vaio, 2004). Parthenocarpic varieties of apple have greater susceptibility to calcium deficiency (Bangerth, 1976), indicating that the number of seeds is an important factor that determines the calcium content in fruits at harvest. Bramlage *et al*. (1990) finds that apple with a higher number of seeds have a larger diameter and a higher concentration of calcium, while with magnesium and potassium there is no such correlation. These authors also find that the internal browning of the fruits increases when the number of seeds is lower, an aspect related to a lower calcium content (Bramlage *et al*., 1990).

Similar results are found in grapes by Boselli *et al*. (1995), who report that fruits with 4 seeds are larger and have a higher concentration of Ca, K and Mg compared to fruits with a lower number of seeds. In this regard, Kubowicz *et al*. (1982) mention that a higher number of seeds per fruit can result in greater transport of auxins from the fruit, which, in turn, can improve the transport of Ca. Copeland and McDonald (2001) indicate that lesser solar radiation results in smaller seeds, due to a reduction in photosynthetic rate that reduces the period of seed filling. In apple trees, as the number of seeds per fruit increases, the fruit size and calcium concentration increases (Bramlage *et al*., 1990).

Seeds and fruit abscission

The effect that seeds have on premature fruit fall is well known in many species, where null or inadequate pollination and/or fertilization can generate early fruit fall (Agustí, 2013). In fruits with varieties that have seeds, the synthesis of GA in the fertilized ovule is the factor that controls initial fruit development (Talón *et al*., 1990), so that its elimination or emasculation, which prevents seed formation, stops fruit development and provokes its abscission. However, in these cases, an application of GA restores growth (Agustí, 2004). Furthermore, the use of a gibberellin synthesis inhibitor, paclobutrazol, causes fruit abscission (Ben-Cheikh *et al*., 1997). This evidence suggests that endogenous GAs are the main responsible factors for fruit set in citrus, but the regulation of ovarian development cannot be attributed exclusively to the seeds (Agustí, 2004). In 'Hass' avocados, a large proportion of fruits are abscised due to the lack of pollen germination and subsequent fertilization; the abortion of ovules and reduction of fruit growth preceding the fruit abscission are related to the accumulation of abscisic acid in fruit tissues (Garner and Lovatt, 2016). These same authors mention that the demonstration of the changes that occur in seed development and hormone concentrations of abscising and persisting fruit during key stages of 'Hass' avocado fruit development will provide fundamental information required for developing and implementing horticultural strategies for decreasing abscission and increasing yield (Garner and Lovatt, 2016).

Persistence and fruit growth are related to seed presence that is demonstrated by existence of development regulatory substances synthesized in seeds (Agustí, 2004). The responsiveness of avocado seedless fruits to ethylene could explain why these fruits have a greater tendency for abscission than seed-bearing fruits (Davenport and Manners, 1982). An apple fruit that contains only a few seeds is more likely to fall from the tree before reaching maturity, especially if water, nutrients, or carbohydrates are scarce (Mantinger, 1997).

CONCLUSIONS

The fruit is a mature ovary that has the functions of formation, protection, and dispersion of seeds, with some exceptions as in the case of parthenocarpic fruits. The seeds, in turn, become an important determinant of fruit development. After pollination and fertilization, seed development begins, and, in turn, the ovary (and, in some cases, the accessory tissues) initiate fruit formation. The hormones produced by seeds are responsible for ensuring the initiation of the fruit, which continues to grow, acquires quality-determining characteristics, and does not fall prematurely. Fruits with an adequate number of developed seeds has better size, shape, and quality. This relationship between seeds and fruits is and must be an important factor to consider for agronomic management of fruit species worldwide in order to guarantee their success. But there are still biochemical and molecular physiological aspects that are unknown and must be studied in order to understand with greater certainty the complex seed-fruit relationship.

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Fitting a logistic growth model to yield traits in lettuce cultivars growing in summer

Ajuste del modelo logístico para caracteres productivos de cultivares de lechuga en condiciones de verano

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Lettuce cultivars experiment view.

Photo: F. Carini

ABSTRACT

The objective of this study was to fit a logistic model to leaf fresh and dry matter and shoot fresh and dry matter in four lettuce cultivars to describe growth in summer. The cultivars Crocantela, Elisa, Rubinela, and Vera were evaluated in the summers of 2017 and 2018 in soil in a protected environment and in a soilless system. Seven days after transplanting, the leaf fresh and dry matter and shoot fresh and dry matter of 8 plants were weighed every 4 days. The model parameters were estimated using R software with the least squares method and iterative process of Gauss-Newton. This study also estimated the confidence intervals of the parameters, verified the assumptions of the models, calculated the goodness-of-fit measures and the critical points, and quantified the parametric and intrinsic nonlinearities. The logistic growth model fit well to the fresh and dry matter in the leaves and shoots in the cultivars Crocantela, Elisa, Rubinela, and Vera and described the growth of lettuce.

Additional key words: *Lactuca sativa*; plant models; crop modelling; non-linear models; vegetable crop.

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RESUMEN

El objetivo de este trabajo fue ajustar el modelo Logístico para las masas fresca y seca de hojas y las masas fresca y seca de parte aérea de cuatro cultivares de lechuga para describir el crecimiento en el verano. Se utilizaron los cultivares de lechuga Crocantela, Elisa, Rubinela y Vera, cultivados en el verano de los años 2017 y 2018, en ambiente protegido y cultivo sin suelo. Después de 7 días del trasplante, se determinó las masas fresca y seca de hojas y las masas fresca y seca de parte aérea de 8 plantas, estas evaluaciones se realizaron cada 4 días. Los parámetros del modelo fueron estimados utilizando el software R, por el método de mínimos cuadrados y proceso iterativo de Gauss-Newton. También se estimaron los intervalos de confianza de los parámetros, verificados los supuestos, calculados los indicadores de calidad del ajuste, los puntos críticos y cuantificados las no linealidades paramétrica e intrínseca. El modelo de crecimiento logístico presentó un ajuste satisfactorio para las masas fresca y seca de hojas y para las masas fresca y seca de parte aérea para las cultivares de lechuga Crocantela, Elisa, Rubinela y Vera, siendo así, indicado para describir el crecimiento de los cultivares de lechuga.

Palabras clave adicionales: *Lactuca sativa*; modelos vegetales; modelación de cultivos; modelos no lineales; cultivo de hortalizas.

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INTRODUCTION

Lettuce is a leafy green vegetable in the Asteraceae family, consumed as raw salads, broths, and soups, that has high vitamin and mineral contents and few calories (Ntsoane *et al*., 2016). In Brazil, lettuce production has economic and social importance, generating jobs and income for family agriculture (Andriolo, 2017).

Since lettuce originated from a temperate climate, temperatures above 30ºC induce flowering, reducing the vegetative phase and number of leaves and affecting the formation of compact heads. The optimum temperature for this crop varies between 15.5 and 18.3°C, with a minimum of 7.2°C and a maximum of 23.9°C (Maynard and Hochmuth, 2007). Genetic improvement techniques have developed cultivars that are tolerant to early bolting with extended cultivation in other seasons and sites.

Lettuce cultivars are classified into groups according to the morphological characteristics of the leaves, head shape, and color, such as iceberg, looseleaf (lollo and oakleaf), butterhead, romaine, and others (Sala and Costa, 2012). The selection of cultivars adapted to the climatic conditions and the crop growing system are determining factors for production success. In addition, in protected environments, during the summer (December to March) in Rio Grande do Sul, high temperatures still affect the quality and palatability of lettuce leaves.

Nonlinear models are widely used in agricultural research (Archontoulis and Miguez, 2015). To describe the growth of plants and fruits, the accumulation of matter over time must be measured. Nonlinear models are important for the proper management and improvement of research methodologies related to lettuce (Terra *et al*., 2010). These tools evaluate the growth rate, stabilization, and reduction of production at the end of the cycle (Mischan and Pinho, 2014).

Empirical models are often used to estimate plant growth and their components, including the logistic model. These model have already been applied to describe the dry matter accumulation in *Allium sativum* (Reis *et al*., 2014), production of strawberry cultivars (Diel *et al*., 2018), production of tomato genotypes (Sari *et al*., 2019), production of *Cucurbita pepo* and *Capsicum annuum* (Lúcio *et al*., 2015), growth of coffee cultivar Rubi MG 1192 (Pereira *et al*., 2014), morphological characters of *Crotalaria juncea* (Bem *et al*., 2017), and growth of green dwarf coconut (Prado *et al*., 2013), cacao (Muniz *et al*., 2017), and Asian pear (Ribeiro *et al*., 2018).

For lettuce, the Gompertz, logistic, and Expolinear models were fit to the leaf dry matter of cultivars Grand Rapids, Regina, and Great Lakes in a hydroponic system in summer (Lyra *et al*., 2003). Studies were also carried out to analyze the growth of lettuce, variety Batavia, in saline soils, using the logistic model (Carranza *et al*., 2009). However, studies that describe growth using nonlinear models for other cultivars, traits, and goodness-of-fit measures were not found.

The objectives of this study were to adjust the logistic model to fit the leaf and shoot fresh and dry matter of four lettuce cultivars (Crocantela, Elisa, Rubinela, and Vera) and to describe the growth of these cultivars in summer.

MATERIALS AND METHODS

Two experiments on the lettuce crop were carried out, one in the summer of 2017 (experiment 1) and the other in the summer of 2018 (experiment 2) in Santa Maria-RS (Brazil) (29°42'S, 53°49'W and 95 m altitude) in a protected environment, of the rain shelter type. The climate of the region is humid subtropical Cfa, with hot summers and an undefined dry season, according to the Köppen classification (Alvares *et al*., 2013).

The evaluated cultivars were: Crocantela (iceberg - green leaves, consistent, crisp, loose, prominent ribs, non-heading); Elisa (butterhead - delicate leaves – loosely-formed head); Rubinela (lollo, loose purple leaves, non-heading), and Vera (lollo - green crisp - consistent, loose leaves, and non-heading). The selection of the genetic material was based on the meteorological characteristics of the cultivation site during the crop cycle and the seed companies' recommendations. Seedlings were produced in 200-cell expanded polystyrene trays filled with commercial Plantmax® substrate in a floating system. Plants with four to five leaves were transplanted on 01/27/2017 (experiment 1) and 09/02/2018 (experiment 2).

The plants were grown on six benches made of corrugated fiber cement sheets, 3.66 m long, 1.10 m wide, 6 mm thick, with six troughs with a depth of 5 cm. The culture channels were waterproofed with 100 μm-thick, clear plastic film and filled with number two washed gravel. The troughs were covered with clear, 100-μm-thick plastic film and filled with number two.washed gravel The benches were raised (0.85 m) on fixed masonry blocks at both ends with a 2% slope. This slope allowed the nutrient solution to return to the 500 L plastic storage tank. The solution was pumped by a low-power submersible motor pump (with a timer) to a PVC pipe (25 mm diameter). This pipe had four drip hoses with pots placed under the drippers at a distance of 30 cm between plants in a row, with a plant density of 11.11 m^{-2}. Each bench had four rows, totaling 44, with 3 L volume pots (11 pots per row) filled with washed sieved coarse sand with 0 dS m^{-1} electrical conductivity.

The macronutrient composition of the nutrient solution was as follows (mmol L^{-1}): 10.36 NO_3^-; 1.0 $H_2PO_4^-$; 3.36 NH_4^+; 1.0 SO_4; 4.0 de K^+; 2.0 Ca^{2+}; and 1.0 Mg^{2+}; and the micronutrients were (mg L^{-1}): 1.0 Fe; 0.50 Mn; 0.22 Zn; 0.26 B; 0.06 Cu, and 0.03 Mo. The electrical conductivity (EC) was maintained at 1.33 dS m^{-1} , and the pH was between 5.5 and 6.5 in both experiments. The EC and pH were monitored during cultivation and corrected when they presented a variation of 20%, higher or lower, in relation to the standard EC and pH.

Seven days after transplantation, evaluations started with eight plants of each cultivar. Then, the evaluations took place every 4 d until the beginning of flowering. Ten evaluations were performed for the two experiments, totaling 80 plants of each cultivar, except for cultivar Elisa in experiment 1, which had 64 plants assessed in eight evaluations. There were 624 plants analyzed in the two experiments for the four cultivars. The fresh leaf matter (FLM, in g/plant), dry leaf matter (DLM, in g/plant), fresh shoot matter (FSM, in g/plant), and dry shoot matter (DSM, in g/plant) were determined in these plants. To obtain the dry matter, the material was packed into paper bags and incubated in a forced circulation oven (60°C ±5°C) to constant matter.

The data on the indoor air temperature were recorded every three hours with a digital data logger (0.1°C resolution and 0.5°C accuracy) installed in a weatherproof shelter. With the data, the daily thermal sum was calculated with the method of Gilmore and Rogers (1958) and Arnold (1959), using equations (1) and (2)

$$STd = (Tmax + Tmin) / 2 - Tb \tag{1}$$

where *Tmax* is maximum daily temperature, °C; *Tmin* is the daily minimum temperature, °C; and *Tb* is the lettuce base temperature = 10°C (Brunini, 1976)

$$ATS = \sum STd \tag{2}$$

where *ATS* is accumulated thermal sum, and $\sum STd$ is the daily thermal sum.

The fitting of the logistic model to each trait (dependent variable), with the repetitions of each evaluation, as a function of the accumulated thermal sum (ATS) (independent variable) was performed using the least squares method and the iterative process of Gauss-Newton. The equation was: $y_i = a/[1+exp(-b -cx_i)]$ where, yi is the i-th observation of the dependent variable with $i = 1,2, ..., n$; x_i is the i-th observation of the independent variable; a is the asymptotic value; b is a location parameter important for maintaining the sigmoidal shape of the model; and c is associated with growth, indicating the growth rate.

The assumptions of normality, independence, and homogeneity of the errors were verified using the Shapiro-Wilk, Durbin-Watson, and Breusch-Pagan tests, respectively. Traits that did not meet the assumptions were Box-Cox transformed with the statistical software Action.

The lower and upper limits of the 95% confidence interval were calculated, and, using the criterion of overlapping of the confidence intervals, the estimates of the parameters (a, b and c) for each trait were compared between the experiments in each cultivar and between the cultivars in each experiment.

The goodness-of-fit of the model was assessed with an adjusted coefficient of determination (R^2 aj), in which the best fit is the one closest to 1, the Akaike Information Criterion (AIC) and the residual standard deviation (RSD), in which the best fit for both is the one closest to zero. The intrinsic nonlinearity (IN) and the parameter-effects nonlinearity (PE) were quantified based on the geometric concept of curvature (Bates and Watts, 1988). The inflection point (IP), the maximum acceleration point (MAP), and the maximum deceleration point (MDP) were calculated according to the equations described by Mischan and Pinho (2014). Inferences about plant growth were made from these critical points. The calculations were made using Microsoft Office Excel® applications and the software R (R Development Core Team, 2018).

RESULTS AND DISCUSSION

In both experiments, the Shapiro-Wilk, Durbin-Watson, and Breusch-Pagan tests had p-values greater than or equal to 0.05 (Tab. 1). Thus, the assumptions of normality, independence, and homogeneity of errors were met for the residuals of the model for the

Table 1. P-value of the Shapiro-Wilk (SW), Durbin-Watson (DW), and Breusch-Pagan (BP) tests applied to Logistic residuals for characteristics as a function of cumulative thermal sum of four lettuce cultivars in two experiments.

Character	Cultivars	SW	DW	BP	SW	DW	BP
		Experiment 1			Experiment 2		
FLM	'Crocantela'	0.92	0.10	0.12	0.17	0.69	0.57
	'Elisa'	0.16	0.35	0.07	0.08	0.47	0.18
	'Rubinela'	0.56	0.57	0.05	0.09	0.20	0.74
	'Vera'	0.22	0.14	0.06	0.90	0.65	0.05
DLM	'Crocantela'	0.42	0.30	0.07	0.28	0.57	0.07
	'Elisa'	0.69	0.22	0.06	0.09	0.16	0.28
	'Rubinela'	0.17	0.84	0.11	0.07	0.29	0.11
	'Vera'	0.14	0.10	0.08	0.08	0.27	0.06
FSM	'Crocantela'	0.89	0.24	0.14	0.19	0.72	0.89
	'Elisa'	0.82	0.47	0.10	0.88	0.33	0.21
	'Rubinela'	0.60	0.84	0.09	0.10	0.24	0.54
	'Vera'	0.42	0.08	0.80	0.96	0.91	0.12
DSM	'Crocantela'	0.30	0.16	0.06	0.19	0.36	0.07
	'Elisa'	0.28	0.18	0.11	0.51	0.10	0.49
	'Rubinela'	0.23	0.66	0.07	0.05	0.32	0.09
	'Vera'	0.06	0.11	0.05	0.07	0.57	0.05

FLM: fresh leaf matter; DLM: dry leaf matter; FSM: fresh shoot matter; and DSM: dry shoot matter.

fresh and dry matter of the leaves and shoots of the lettuce cultivars. Similar results were found for a tomato crop, indicating that the estimation of the parameters with the method of ordinary least squares is adequate (Carini *et al.*, 2019; Sari *et al*., 2019).

For each trait of the logistic model, the estimates of the parameters (*a*, *b*, and *c*) were compared between the experiments (Tab. 2) and between the cultivars (Tab. 3) with the criterion of overlapping confidence intervals. As an illustration of the use of the criterion of overlapping 95% confidence intervals (CI), the DLM of cv. Crocantela was selected to compare the estimate of parameter a with the logistic model between experiments 1 and 2 (Tab. 2). The estimate of parameter a (361.4624) in experiment 1 was found to lie outside the confidence interval of the estimate of parameter *a* in experiment 2 (375.3193 to 407.5963). However, the estimate of parameter *a* (391.4578) in experiment 2 is within the confidence interval of the estimate of parameter *a* of experiment 1 (312.2015 to 410.7332). Thus, when at least one of the estimates is within the CI of the other, the effect is non-significant. On the other hand, when two estimates lie outside the confidence interval, they differ between the experiments.

Table 2. Estimation of the parameters *a*, *b*, and *c*, lower limit (LL) and upper limit (UL) of the confidence interval (CI95%) of the logistic model for the traits as a function of accumulated thermal sum (in ºC) of lettuce cultivars (Crocantela, Elisa, Rubinela, and Vera) in two experiments in the summer.

Character	Parameter	Estimates	CI95%		Estimates	CI95%	
			LL	UL		LL	UL
		Experiment 1			Experiment 2		
'Crocantela'							
FLM	a^{NS}	361.4624	312.2015	410.7232	391.4578	375.3193	407.5963
	b *	-5.2352	-6.3805	-4.0899	-6.7770	-7.6323	-5.9217
	c *	0.0097	0.0071	0.0124	0.0160	0.0138	0.0182
DLM	a^{NS}	22.2816	12.9211	31.6422	15.2970	14.3764	16.2177
	b *	-4.9474	-5.9063	-3.9886	-7.5154	-9.2015	-5.8292
	c *	0.0073	0.0048	0.0099	0.0182	0.0139	0.0226
FSM	a^{NS}	476.9038	394.2911	559.5165	457.3523	436.4193	478.2853
	b *	-5.1544	-6.1619	-4.1469	-6.6278	-7.4289	-5.8267
	c *	0.0089	0.0066	0.0112	0.0152	0.0131	0.0172
DSM	a^{NS}	29.2298	14.6145	43.8450	18.0644	16.9789	19.1500
	b *	-5.1348	-6.0036	-4.2660	-7.1991	-8.6089	-5.7893
	c *	0.0072	0.0047	0.0097	0.0169	0.0133	0.0205
'Elisa'							
FLM	a *	185.2778	169.8337	200.7219	244.3639	222.4487	266.2790
	b^{NS}	-6.6950	-8.7591	-4.6309	-8.1763	-11.2348	-5.1177
	c^{NS}	0.0179	0.0121	0.0238	0.0207	0.0128	0.0287
DLM	a *	8.9630	7.2465	10.6795	12.6729	11.5186	13.8272
	b *	-4.8269	-6.6855	-2.9683	-7.9188	-10.8457	-4.9919
	c *	0.0119	0.0064	0.0173	0.0199	0.0123	0.0275
FSM	a *	300.6402	275.2325	326.0478	376.2930	353.6540	398.9319
	b *	-5.9503	-7.0829	-4.8177	-8.4225	-10.4071	-6.4378
	c *	0.0139	0.0109	0.0169	0.0195	0.0146	0.0244
DSM	a *	12.0021	9.5491	14.4550	17.3384	15.9877	18.6890
	b *	-5.0590	-6.7237	-3.3943	-7.7846	-10.0156	-5.5535
	c *	0.0115	0.0068	0.0161	0.0183	0.0127	0.0240

Continues...

Table 2.

Character	Parameter	Estimates	IC95%		Estimativa	IC95%	
			LL	UL		LL	UL
		Experiment 1			Experiment 2		
‘Rubinela’							
FLM	a^{NS}	290.3400	231.9672	348.7129	299.5631	280.2090	318.9172
	b *	-4.5073	-5.1155	-3.8992	-6.5113	-7.2516	-5.7709
	c *	0.0073	0.0057	0.0090	0.0138	0.0119	0.0157
DLM	a^{NS}	24.2729	5.4084	43.1373	11.2546	10.3438	12.1655
	b *	-4.5282	-4.9548	-4.1016	-7.1432	-8.8810	-5.4055
	c *	0.0055	0.0039	0.0071	0.0164	0.0120	0.0208
FSM	a^{NS}	440.1027	318.3813	561.8241	349.3106	326.0222	372.5991
	b *	-4.6709	-5.1383	-4.2035	-6.4519	-7.1002	-5.8036
	c *	0.0068	0.0054	0.0082	0.0133	0.0117	0.0150
DSM	a^{NS}	33.9705	1.7778	66.1632	12.8154	11.7420	13.8889
	b *	-4.8280	-5.3390	-4.3170	-6.9138	-8.4208	-5.4068
	c *	0.0055	0.0040	0.0070	0.0155	0.0117	0.0194
‘Vera’							
FLM	a *	418.9802	382.8992	455.0612	355.1664	323.8265	386.5064
	b *	-5.1416	-5.6644	-4.6188	-6.5101	-7.7919	-5.2284
	c *	0.0090	0.0078	0.0102	0.0144	0.0111	0.0178
DLM	a *	29.6956	20.5754	38.8158	14.6730	13.5342	15.8117
	b *	-4.9483	-5.4720	-4.4246	-7.2819	-9.1636	-5.4002
	c *	0.0070	0.0055	0.0085	0.0171	0.0123	0.0219
FSM	a *	612.9969	536.9193	689.0745	440.9334	399.6508	482.2160
	b *	-5.1509	-5.6253	-4.6766	-6.5082	-7.6124	-5.4040
	c *	0.0082	0.0070	0.0093	0.0139	0.0110	0.0167
DSM	a *	40.0535	25.5274	54.5796	18.0346	16.5610	19.5082
	b *	-5.1771	-5.6506	-4.7036	-7.1899	-8.8487	-5.5310
	c *	0.0070	0.0055	0.0084	0.0162	0.0120	0.0204

FLM: fresh leaf matter, as g/plant; DLM: dry leaf matter, as g/plant; FSM: fresh shoot matter, as g/plant; and DSM: dry shoot matter, as g/plant. Comparison of the estimates of the parameters (*a*, *b*, and *c*) between the experiments. *Significant effect at 0.05 probability level. ^{NS}: Non-significant.

Table 3. Comparison of the estimates of the parameters (*a*, *b*, and *c*) of the logistic model for the traits as a function of the accumulated thermal sum, based on the confidence interval (CI95%) between lettuce cultivars (Crocantela, Elisa, Rubinela and Vera) in two experiments in the summer.

Cultivars	Cultivars	FLM	DLM	FSM	DSM	FLM	DLM	FSM	DSM
		Experiment 1				Experiment 2			
a									
‘Crocantela’	‘Elisa’	*	*	*	*	*	*	*	NS
‘Crocantela’	‘Rubinela’	*	NS	NS	NS	*	*	*	*
‘Crocantela’	‘Vera’	*	NS	*	NS	*	NS	NS	NS
‘Elisa’	‘Rubinela’	*	NS	*	NS	*	*	*	*
‘Elisa’	‘Vera’	*	*	*	*	*	*	*	NS
‘Rubinela’	‘Vera’	*	NS	*	NS	*	*	*	*

Continues...

Table 3.

b									
'Crocantela'	'Elisa'	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}
'Crocantela'	'Rubinela'	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}
'Crocantela'	'Vera'	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}
'Elisa'	'Rubinela'	*	^{NS}	*	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}
'Elisa'	'Vera'	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}
'Rubinela'	'Vera'	*	^{NS}	*	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}
c									
'Crocantela'	'Elisa'	*	^{NS}	*	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}
'Crocantela'	'Rubinela'	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}
'Crocantela'	'Vera'	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}
'Elisa'	'Rubinela'	*	*	*	*	^{NS}	^{NS}	*	^{NS}
'Elisa'	'Vera'	*	^{NS}	*	^{NS}	^{NS}	^{NS}	*	^{NS}
'Rubinela'	'Vera'	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}

FLM: fresh leaf matter, as g/plant; DLM: dry leaf matter, as g/plant; FSM: fresh shoot matter, as g/plant; and DSM: dry shoot matter, as g/plant. Comparison of the parameters estimates (*a*, *b* and *c*) between the experiments: * Significant effect at 0.05 probability of error. ^{NS}: Non-significant.

In the logistic model, the behavior of the estimates of parameter *a* of the cultivars Crocantela and Rubinela was the same, with asymptotic values that did not differ between all traits. The opposite behavior was found for parameters *b* and *c*, which were different between the experiments (Tab. 2). The traits DLM, FSM, and DSM of cv. Elisa showed differences for all parameters, and the asymptotic values in experiment 2 were higher than those in experiment 1, indicating that the plants had a greater fresh matter production in experiment 2. All traits of cv. Vera differed in relation to the parameters *a*, *b*, and *c*. These findings showed that the growth models were different between experiments 1 and 2. Different models were also selected for different experiments for the production of tomato genotypes (Sari *et al*., 2019).

The comparison between cultivars in each experiment showed that, for the logistic model, in experiment 1, the traits DLM, FSM and DSM did not differ between 'Crocantela' and 'Rubinela', and the traits DLM and DSM did not differ between 'Crocantela' and 'Vera' or between 'Rubinela' and 'Vera' (Tab. 3). In experiment 2, the estimates of DLM, FSM and DSM did not differ between 'Crocantela' and 'Vera', and DSM did not differ between 'Crocantela' and 'Elisa' or between 'Elisa' and 'Vera'. The other comparisons showed a difference in at least one of the three parameters of the logistic model. Differences predominated for the logistic model, indicating the need for specific models for each trait and cultivar. Different models were also needed to describe production in *Cucurbita pepo* and *Capsicum annuum* (Lúcio *et al*., 2015) and fruit production estimates in *Lycopersicon esculentum* var. *cerasiforme* (Lúcio *et al.,* 2016).

Goodness-of-fit measures were used to determine the model that best fit the original data. The logistic model showed acceptable goodness-of-fit values (high R^2, low AIC, and intermediate RSD) that were close to each other (Tab. 4). Lyra *et al*. (2003) also reported high coefficients of determination but did not use other goodness-of-fit measures, which was seen as a limitation in the study.

Although the models showed satisfactory goodness-of-fit, a small overestimation occurred for the logistic model in 'Rubinela' in Experiment 1, with an asymptotic value of 440.1027 for FSM, and the maximum value of 368.30 g/plant was observed in the data set. The tendency for overestimations in the logistic model was also reported in the modeling of production during the formation of sugarcane (Batista *et al*., 2013). However the use of models to describe the accumulation of dry mass in garlic plant accessions did not present an overestimation of parameters for the Logistic model but did for the Brody, von Bertalanffy and Mitscherlich models (Puiatti *et al*., 2013).

Intrinsic nonlinearity (IN) and parameter-effects nonlinearity (PE) are used to help in the determination of the model. The logistic model presented reduced values of IN and PE for all traits, cultivars, and experiments (Tab. 4). The lower values of IN and PE indicated better suitability of the logistic model. These criteria were also adopted to indicate the most appropriate model to describe production in strawberries (Diel *et al*., 2018) and salad tomato genotypes (Sari *et al*., 2019).

Table 4. Coefficient of determination adjusted (R^2 aj), Akaike information criterion (AIC), residual standard deviation (RSD), intrinsic nonlinearity (IN), nonlinearity of the parameter effect (PE), inflection point (IP) , maximum acceleration point (MAP), and maximum deceleration point (MDP) of the Logistic model for characters as a function of the accumulated thermal sum (in °C) of lettuce cultivars (Crocantela, Elisa, Rubinela and Vera) in two experiments.

Statistic		FLM	DLM	FSM	DSM	FLM	DLM	FSM	DSM
		Experiment 1				Experiment 2			
Crocantela									
R^2 aj		0.884	0.844	0.896	0.861	0.974	0.927	0.976	0.944
AIC		7.668	1.727	7.933	1.926	6.488	1.139	6.674	1.147
RSD		44.576	2.285	50.907	2.525	24.712	1.705	27.115	1.711
PE		1.140	4.479	1.520	5.838	0.355	0.468	0.426	0.529
IN		0.169	0.173	0.154	0.166	0.101	0.175	0.098	0.157
IP	x	537.368	674.108	579.890	712.798	423.085	412.157	436.856	426.523
	y	180.731	11.141	238.452	14.615	195.729	7.649	228.676	9.032
MAP	x	402.188	494.667	431.726	529.982	340.868	339.933	350.051	348.498
	y	76.386	4.709	100.782	6.177	82.725	3.233	96.650	3.817
MDP	x	672.548	853.549	728.054	895.615	505.303	484.382	523.661	504.547
	y	285.076	17.573	376.122	23.053	308.733	12.064	360.702	14.247
Elisa									
R^2 aj		0.864	0.722	0.934	0.784	0.814	0.817	0.935	0.895
AIC		6.579	1.121	6.589	1.308	7.786	1.836	7.412	1.770
RSD		29.039	1.895	29.195	2.081	47.308	2.415	39.349	2.338
PE		0.666	1.558	0.677	1.705	0.593	0.620	0.560	0.675
IN		0.243	0.275	0.147	0.241	0.275	0.276	0.193	0.230
IP	x	373.847	405.774	429.476	441.149	394.710	397.842	431.857	424.509
	y	92.639	4.482	150.320	6.001	122.182	6.336	188.146	8.669
MAP	x	300.309	295.063	334.422	326.310	331.133	331.678	364.331	352.692
	y	39.154	1.894	63.533	2.536	51.640	2.678	79.520	3.664
MDP	x	447.386	516.485	524.530	555.989	458.286	464.007	499.383	496.326
	y	146.124	7.069	237.107	9.466	192.724	9.995	296.773	13.674
Rubinela									
R^2 aj		0.924	0.903	0.941	1.530	0.977	0.916	0.981	0.929
AIC		6.303	0.352	6.487	0.498	5.643	0.601	5.694	0.639
RSD		22.542	1.150	24.732	1.237	16.199	1.303	16.613	1.327
PE		1.954	13.526	3.135	19.553	0.665	0.755	0.696	0.816
IN		0.108	0.101	0.093	0.096	0.091	0.197	0.081	0.176

Continues...

Table 4.

IP	x	613.956	828.298	688.861	882.761	471.953	435.302	483.970	445.222
	y	145.170	12.136	220.051	16.985	149.782	5.627	174.655	6.408
MAP	x	434.570	587.400	494.637	641.966	376.497	355.047	385.182	360.414
	y	61.356	5.129	93.005	7.179	63.305	2.378	73.818	2.708
MDP	x	793.342	1069.197	883.086	1123.557	567.410	515.556	582.758	530.029
	y	228.984	19.143	347.098	26.792	236.258	8.876	275.493	10.107
Vera									
R^2 aj		0.970	0.938	0.971	0.946	0.937	0.907	0.951	0.925
AIC		6.375	1.096	6.838	1.332	7.120	1.280	7.222	1.407
RDP		23.421	1.668	29.548	1.877	33.880	1.829	35.650	1.948
PE		0.750	3.548	1.180	4.631	0.866	0.680	0.959	0.796
IN		0.080	0.102	0.076	0.098	0.159	0.208	0.136	0.187
IP	x	573.692	704.507	629.627	743.069	450.795	425.772	469.360	443.158
	y	209.490	14.848	306.498	20.027	177.583	7.336	220.467	9.017
MAP	x	426.748	517.007	468.647	554.045	359.602	348.770	374.384	361.985
	y	88.541	6.275	129.541	8.464	75.055	3.101	93.180	3.811
MDP	x	720.637	892.007	790.606	932.093	541.989	502.774	564.336	524.331
	y	330.439	23.420	483.455	31.589	280.111	11.572	347.753	14.223

FLM: fresh leaf matter, as g/plant; DLM: dry leaf matter, as g/plant; FSM: fresh shoot matter, as g/plant; and DSM: dry shoot matter, as g/plant.

Analyzing the five goodness-of-fit measures (R^2, AIC, RSD, IN, and PE) showed that the logistic model was suitable for all traits and experiments for the cultivars Crocantela, Elisa, Rubinela, and Vera, and the most suitable for describing growth in lettuce cultivars. The cultivar Crocantela in experiment 2 served as example of the shape of the logistic growth curve and the respective critical points for each trait (Fig. 1).

Critical points are used to describe crop growth (Tab. 4). The logistic model, for most of the cultivars in experiment 2, showed that the inflection point (IP) coincided with the plant stage closest to harvest, with the appearance of senescent outer leaves, which in practice is one of the criteria used for the commercial classification of the product. Also, the maximum acceleration point occurred at the beginning of the curve in experiment 2, with small plants still showing young leaves. In general, among the cultivars, cv. Elisa reached IP with the lowest ATS, independent of the experiment. However, 'Rubinela' required a greater accumulated thermal sum and showed lower values for the traits than the cultivars Vera (experiment 1) and Crocantela (experiment 2). These results indicate that the cultivars Vera and Crocantela were able to use the accumulated thermal sum efficiently. The maximum deceleration points (MDP) referred to the final growth stage of the cultivars, in which the crop was close to the beginning of flowering. Therefore, the inflection point is an alternative for future projections related to crop planning.

In this study, the logistic model described the growth of the lettuce satisfactorily in order to assist in the selection of promising cultivars. In addition, the logistic model was used to describe the growth curve of pruned coffee trees (Pereira *et al*., 2016), the accumulation of macronutrients in an onion crop (Pôrto *et al*., 2006), the production of strawberry cultivars from different seedling origins grown on organic substrates (Diel *et al*., 2018), the production of tomato genotypes (Sari *et al*., 2019), and the length, diameter, and matter of cocoa fruits (Muniz *et al*., 2017) and Asian pears (Ribeiro *et al*., 2018).

Simulation and prediction (parameters *a*, *b*, and *c*) can be used in the research or production of the cultivars Crocantela, Elisa, Rubinela, and Vera in summer. However, the thermal sum of the growing site should be used to achieve conditions close to the real ones. Therefore, these models are a reference for further research, and the obtained values should maintain the pattern of the growth curve.

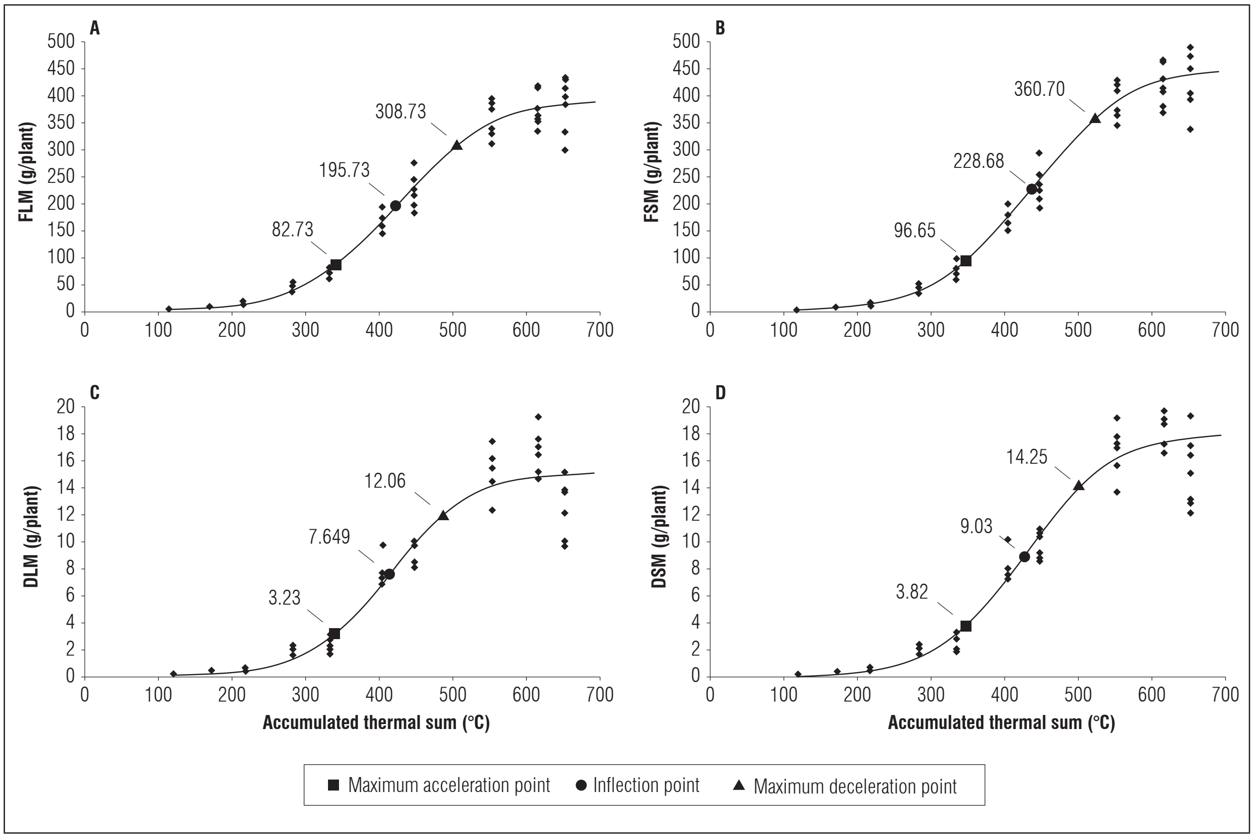

Figure 1. Logistic model plot for fresh leaf matter (FLM, as g/plant), dry leaf matter (DLM, as g/plant), fresh shoot matter (FSM, as g/plant), and dry shoot matter (DSM, as g/plant) as a function of the accumulated thermal sum (ATS, as °C) for the cultivar Crocantela in experiment 2.

CONCLUSION

The logistic growth model fit well to the fresh and dry matter of the leaves and shoots of the cultivars Crocantela, Elisa, Rubinela, and Vera and describes the growth of lettuce.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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Partial molecular characterization of begomoviruses isolated from weeds collected in tomato crops in the southeast of Valle del Cauca, Colombia

Caracterización molecular parcial de begomovirus aislados de arvenses colectadas en cultivos de tomate en el sureste del Valle del Cauca, Colombia

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***Amaranthus dubius* is an alternative host to PYMV.**

Photo: K. Lopez-Lopez

ABSTRACT

Weeds are a source of new viruses but are often neglected during diversity studies. Eleven weed samples previously were collected along the edges of tomato crops in four municipalities (Florida, Ginebra, Cerrito and Candelaria) in the southeast of Valle del Cauca. These samples were positive for begomoviruses, but their molecular characterization had not been done until now. For each sample, DNA fragments were cloned, sequenced and analyzed. Nucleotide sequence analysis of viral fragments showed the presence of six different begomoviruses: two viruses isolated from *Lantana camara*, *Desmodium* sp. and *Amaranthus dubius*, which were previously identified as potato yellow mosaic virus (PYMV) and passionfruit leaf distortion virus (PLDV), respectively; four viruses isolated from *L. camara*, *A. dubius*, *Rivina humilis*, *Desmodium* sp., *Rhynchosia minima*, *Hybanthus attenuatus*, *Verbena* sp., *Croton hirtus* and *Caesalpinia* sp., showed the highest nucleotide sequence identity (89%) with the bean chlorotic mosaic virus (BChMV), datura leaf distortion virus (DaLDV) and rhynchosia golden mosaic Yucatan virus (RhGMYV). The cloned virus fragments isolated from these weeds could be new begomoviruses which have not been previously reported before, according with the ICTV species demarcation criteria established for the *Begomovirus* genera (≥91 % sequence identity). In this study, mixed infections of begomoviruses were founded in *Desmodium* sp. and *A. dubius*. Finally, this paper reports for the first time the identification of three alternative begomovirus weeds: *A. dubius* for PYMV and *L. camara* and *Desmodium* sp. for PLDV, both viruses which infect tomato and passion fruit crops, respectively.

Additional key words: geminivirus; hosts; Solanaceae; weed.

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RESUMEN

Las arvenses son una fuente de nuevos virus, pero a menudo se descuidan durante los estudios de diversidad. Previamente, se recolectaron once muestras de arvenses a lo largo de los bordes de un campo de cultivo de tomate ubicado en cuatro municipios (Florida, Ginebra, Cerrito y Candelaria) en el sureste del Valle del Cauca. Estas muestras fueron positivas para begomovirus, pero su caracterización molecular no se había realizado hasta ahora. Para cada muestra, se clonaron, secuenciaron y analizaron fragmentos de DNA. El análisis de la secuencia de nucleótidos de los fragmentos virales mostró la presencia de seis begomovirus diferentes: dos virus aislados de *L. camara, Desmodium* sp. y *A. dubius* fueron descritos previamente como el virus del mosaico amarillo de la papa (PYMV) y el virus de distorsión de la hoja de maracuyá (PLDV), respectivamente; otros cuatro virus que se aislaron de *L. camara, A. dubius, R. humilis, Desmodium* sp., *R. minima, H. attenuatus, Verbena* sp., *C. hirtus* y *Caesalpinia* sp., mostraron su mayor identidad de secuencia de nucleótidos (89%) con el virus del mosaico clorótico del frijol (BChMV), el virus de la distorsión de la hoja de la datura (DaLDV) y el virus del mosaico dorado de rhynchosia de Yucatán (RhGMYV). Los fragmentos de virus clonados de estas malezas podrían ser begomovirus nuevos que no se reportaron anteriormente, esto de acuerdo con el criterio de demarcación de especies de ICTV para el género *Begomovirus* (≥ 91% de identidad de secuencia). Este análisis también encontró la presencia de infecciones mixtas de begomovirus en las arvenses *Desmodium* sp. y *A. dubius*. Finalmente, este artículo reporta por primera vez tres arvenses hospederas alternativas de begomovirus que infectan cultivos de tomate y maracuyá: *A. dubius* para PYMV, y *L. camara* y *Desmodium* sp. para PLDV, respectivamente.

Palabras clave adicionales: geminivirus; hospederos; Solanaceae; arvense.

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INTRODUCTION

The *Geminiviridae* family is characterized by the presence of single-stranded, circular DNA genomes that are encapsidated in twinned isometric particles of about 18x30 nn. This family is classified into nine genera based on genome organization, vector transmission and host range: *Curtovirus, Mastrevirus, Topocuvirus, Becurtovirus, Eragrovirus, Turncurtovirus, Capulavirus, Grablovirus* and *Begomovirus* (Zerbini *et al.,* 2017). The begomovirus genomes endemic to the Western Hemisphere (>322 species) consist of DNA-A and DNA-B components, each of 2.5-2.6 kb, are transmitted by members of a whitefly cryptic species complex [*Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae)], and cause economically important diseases in most tropical and subtropical regions of the world (Brown *et al*., 2015; Gilbertson *et al*., 2016).

Many scientific reports suggest that weeds are potential sources of primary inoculum and play an important role as reservoirs and alternative hosts that spread viruses to new host crops and lead to major yield losses (Barreto *et al*., 2013; Prajapat *et al*., 2014; Vaca-Vaca *et al*., 2018). Therefore, the epidemiology of the geminivirus is incomplete without the study of weeds with crop-infecting begomoviruses that may have arisen from endemic weed-infecting viruses; there are known weed-infecting begomoviruses that can infect crops (Sanchez-Campos *et al*., 2000; Andrade *et al*., 2006). Wild hosts, such as weeds, are sites where begomoviruses carry out processes of genetic recombination or pseudo-recombination, decisive steps in the evolution of viruses. In addition to the processes of competition and selection, they can generate new variants of viruses with attenuated or higher pathogenicity in a short period of time (Padidam *et al*., 1999; Da Silva *et al*., 2011). Studies on viruses are of special importance to finding virus control measures in crops (Gallo *et al*., 2018).

Research on bipartite begomoviruses in Colombia is still very limited. Right now in Colombia, there are five reported virus species: bean dwarf mosaic virus (BDMV) (Gilbertson *et al*., 1991), bean leaf crumple virus (BLCrV) (Carvajal-Yepes *et al*., 2017), potato yellow mosaic virus (PYMV) (Martínez *et al*., 2008; Vaca-Vaca *et al*., 2012), and passionfruit leaf distortion virus (PDLV) (Vaca-Vaca *et al*., 2017), which have been isolated from beans, tomato and passion fruit crops, respectively. Recently, a new begomovirus was detected in chili peppers (*Capsicum* spp.) in Valle del Cauca but its identity is being studied (Vaca-Vaca *et al.*, 2019b).

In addition, begomoviruses detection has been done in weeds collected from tomato crops in Valle del Cauca, such as *Rivina humilis, Lapoerta aestuans, Lantana camara, Amaranthus dubius, Desmodium* sp., *Momordica charantia*, *Hybanthus attenuates*, *Verbena* sp., *Croton hirtus*, *Rhynchosia minima* and *Caesalpinia* sp. (López-López *et al.,* 2012; 2014). In 2018 and 2019, the complete genomes of begomoviruses were found in *C. hirtus* and *R. minima*, both new begomoviruses with the proposed names *Croton golden mosaic virus* (CroGMV) and *Rhynchosia golden mosaic Colombia virus* (RhGMCV), respectively. (Vaca-Vaca *et al*., 2018; López-López *et al*., 2019). Recently, new begomovirus hosts were identified in a study conducted in Valle del Cauca on weeds associated with chili pepper crops: *Parthenium hysterophorus, Malvastrum* sp., *Sida acuta*, *Acalypha* sp., *Euphorbia hirta*, *R. minima* and *R. humilis* (Vaca-Vaca *et al*., 2019a).

The increasing occurrence of begomoviruses in Colombia demands efforts to study their diversity in order to anticipate and monitor outbreaks, as well as to understand the evolutionary forces driving the emergence of novel begomoviruses in crop species previously reported as unaffected by these pathogens. Previously, a weed survey was conducted by the IPMA research group in tomato crops infected with PYMV in Valle del Cauca, where *L. camara, A. dubius, R. humilis, Desmodium* sp., *R. minima*, *H. attenuatus, Verbena* sp., *C. hirtus* and *Caesalpinia* sp. were identified as begomovirus hosts, but the identity of these viruses is still unknown to date (López-López *et al*., 2014). Therefore, the aim of this research was to characterize begomoviruses present in the weeds samples collected in 2014 along the edges of a tomato crop field located in four municipalities (Florida, Ginebra, Cerrito and Candelaria) in the Southeast of Valle del Cauca. The information obtained in this research could be useful for designing future effective strategies for the control of geminiviral diseases in crops.

MATERIALS AND METHODS

Sample collection: Weed samples were collected in tomato crops located in the southeast of Valle del Cauca (Colombia), and begomoviruses were detected with PCR (López-López *et al*., 2014). Eleven begomovirus positive weed samples were used in this research: four weed samples (leaves) were collected in the municipality of Florida: *H. attenuates* (AT18), *Verbena* sp. (AT19), *C. hirtus* (AT20) and *L. camara* (AT29); one sample was collected in the municipality of Ginebra: *A. dubius* (AT32); another five weeds were collected in the municipality of Cerrito: *R. minima* (two samples AT35 and AT36), *R. humilis* (AT38), *Desmodium* sp. (AT40) and *Caesalpinia* sp. (AT41); and the last sample was collected in the municipality of Candelaria: *R. humilis* (AT61).

Amplification and cloning of partial DNA-A component: Plant total DNA was extracted from leaf samples using the Dneasy Plant mini kit (Qiagen®). A 1.2 kb fragment was amplified with PCR with universal primers for members of the *Begomovirus* genus (Rojas *et al.,* 1992). This fragment spans the 5´ region of AC1 (Rep) and coat protein (AR1) genes, and the intergenic region (IR) of the begomovirus. For each sample, a 1.2 kb fragment was cloned in pGEM T-Easy Vector (Promega®) and transformed in *E. coli* One Shot® (Invitrogen), according to standard procedures. Five clones of each fragment were completely sequenced at Macrogen Inc. (South Korea) with primer walking.

Sequence nucleotide analysis: Contigs of viral fragments were assembled using CLC Main Workbench, version 7.5 (Qiagen®), and were initially analyzed using the BLASTn algorithm (Zhang *et al*., 2000) to determine the viral species with greatest identity. The 1.2 kb fragments of the 5' region of both the AC1 (Rep) and coat protein (Cp) genes and the entire intergenic region (IR) of all the clones were aligned using the MUSCLE algorithm in MEGA 7.0 (Kumar *et al*., 2016). To establish the identity of each sequence, the pairwise percentage nucleotide identity was calculated using Sequence Demarcation Tool Version 1.2 (SDTv1.2) (Muhire *et al*., 2014). Thirteen nucleotide sequences obtained in this study, together with the begomovirus sequences of the Abutilon and Squash clades, were aligned using the ClustalW algorithm with the default parameters. A phylogenetic tree was constructed with the neighbor-joining method with 2000 bootstrap replicates using MEGA 7.0. Phylogenetic trees were rooted with the sequence of a bipartite begomovirus, *African cassava mosaic virus* (ACMV, AF112352), from the Eastern Hemisphere (Nigeria) and a monopartite begomovirus, *Tomato yellow leaf curl virus* (TYLCV, X15656).

Confirmation of mixed viral infection with PCR: In order to confirm mixed viral infection in the samples of *Desmodium* sp. (AT40) and *A. dubius* (AT32), specific primers were designed using CLC Main Workbench, version 7.5 (Qiagen®), based on the nucleotide sequences PLDV (KT899302) and PYMV (JQ045706) deposited in the GenBank database. Following this strategy, the oligonucleotides PYMV-1: 5'-TTG-GGC-CAA-GAA-TAA-ACA-AGG-CCG-3'/ PYMV-2: 5'-ACC-ACC-TGT-GAC-CTT-GCC-ATA-GAA-3'; and MaraAF23-CP: 5'-TCC-GCT-CTC-TCT-TTC-TCT-TT -3'/ MaraAR405-CP: 5'-GGA-TAT-GTC-GTG-ACG-CTG-3' were designed and using these pairs of primers, yield fragments of 500 bp and 467 bp of PYMV and PLDV, respectively.

RESULTS AND DISCUSSION

Six begomoviruses isolated from weeds in tomato crops were identified

From each weed, five clones were obtained and fully sequenced with primer walking. Each amplified clone carried a fragment of approximately 1.2 kb of DNA-A. The nucleotide sequences were assembled in CLC® Main Workbench v7 (Qiagen) and analyzed. Nine weeds presented five identical DNA fragments (100% between themselves), meaning each weed contained a unique virus, with the exception of *A. dubius* (AT32) and *Desmodium* sp. (AT40), which presented two different fragments, indicating two viruses in each weed. The analysis yielded a total of thirteen DNA fragments (Tab. 1). The analysis of these thirteen nucleotide sequences with SDTv1.2 generated a matrix of percentages of identity that showed the presence of six viral group clusters with a higher percentage of identity (> 94%) (Fig. 1).

Group (I) consisted of one virus isolated from *C. hirtus* (FR-20-Cr), which was different from other DNA fragments. Group (II) consisted of begomoviruses isolated from *H. attenuatus* (FR-18-Hyb) and *R. humilis* (FR-61-Riv), which shared a higher identity (99%). Group (III) had begomoviruses isolated from of *L. camara* (FR-29-Lan) and *Desmodium* sp. (FR-40.10-Des). Group (IV) contained one begomovirus isolated from *Verbena* sp. (FR-19-Ver). Group (V) corresponded to the isolate FR-32.1-Am, obtained from *A. dubius*. Finally, Group (VI) had the highest identity (99-100%) of the nucleotide sequences presented by the begomoviruses isolated from *A. dubius* (FR-32.6-Am), *R. minima* (FR-35-Rhy and FR-36-Rhy), *R. humilis* (FR-38-Riv), *Desmodium* sp. (FR-40.5-Des) and *Caesalpinia* sp. (FR-41-Cae).

The nucleotide sequences were analyzed using the BLASTn algorithm for sequence identity searches. The results of this analysis are shown in Tab. 1. DNA fragments isolated from *H. attenuatus* (FR-18-Hyb), *C. hirtus* (FR-20-Cr), and *R. humilis* (FR-61-Riv), which were collected in the municipalities of Florida and Candelaria, respectively, showed the highest percentage of identity (89%) for *Bean chlorotic mosaic virus* (BChMV, JN848772), a begomovirus isolated

		(1)	(2)	(3)	(4	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
I	FR - 20 - Cr (1)	100	77	77	74	74	69	67	57	57	57	57	56	57
II	FR - 18 - Hyb (2)		100	99	86	86	80	70	60	60	60	60	61	60
	FR - 61 - Riv (3)			100	86	86	80	70	60	60	60	60	61	60
III	FR - 29 - Lan (4)				100	97	80	70	60	60	60	60	61	60
	FR - 40.10 - Des (5)					100	80	70	60	60	60	60	61	60
IV	FR - 19 - Ver (6)						100	74	62	62	62	62	62	62
V	FR - 32.1 - Am (7)							100	59	59	59	59	59	59
VI	FR - 32.6 - Am (8)								100	99	99	99	99	99
	FR - 35 - Rhy (9)									100	99	99	99	99
	FR - 36 - Rhy (10)										100	99	99	99
	FR - 38 - Riv (11)											100	99	99
	FR - 40.5 - Des (12)												100	99
	FR - 41 - Cae (13)													100

Figure 1. Identification of six viral groups with the highest percentage of identity (>94%) between the thirteen fragments isolated from weeds in this study. Percentages of nucleotide identity was calculated with SDTv1.2. For details and the nomenclature of each sample, see Tab. 1. The groups of begomoviruses are indicated with Roman numerals (I-IV).

from bean in Venezuela (Fiallo-Olivé *et al*., 2013b). This result is remarkable since the FR-20-Cr fragment is composed of a separate identity group (group I) of the FR-18-Hyb and FR-61-Riv fragments (group II), which would indicate that both begomoviruses are related to BChMV but are different viral entities (Fig. 1). The FR-19-Ver fragment isolated from *Verbena* sp. showed the highest percentage of identity (89%) for the begomovirus datura leaf distortion virus (DaLDV-JN848773), isolated from the weed *Datura stramonium* in Venezuela (Fiallo-Olivé *et al.,* 2013a).

Meanwhile, a DNA fragment (FR-29-Lan) isolated from *L. camara* and one (FR-40.10-Des) isolated from *Desmodium* sp. showed the highest value of identity (99%) for PDLV (KT899302), a begomovirus that affects passion fruit crops in Valle del Cauca (Vaca-Vaca *et al*., 2017). FR-32.1-Am, a DNA fragment isolated from *A. dubius,* showed the highest value of identity (99%) for PYMV (JN604019), a begomovirus isolated from tomato crops in Valle del Cauca (Martínez *et al*., 2008; Vaca-Vaca *et al*., 2012). These results showed for the first time the identification of three weeds that can be alternative hosts for begomoviruses that infect tomato and passion fruit crops in Valle del Cauca: *L. camara* collected in Florida and *Desmodium* sp. in Cerrito, both reservoirs for PLDV, while *A. dubius,* collected in Ginebra, is a host for PYMV. Similar results have been reported in Brazil, where *Nicandra physaloides, Euphorbia heterophylla, Crotalaria* spp. and *Sida* spp. weeds are natural reservoirs for the tomato severe rugose virus (ToSRV), a begomovirus that infects tomato crops (Barreto *et al*., 2013). Another example is the tomato yellow spot virus (ToYSV), a begomovirus that severely affects tomato crops at Brazil, which was identified in the alternative host *Sida* spp. (Ferro *et al*., 2017).

Finally, the DNA fragments belonging to group VI (FR-32.6-Am, FR-35-Rhy, FR-36-Rhy, FR-38-Riv, FR-40.5-Des and FR-41-Cae) presented a highest identity (89-90%) for *Rhynchosia golden mosaic Yucatan virus* (RhGMYV, EU021216), a begomovirus previously detected in *R. minima* in Yucatán, México

Table 1. Percentage of nucleotide identity for thirteen begomoviral fragments isolated from weeds in this study compared with begomoviruses deposited in the GenBank, calculated with the BLASTn program.

Weeds	Collection site	Name clone / isolate	Insert size (bp)	GenBank accession number	Begomoviruses related in the GenBank database / Genbank accession number	Identity percentage
Hybanthus attenuatus	Florida	FR-18-Hyb	1154	KX216473	*Bean chlorotic mosaic virus*-BChMV /JN848772	89%
Verbena sp.	Florida	FR-19-Ver	1180	KX216474	*Datura leaf distortion virus*-DaLDV /JN848773	89%
Croton hirtus	Florida	FR-20-Cr	1110	KX216475	*Bean chlorotic mosaic virus*-BChMV /JN848772	89%
Lantana camara	Florida	FR-29-Lan	1153	KX216476	*Passion fruit leaf distortion virus*-PDLV /KT899302	99%
Amaranthus dubius	Ginebra	FR-32.1-Am	1138	KX216477	*Potato yellow mosaic virus*-PYMV /JN604019	99%
Amaranthus dubius	Ginebra	FR-32.6-Am	1095	KX216482	*Rhynchosia golden mosaic Yucatan virus* -RhGMYV /EU021216	89%
Rhynchosia minima	Cerrito	FR-35-Rhy	1138	KX216483	*Rhynchosia golden mosaic Yucatan virus* -RhGMYV /EU021216	90%
Rhynchosia minima	Cerrito	FR-36-Rhy	1138	KX216484	*Rhynchosia golden mosaic Yucatan virus* -RhGMYV /EU021216	89%
Rivina humilis	Cerrito	FR-38-Riv	1117	KX216485	*Rhynchosia golden mosaic Yucatan virus* -RhGMYV /EU021216	90%
Desmodium sp.	Cerrito	FR-40.10-Des	1138	KX216478	*Passion fruit leaf distortion virus*-PDLV /KT899302	99%
Desmodium sp.	Cerrito	FR-40.5-Des	1139	KX216486	*Rhynchosia golden mosaic Yucatan virus* -RhGMYV /EU021216	89%
Caesalpinia sp.	Cerrito	FR-41-Cae	1138	KX216934	*Rhynchosia golden mosaic Yucatan virus* -RhGMYV /EU021216	90%
Rivina humilis	Candelaria	FR-61-Riv	1155	KX216479	*Bean chlorotic mosaic virus*-BChMV /JN848772	89%

(Hernández-Zepeda *et al*., 2010). It is remarkable that all these viral fragments were isolated from weeds collected in the same tomato crop located in the municipality of Cerrito. The nucleotide sequences of each viral fragment were deposited in the Genbank database (are described in Tab. 1).

In summary, six different begomoviruses were identified in thirteen DNA fragments obtained from eleven weeds collected in the municipalities of Florida, Ginebra, Cerrito and Candelaria (Tab. 1 and Fig. 1). Four of these viruses could be new begomoviruses not previously reported, based on the sequence identity for all other known begomoviruses to date and in accordance with the ICTV species demarcation criterion for the genus *Begomovirus* (≥91 % sequence identity) (Brown *et al*., 2015). In order to confirm this assumption, it is necessary to obtain the complete genome of each weed's begomovirus (Tab. 1). This result confirmed the relevance of weeds as reservoirs for new unidentified geminiviruses that could become a limiting factor for economically important crops such as tomatoes. Further studies are required to explore the infectivity, geographical distribution and host range of new begomoviruses. Recently, our research group reported the complete genome sequence of

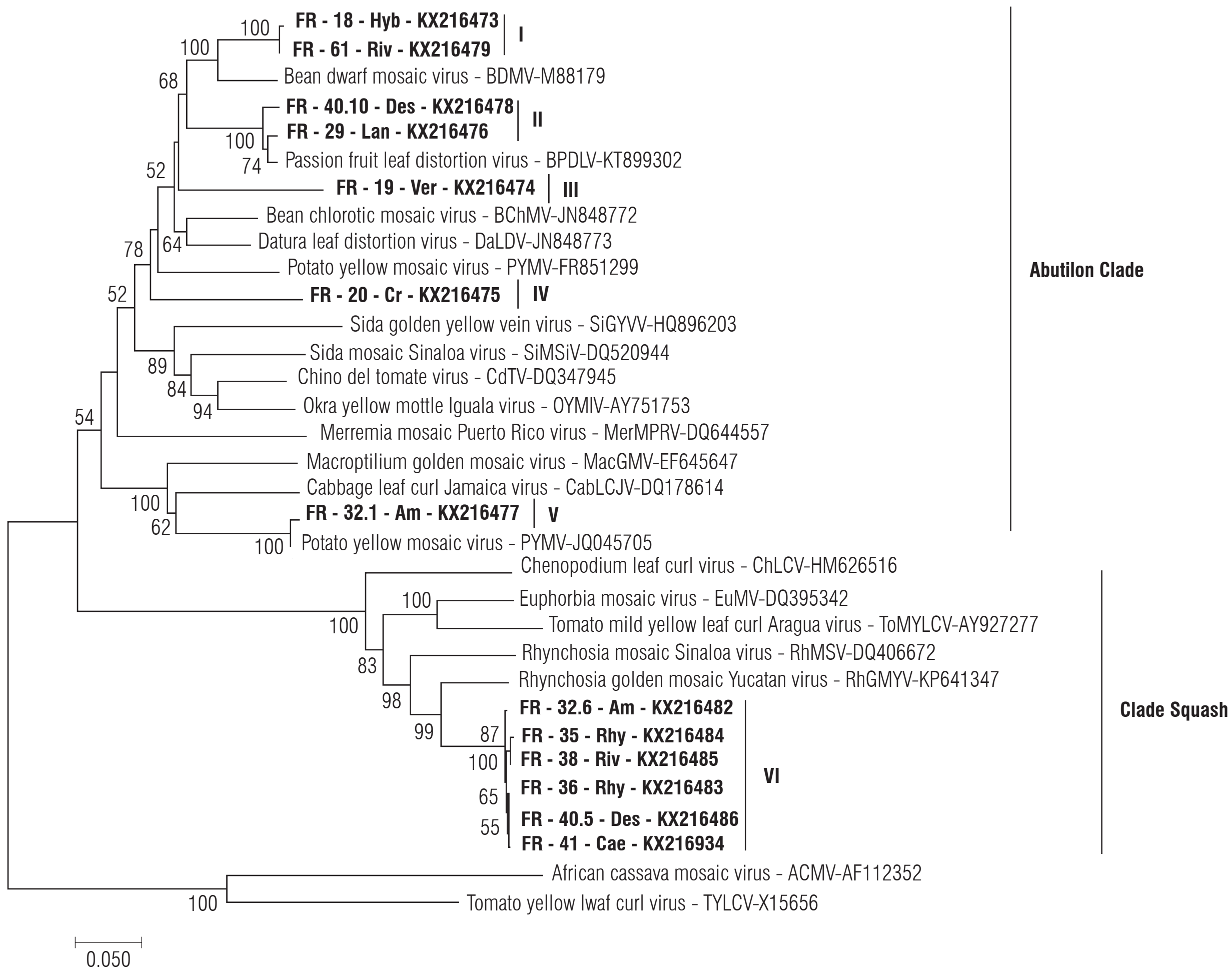

Figure 2. Phylogenetic relationships of thirteen begomoviruses isolated from weeds in this study with the most closely related begomoviruses of the Abutilon and Squash clades. Bold letters indicate the viral fragments isolated in this study (see Tab. 1 for details). The Roman numerals show the six viral groups identified in this study (see Fig. 1 for details). The bar below each tree indicates the nucleotide substitutions per site. The bootstrap consensus of the tree was inferred from 2000 replicates. Only bootstrap values above 50% are shown. ACMV (AF112352) and TYLCV (X15656) were used as an outgroup.

begomoviruses isolated from *C. hirtus* and R. *minima*, confirming them as new begomoviruses (Vaca-Vaca *et al*., 2018; López-López *et al*., 2019).

The phylogenetic analysis showed that the thirteen DNA fragments were placed in two separated clades of begomoviruses: Squash leaf curl virus (clade Squash) and Abutilon mosaic virus (clade Abutilon) (Fig. 2).

The DNA fragments obtained from *A. dubius* (FR-32.6-Am), *R. minima* (FR-35-Rhy and FR-36-Rhy,), *R. humilis* (FR-38-Riv), *Desmodium* sp. (FR-40.5-Des) and *Caesalpinia* sp. (FR-41-Cae) were placed together with viruses belonging to the Squash clade. Meanwhile, the DNA fragments FR-18-Hyb, FR-19-Ver, FR-20-Cr, FR-29-Lan (PLDV), FR-32.1-Am (PYMV), FR-40.10-Des (PLDV) and FR-61-Riv were placed together with viruses belonging to the Abutilon Clade. In the particular case of DNA fragments, FR-18-Hyb and FR-61-Riv were most related to BDMV isolates, a begomovirus previously reported as affecting bean crops in Colombia (Gilbertson *et al.,* 1991). The phylogenetic analysis showed that the DNA fragments FR-19-Ver and FR-20-Cr were placed on a monophyletic branch together with other begomoviruses and were closely related to isolates previously reported in tomato, bean and passion fruit crops in Colombia and Venezuela (Martínez *et al*., 2008; Vaca-Vaca *et al*., 2012; Fiallo-Olivé *et al*., 2013b; Vaca-Vaca *et al*., 2017). These results are in accordance with the pairwise sequence identity data (Tab. 1).

It is believed that the emergence of crop-infecting begomoviruses was the result of the horizontal transfer of indigenous viruses that infect weed hosts by the polyphagous *B. tabaci* biotype B vector. Geminiviruses exhibit high mutation rates and recombination frequencies, both within and between species, resulting in adaptative evolution. Recombination mechanisms are common among geminiviruses and contribute greatly to the evolutionary potential and local adaptation (Vaca-Vaca *et al*., 2012; Fargette *et al*., 2006; Padidam *et al*., 1999). For example, a number of natural begomovirus recombinants have been responsible for severe diseases and great economic losses in cassava in East Africa (Padidam *et al*., 1999). Another evolutionary mechanism that drives new geminivirus evolution and emergence is pse-udorecombination, which is generally restricted to the DNA genome components of closely related species. In some cases, recombinants and pseudo-recombinants have exhibited a new pathogenic phenotype that could be more virulent (Sanz *et al*., 2000; García-Andrés *et al*., 2006).

Mixed infection by begomoviruses in *Desmodium* sp. and *A. dubius*

The sequence analysis detected mixed infections in two weeds, *Desmodium* sp. and *A. dubius* (Tab. 1). *Desmodium* sp., collected in the municipality of Cerrito, had two begomoviruses, which were present in two different DNA fragments: one of which presented the highest identity for PLDV (99%), while the other one was more related to RhGMYV (89%). In the case of *A. dubius*, collected in the municipality of Ginebra, two different begomoviruses were present: one DNA fragment of these showed the highest identity with PYMV (99%), while the other DNA fragment one was related to RhGMYV (89%). The presence of PLDV and PYMV was corroborated by PCR using specific primers in *Desmodium* sp. and *A. dubius*. These specific primers amplified the coat protein gene (CP) for PDLV (500 bp) and PYMV (467 bp), respectively (Fig. 3). As a consequence, in *Desmodium* sp. and *A. dubius,* there were mixed infections by at least two begomoviruses. Mixed infections by different begomoviruses are common in non-cultivated host facilitating recombination events that may

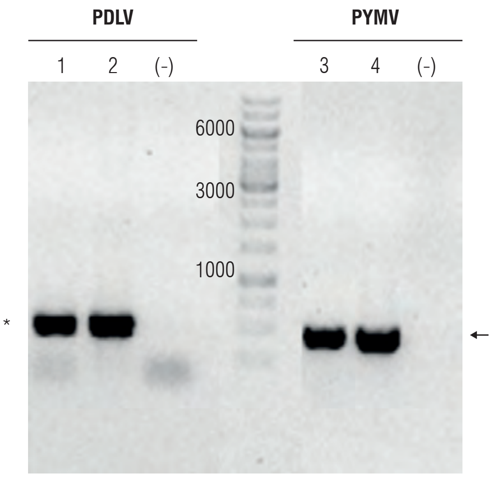

Figure 3. Detection of PLDV and PYMV with PCR in *Desmodium* sp. and *A. dubius,* respectively. Gel 1% agarose. M, molecular weight marker gene 1 kb DNA. 1. *Desmodium* sp. (sample AT40); 2. Passion fruit infected with PLDV (positive control); 3. *A. dubius* (sample AT32); 4. Tomato infected with PYMV (positive control). (-). Negative control. The asterisk indicates the expected 0.5 kb fragment for PLDV. The arrow indicates the expected 0.46 kb fragment for PYMV.

contribute to the frequent emergence of new species (Monde *et al*., 2010). Both viruses can interact in mixed infections, inducing more severe symptoms in crops, as reported by Jordá *et al*. (2007). Biologically, there are side benefits of mixed infections that can render some advances for begomoviruses, such as: a) increased rates of viral replication (Rentería-Canett *et al*., 2011); b) change or modification of tissue tropism (Alves-Junior *et al*., 2009); and c) efficient viral systemic movement and enhanced host begomoviral symptoms (Fondong *et al*., 2013).

CONCLUSION

This study provided the partial molecular characterization of begomoviruses isolated from eleven weeds frequently found along the edges of tomato crop fields in four municipalities (Florida, Ginebra, Cerrito and Candelaria) in the southeast of Valle del Cauca. The results showed four probable new begomoviruses in *H. attenuates*, *Verbena* sp, *C. hirtus, L. camara, A. dubius, R. minima*, *Desmodium* sp., *Caesalpinia* sp. and *R. humilis*. These begomoviruses are more related to BDMV, BChMV, PLDV, DaLDV and RhGMYV, which were previously reported in crops and weeds in Colombia, Venezuela and Mexico.

PLDV and PYMV were detected in *L. camara*, *Desmodium* sp, and *A. dubius*, the first report of these weeds as alternative hosts for these viruses. Finally, there was evidence of mixed infections by two begomoviruses in *A. dubius* (PYMV/related RhGMYV) and *Desmodium* sp. (PDLV/related RhGMYV). Many studies have demonstrated that mixed infections by begomoviruses are the main source of novel recombinant geminiviruses that will constrain crop production in the future. As a consequence, the close monitoring and eradication of weeds such as *L. camara*, *A. dubius* and *Desmodium* sp. could reduce the incidence of begomoviruses in tomato and passion fruit crops. Further studies are required to explore the infectivity, geographical distribution and host range of this new, infecting begomovirus.

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BLUP (Best Linear Unbiased Predictors) analysis for the selection of superior yellow diploid potato genotypes (*Solanum tuberosum* group Phureja)

Análisis BLUP (Best Linear Unbiased Predictors) en etapas avanzadas de selección en papa criolla (*Solanum tuberosum* Grupo Phureja)

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Field evaluation of promising yellow diploid potato genotypes.

Photo: L.E. Rodríguez

ABSTRACT

One of the major challenges that breeders face is the differential response of genotypes from one environment to another, known as the genotype × environmental interaction (GxE). The optimal procedure with the restricted maximum likelihood/best linear unbiased predictor (REML/BLUP) allows simultaneous estimation of genetic parameters and prediction of genotypic values. BLUP predictors are an alternative to the narrowing of biased values, which are based on variances of genotype to determine the response value, as a complement to the selection index (SI). The ESIM (Eigenvalue Selection Index) selects genotypes based on two or more variables or selection characters as long as the economic matrix possesses the appropriate values for highlighting the desired response variable. Three stages of selection were evaluated in an advanced diploid potato improvement program. BLUP values were obtained for the yield and specific gravity variables, used to determine the genetic parameters and the SI. The genetic gain for yield corresponded to 1.228 kg/plant with a heritability (H^2) = 0.82, while the GA for GE was 0.02 with an H^2 = 0.935. The SI from the BLUP values selected in the final stages of the three new cultivars (Criolla Dorada, Criolla Ocarina and Criolla Sua Pa) was registered at the Instituto Colombiano Agropecuario (ICA). Although BLUE and BLUP are highly correlated, the BLUP/ESIM analysis has an advantage as a predictor because it reduces responses to the environmental effect, efficiently selecting genotypes with a high agronomic potential.

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Additional key words: potato breeding; selection index; ESIM; genetic parameters.

RESUMEN

Uno de los principales desafíos que enfrentan los mejoradores es la respuesta diferencial de los genotipos de un entorno a otro, conocida como la interacción genotipo × ambiente (GxE). El procedimiento óptimo restringido de máxima verosimilitud/mejor predictor imparcial lineal (REML / BLUP) permite la estimación simultánea de parámetros genéticos y la predicción de valores genotípicos. Los predictores BLUP se presentan como una alternativa al estrechamiento de los valores sesgados. Se basa en las variaciones por genotipo para determinar su valor de respuesta como complemento al índice de selección (SI). El índice de selección (IS) ESIM (Eigenvalue Selection Index) permite seleccionar genotipos basados en dos o más variables o caracteres de selección siempre y cuando la matriz económica posea los valores adecuados para resaltar la variable respuesta deseada. Se evaluaron tres etapas de selección en un programa avanzado de mejoramiento de papa diploide, se obtuvieron los valores BLUP para las variables rendimiento y gravedad específica (GE), y a partir de ellos los parámetros genéticos y el IS. La ganancia genética para rendimiento correspondió a 1.228 kg/planta con una heredabilidad (H^2)=0,82, mientras que la GA para GE fue 0,02 con una H^2 = 0,935. El IS a partir de los valores BLUP seleccionó en las etapas finales tres nuevos cultivares (Criolla Dorada, Criolla Ocarina y Criolla Sua Pa) que fueron registradas ante el Instituto Colombiano Agropecuario (ICA). Aunque BLUE y BLUP están altamente correlacionadas, el análisis BLUP/ESIM da ventaja al predictor pues reduce las respuestas con efecto ambiental, seleccionando eficientemente genotipos con alto potencial varietal.

Palabras clave adicionales: mejoramiento de papa; índice de selección; ESIM; parámetros genéticos.

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INTRODUCTION

The yellow diploid potato (*Solanum tuberosum* L. grup Phureja) (Huamán and Spooner, 2002) is one of the most important plant genetic resources in Colombia. This potato is known for its nutritional value, color, flavor, texture and rapid cooking (Rivera *et al.*, 2011; Peña *et al.*, 2015; Gómez *et al.,* 2018). It poses good efficiency potential, higher early blooming, an absence of tuber dormancy, resistance to biotic and abiotic factors, specific adaptations to different environments and an unexplored source of genes usable in plant breeding programs (Rodríguez, 2013). Individuals from the Phureja group have been characterized as a good source for iron and zinc (Burgos *et al.*, 2007).

Efficiency and nutritional quality are influenced by environmental effects (Bonierbale *et al.* 2004, Rodríguez-Pérez, 2010; Hammond *et al.*, 2011; PGSC, 2011; Li *et al.*, 2013) and genetic expression. Likewise, they control the weight and starch content. These variables, when estimated, can increase the accuracy of selection in advanced breeding stages (Rivera *et al.*, 2011; Li *et al.*, 2013). To do so, it is necessary to rigorously discriminate characteristics of high heritability and easy phenotypical characterization, such as shape and color. Multiplication rates and late selection occurs for variables such as efficiency and characteristics with low heritability in late stages of selection (Poehlman and Allen, 2003).

The success of a plant breeding program depends on the capacity to deliver genotypes that guarantee high performance in terms of efficiency and/or quality across a range of environmental conditions. The genotype by environment interaction (GXE) is the result of a differential response of genotypes across environments. GXE analyses and estimations have the potential to generate information on genotype characteristics, identifying superior ones for specific environmental conditions. This potato has wide adaptability and a high efficiency potential, as determined by the GXE. The most desirable cultivar is the one combining high efficiency and stability.

Federer and Raghavarao (1975) pointed out that the analysis of variance is useful for estimating the variance components associated with genotypes, environments and their interaction. This provides information on the stability of genotypes that, calculated across heritability in a broad sense (H^2), enables the establishment of new genotypes and possible parental plants in a breeding program. One option for estimating the average value of genotypes or families is a minimum-quadratic estimation (Littell *et al.*, 2006). Under the assumption of normality and independence from errors, it provides the best linear unbiased estimate (BLUE) (Littell *et al.*, 2006; Ticona-Benavente and Silva, 2015).

For the improvement of the potato, an assessment of new genotypes through the establishment of augmented designs that enable the assessment of hundreds of genotypes without the use of replicas is recommended (Federer, 1998; Benavente *et al.*, 2011). Moreover, within the predictive power of BLUE, heritability or information from parental plants are not considered, thereby removing power from the selection analysis (Ticona-Benavente and Silva, 2015). The removal of assumed presuppositions within BLUE led Henderson (1953,1984) and Patterson and Thompson (1971) to structure the basis for the best linear unbiased predictor (BLUP) (Henderson, 2012; Ticona-Benavente and Silva, 2015), used to estimate random effects in a mixed model (Piepho *et al.*, 2008). This allowed predictions of a more accurate genetic value in the search for new varieties and genetic values in parental selection (Piepho *et al.*, 2008; Ticona-Benavente and Silva, 2015).

BLUP values show predictive accuracy when compared to other procedures since the pedigree information is often included via the numerator relation matrix, which is often susceptible to analysis via a simple mixed model. The estimation of genetic values is mainly based on models with fixed effects, eliminating variations and bringing the response value closer to the genotypic response (Piepho *et al.*, 1994; Piepho, 2008). Some studies on selection in advanced stages (close to obtaining a variety) based on BLUP methodologies have been carried out in species such as beans (Ferreira *et al.*, 2008), yams (Borges *et al.*, 2010), oil palm (Flori and Hamon, 2001), sugar cane (Barbosa *et al.*, 2014), sorghum (Filho and Tardin, 2014), corn (Bernardo, 1995,1996; Olivoto *et al.,* 2017), and cassava (Ceballos *et al.*, 2016).

In plant breeding, besides BLUP, the implementation of a Selection Index (SI) enables the selection of individuals for incorporation in a later stage of assessment. This process is carried out based on the phenotypic values observed for several traits of each candidate individual (Cerón-Rojas *et al.*, 2006). As defined by Smith (1936), SI originally assigns subjective economic weight to each trait, with the disadvantage that it requires large amounts of information, making weight assignment difficult. Thus, the Eigen value selection index method (ESIM) appears to be an alternative for the selection of genotypes in advanced stages of a selection program since it takes into account the proportion of traits contributing to the SI, which can involve two or more variables in the construction of the index with differential economic values, prioritized by relevant variables according to the species (Cerón-Rojas *et al.*, 2006, 2016).

Although some work has been done in simulated data for potatoes (Slater *et al.*, 2014; Ticona-Benavente and Silva, 2015), the estimation of BLUP and the application of ESIM are pioneering methods for the analysis of selection programs for the diploid potato. The objective of this study was the implementation of BLUP and ESIM as a strategy for the selection of superior families and genotypes in three advanced selective stages in the yellow diploid potato breeding program at the Universidad National de Colombia.

MATERIALS AND METHODS

Advanced lines of yellow diploid potato genotypes (*Solanum tuberosum* Group Phureja) were developed with hybridization obtained from crosses of different diploid parents (Tab. 1) cultivated and selected in different years and locations in a process of participative selection research with small farmers.

With materials from familiar selection obtained in two stages and after characterization based on variables of shape and color, three additional selection stages were established: three (SS3), four (SS4) and five (SS5), which facilitate advancement in the obtained population. SS3 corresponded to the establishment of four evaluations of diploid potato using 100 advanced genotypes, chosen with participative selection processes with farmers in the municipalities of Facatativa (Cundinamarca), Medellin (Antioquia), Pasto (Nariño) and Chipaque (Cundinamarca). SS4 corresponded to the evaluation of 30 genotypes selected in SS3 in two municipalities, Facatativa (Cundinamarca) and Pasto (Nariño). SS5 corresponded to the evaluation of eight genotypes and two controls that were evaluated in eleven settlements that represent the "papa criolla" productive system in Colombia, (Carlosama, Cumbal, Ipialpud, Jamondino, Mosquera, Obonuco, Tuquerres, in Nariño and San Ramon, Santa Barbara, Subachoque and Zipaquira, in Cundinamarca), located between 2400 and 2800 m, during two consecutive semesters.

For the three stages of selection, a full block design was randomly established, with four repetitions. The experiment unit was made up of 40 m^2 plots with a distance of 1.0 m between furrows and 0.3 m between sites. Each plant was fertilized with 30 g of the compound N-P-K (10-20-20). The evaluated variables were tuber yield (TY) and specific gravity (SG), according to Araujo *et al*. (2020).

Bearing in mind that the data corresponded to three multi-environmental evaluations in order to obtain the mean of each genotype in each settlement. The BLUPs (Henderson, 1984) were calculated assuming the genotypes were random effects and the settlements were fixed effects.

The value for heritability (H^2) was obtained in a broad sense for each assay and each settlement. The settlements where heritability was under 0.5 were eliminated since high variance values might imply an elevated error value. The calculations were made using the settlements where heritability was H^2= 0.5; meaning a genetic gain (GG) and its percentage (GAM) significant, following Poehlman and Allen's (2003) procedure.

For the determination of the ESIM specific values of SI were used in each stage, from the matrix of correlation between efficiency and specific gravity (Cerón-Rojas, 2006, 2008). To obtain the individual index for each genotype, the corresponding BLUP values were used, along with the MIXED procedure in SAS, 9.4. In order to prove that the effect of settlement was statistically meaningful, Ward's method of variance cluster was used with an ANOVA analysis to classify the settlements according to the TY variable. For the classification of genotypes, the quadrant method (Francis and Kanenberg, 1978), based on the mean and CV of the cultivar across the environments was used.

Finally, the correlation values between the BLUP and BLUE of response variables were used as a method

Table 1. Genealogy of 25 generations of F1 families; parentheses indicate the number of genotypes evaluated by family.

Family Code (N° of seeds)	Genealogy	Family Code (N° of seeds)	Genealogy
08-001 (125)	{*S. bukasovii* 473452-15 × Criolla Guaneña}	08-056 (70)	{UN-col 38 × Criolla Latina}
08-028 (183)	{*S. bukasovii* 473452-12 × Guaneña}	08-041 (75)	{Criolla Colombia × Criolla Galeras}
09-004 (190)	{*S. bukasovii* 265865-9 × 98-71.9}	08-042 (90)	{Criolla Colombia × Criolla Latina}
08-045 (20)	{*S. stenotomum* 195188-7 × Group phureja 704218}	08-051 (100)	{Criolla Latina × Criolla Colombia}
08-057 (20)	{*S. stenotomum* 195188-10 × Group phureja 704218}	08-046 (130)	{Criolla Galeras × Criolla Colombia}
08-048 (95)	{*S. stenotomum* 195188-12 × Criolla Colombia}	09-003 (20)	{98-71-9 × Criolla Colombia}
08-052 (96)	{S. *stenotomum* 703285 × Criolla Colombia}	08-044 (5)	{Criolla Guaneña × Criolla Latina}
08-049 (45)	{Latina × *S. stenotomum* (*gon* 1)}	08-004 (65)	{Criolla Guaneña × Criolla Galeras}
08-047 (85)	{Criolla Colombia × *S. stenotomum* 703315}	08-055 (65)	{Criolla Latina × UN-col 38}
08-043 (85)	{Criolla Colombia × *S. stenotomum* 703285-1}	Controls	
09-001 (155)	{*S. goniocalyx* 704481 × Criolla Colombia}		
09-002 (120)	{*S. goniocalyx* 703825 × Criolla Colombia}	98-71.9;26	{Criolla Colombia × *S. gon* (Amarilla Tumbay)}
09-005 (100)	{*S. goniocalyx* 703279 × Criolla Colombia}	C. Galeras	{Criolla Colombia × *S. gon* (Amarilla Tumbay)}
08-040 (95)	{UN-col 3 × Criolla Latina}	C. Guaneña	{*S. gon* (Amarilla Tumbay) × (Criolla Colombia)}
08-039 (85)	{UN-col 30 × UN-col 62}	C. Latina	{Criolla Colombia × *S. gon* (Amarilla Tumbay)}
08-054 (65)	{UN-col 38 × Criolla Galeras}	C. Colombia	{Clonal selection of round and yellow Genotypes

for the verification of the impact of using variance components as fixed or random effects in each of the stages of an advanced breeding program.

RESULTS AND DISCUSSION

For the TY variable, based on the BLUP values, the predicted mean for SS3 was 1.489 kg/plant, SS4 was 0.429 kg/plant and SS5 was 0.929 kg/plant (Tab. 2).Non-BLUP average values for SS3, Es4 and Es5 were 1.520, 0.443, and 0.964 kg/plant, respectively.

The changes between the observed values and the predicted BLUP values for TY were rather similar, in accordance with Barbosa *et al.* (2014). Although the GAM value of the analyzed population decreased, 64.55% (SS3) 26.56% (SS4), and 16.55% (SS5), (Tab. 2), it indicated that it is a logical consequence of superior genotypes in advanced stages. This means that, as a response to the selection of values observed for predicting familiar or genetic values, it is a relative improvement for the heritability value and a constant decrease in variation (as occurred in this study), where the value of heritability for TY in two states shifted from 0.63 to 0.82 and CV decreased from 36.78 a 3.70%, confirming the report by Ticona-Benavente and Pinto (2012) (Tab. 2).

Bearing in mind that the selection states are consecutive, the TY gain is guaranteed, based on the addition of the GG values at the end of stage five, to be 1.228 kg/plant, which is high since the genetic advancement of potatoes consists of the multiplication of material in a vegetative manner that may be related to the environmental effect. This produces a positive consequence in the response variable for some environments, such as a better offer for the genotypes (Cotes *et al.*, 2000; Rivadeneira *et al.*, 2016).

One of the criteria for this type of analysis is values for inheritability under 0.5 (Carlosama 12A, Cumbal 12A, San Ramón 12B, Túquerres 12A, Túquerres 12B) that were not taken into account, which is the reason why only 17 settlements were included for TY. For SS3, the value H^2=0.63 for the efficiency characteristic indicated that it is related to an effective selection of superior families during the first few generations, as reported by Ticona-Benavente and Silva (2015).

The high value for H^2 = 0.82 in SS5 corresponds to a value across all settlements. This value may be related to an efficient selection pressure, which led to the proper selection of superior individuals, ensuring high heritability for superior individuals. The values for thee genetic parameters may increase based on the efficiency of the BLUP methodology, keeping in mind that the selection of individuals must have an organoleptic characteristics component (special shape and color). This affects final selection, which is focused on obtaining genotypes with a high commercial potential in the tetraploid potato (Ticona-Benavente and Silva, 2015).

Table 2. Genetic parameters for tuber yield and specific gravity.

Parameter	Tuber yield			Specific gravity		
	Selection stage					
Factors	Three	Four	Five	Three	Four	Five
Repetitions	3	3	4	3	3	4
Settlements	4	2	17	4	2	21
Settlement variance	1372078.60	8993.69	101596,05	1.29E-05	1.90e-04	9.05E-05
Genotypes variance	241353.89	8051.51	2764,89	2.16E-05	1.97E-05	1.32E-05
Genot. Variance x Sett.	405231	2,76E-13	6614.08	1.62E-05	1.03E-06	8.27E-06
Error variance	470416.77	39568.34	14003.75	1.66E-04	1.84E-04	4.36E-05
General mean	1.489	0.429	0.929	1.072	1.082	1.087
CV (%)	36.78	28.69	3.70	0.572	0.773	0.124
H^2	0.630	0.540	0.820	0.546	0.386	0.935
GG	0.961	0.114	0.153	0.006	0.007	0.007
GAM (%)	64.55	26.56	16.55	56.30	64.800	66.10

CV: Variation quotient; H^2: heritability in wide sense; GG: Genetic gain; GAM: GG in %.

Additionally, efficiency is considerably affected by the environment and might also be influenced by the inclusion of diversity generated in the population by introducing wild parents and bred diploids.

For the variable SG, the mean predicted for SS3 was 1.072, SS4 was 1.082 and SS5 was 1.087, using BLUP values. The average Non-BLUP values without prediction for Es3, Es4 and SS5 were 1.080, 1.081, and 1.088, respectively. It was evident that the adjustment with the BLUP methodology determined that the average predicted value for SG would change, decreasing from 1.083 to 1.080. (Tab. 2). However, individual responses of selection display higher variability, which might imply the incorporation of characteristics such as shape and color (Robinson, 1991; Piepho *et al*., 2008), generating a better estimation under the BLUP proposal.

In agreement with the variable TY for SG in SS5, the analysis of information was carried out based on 21 settlements since heritability in Carlosama 12A was lower than 0.5. As a result, the values for H^2 for the SG characteristic ranged between 0.38 and 0.93. The superior values matched the ones reported by Ticona-Benavente and Silva (2015).

The GAM values showed no meaningful advancement within the selective states, indicating that, for the SG variable performing familiar selection, a greater advancement was achieved in the early stages of selection by decreasing the selective pressure to values of 50%. This would negate the need for exhaustive selection in later stages, indicating that predicted values might enable an increase in GG so long as pressure decreases to 30% (Ticona-Benavente and Silva, 2015).

Selection index

Using a selection pressure near 10% as the criterion for a population susceptible to improvement based on an analysis of specific values for SS3, SS4 and SS5, the top 10 genotypes were obtained (Tab. 3).

The families that provided the selected individuals were 08-051 (Criolla Latina × Criolla Colombia), 08-046 (Criolla Guaneña × Criolla Colombia), and 08-004 (Criolla Guaneña × Criolla Galeras). Criolla Galeras and Criolla Colombia were highlighted as parents that enabled the selection of individuals that became new cultivars, implying that they had a high, unexplored potential for improvement leading to an increase in efficiency or even the improvement of complex characteristics.

The reduction of superior families occurred mainly because phenotypical resemblance is a tendency that genetically related individuals have towards sharing a greater number of genes with each other than with the general population because of the variation for efficiency potential among and within families in a population of diploid potatoes. They are thus grouped according to the principle that phenotypic resemblance among related individuals increases efficiency or even the improvement of complex characteristics.

Table 3. Selection index for selection stages three, four and five.

SS3		SS4		SS5	
Genotype	SI	Genotype	SI	Genotype	SI
08-058-65	2.548224	08-048-86	1.148919	08-004-16	1.252889
08-058-12	1.892389	08-051-64	1.136598	08-051-90	0.707345
08-056-69	1.871687	08-058-69	1.105712	08-051-64	0.503355
08-053-77	1.820245	08-040-120	1.08126	Galeras	0.44831
08-051-53	1.70215	08-048-79	1.039077	08-046-191	-0.10097
08-056-15	1.604584	08-051-68	1.03866	08-046-187	-0.16004
Guaneña	1.490208	08-046-191	0.982446	Colombia	-0.35929
08-053-57	1.40364	08-042-7	0.918086	08-051-68	-0.43807
08-055-2	1.334886	08-004-16	0.857351	08-051-70	-0.7391
08-053-53	1.320219	08-051-70	0.468743	08-004-59	-1.11443

The main particularity of genetic improvement in potatoes lies in vegetative multiplication, which guarantees homogeneity in tubers obtained with an identical genetic base. Therefore, when a desirable genotype is identified, it can be multiplied and maintained generation after generation without genetic segregation, offering a great advantage over crops that only multiply through botanic cuttings or sexual seeds.

In order to establish the effect of GXE on advanced genotypes on SS5, it was initially proven that its effect was statistically meaningful via ANOVA analysis. In order to classify the settlements by the variable TY, a grouping method was applied (Fig. 1.), resulting in similarities and creating four major groups: San Ramon 12A and Zipaquira 12B, which corresponded to group 1, an external group; Subachoque 12 A, Mosquera 12 A and 12B, Cumbal 12A and Ipialpud 12B, corresponding to group 2; Zipaquira 12A, Subachoque 12B, Santa Barbara 12B, Ipialpud 12 A and Obonuco 12 A, corresponding to group 3, and Jamondino 12A, Santa Barbara 12B, Obonuco 12B, Carlosama 12B and Jamondino 12B in group 4. This grouping indicated similarities among the settlements and, therefore, their conditions.

Francis and Kanenberg's (1978) quadrant method identified superior genotypes as those possessing a high mean and low CV for the RT variable (Fig. 2).

Volpato *et al.* (2019) indicated that BLUP values enable an accurate estimate of the genetic parameters in multi-trait, multi-environment evaluations (MTME) that, under the quadrant method, identified the genotypes 08-051-90, 08-004-16, and 08-053-57 as having a high mean for the TY variable and acceptable CV, along with round, yellow tubers with shallow buds, good flavor and texture. All these conditions are appreciated in the improvement of the yellow diploid potato, making them suitable phenotypes for registry as new cultivars for Colombia.

BLUE *vs*. BLUP contrast

The TY and SG variables for SS3, SS4 and SS5 showed a high degree of correlation between the BLUP vs. BLUE values and were highly meaningful ($\alpha=0.05$) for the response variables (Tab. 4).

High correlations for advanced stages of the associated variable responses indicated that there was no higher relevance if the effects are treated as fixed or random. This is why a BLUP methodology is advised since it provides more modest responses, which have a low tolerance effect on extreme genotypic responses, and provides a view of possible behaviors in further testing, as expressed by Vittorazzi *et al.* (2017) who indicated that the application of BLUP results in a higher estimated gain than those applied from classic biometric selection index methodologies, and BLUP should be incorporated in the progenie section.

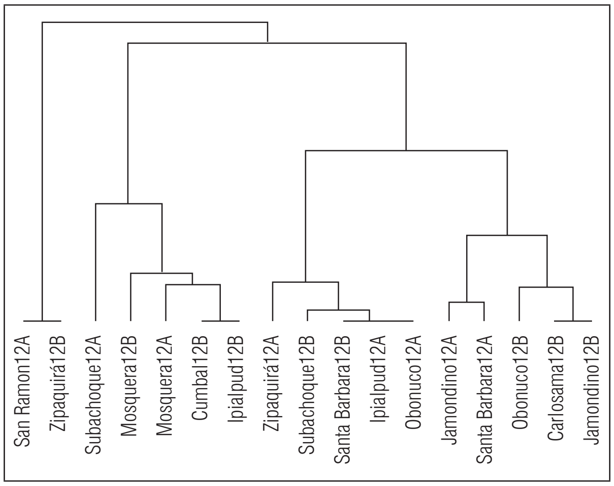

Figure 1. Dendogram for settlements, Ward's distance , variance clustering method.

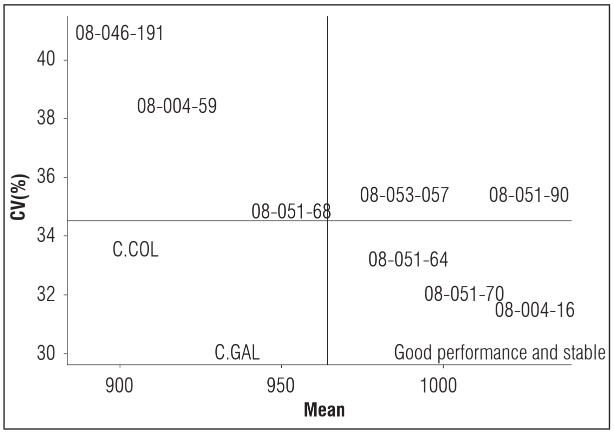

Figure 2. Evaluation of advanced genotypes SS5. Quadrant method.

Table 4. Correlations for SG and TY for the different stages of selection.

SS3				
	BLUP_TY	BLUE_TY	BLUP_SG	BLUE_SG
BLUP_TY	1.00			
BLUE_TY	0.994 (<.0001)	1.00		
BLUP_SG	0.160 (0.1027)	0.170 (0.0835)	1.00	
BLUE_SG	0.169 (0.0861)	0.188 (0.0563)	0.992(<.0001)	1.00
SS4				
BLUP_TY	1.00			
BLUE_TY	0.990 (<.0001)	1.00		
BLUP_SG	-0.344 (0.0535)	-0.366 (0.0395)	1.00	
BLUE_SG	-0.351 (0.0490)	-0.385 (0.0298)	0.977 (<.0001)	1.00
SS5				
BLUP_TY	1.00			
BLUE_TY	1.00 (<.0001)	1.00		
BLUP_SG	-0.481 (0.1591)	-0.481 (0.1591)	1.00	
BLUE_SG	-0.481 (0.1591)	-0.481 (0.1591)	1.00 (<.0001)	1.00

Correlation above, meaningfulness aside.

CONCLUSION

The implementation of BLUP values and ESIM in advanced selection stages is a tool that can improve the selection methodology because it facilitates the integration of response variables that are corrected and organized within an index, allowing discrimination of the best genotypes.

For selection stage five, an efficient statistical support was achieved. It corroborated the description for obtaining genotypes as registered varieties, indicating that the technique has a high potential for discriminating between genotypes and within them to select superior genotypes.

The GXE analysis facilitated the characterization of the settlements and showed the better ones, making this analysis an additional tool that provides an even more restrictive discrimination, associating a superior behavior with a better environment.

H^2 is a genetic parameter that, once obtained from the BLUP values, can be used in advanced selection stages for the variables TY and SG.

Genetic improvement in diploid potatoes is an option for developing genotypes with a high agronomic value, high efficiency potential and superior characteristics in color, texture, flavor and early blooming, taking advantage of heterotic values present in diploid populations and thus revealing a seldom-explored path for global improvement programs.

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DEDICATION

This paper and my whole life as a researcher are dedicated to the loving memory of Dr. José C. Pacheco Maldonado (1950-2019).

My father devoted his whole life to education. He started his career as a high school teacher and subsequently dedicated 40 years to his beloved Universidad Pedagógica y Tecnológica de Colombia- “UPTC” as a professor of genetics.

During the 1980s, he founded the research group BIOPLASMA-UPTC, where his main research area was plant tissue culture. His contributions left a lasting impact on this field over the course of the following decades.

His work focused on the protection of endangered species such as frailejones (*Espeletia sp*.) and the use of biotechnology in the propagation and improvement of species with agricultural potential.

The best dad and the best professor. Your light still guides my way. Mom and I will always remember you. Thanks for all your love “Papío”.

José E. Pacheco (son)

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Growth and dry matter partitioning in potatoes as influenced by paclobutrazol applied to seed tubers

Crecimiento y partición de materia seca de papa influenciada por paclobutrazol aplicado al tubérculo-semilla

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Potato production field.

Photo: F.F. Araújo

ABSTRACT

Under cultivation conditions that favor the growth of plant shoots, the adoption of management practices that reduce the size of potato plants can be an alternative for increasing tuber yield. The objective of this study was to evaluate the effect of paclobutrazol on vegetative growth and dry matter partitioning in the potato plant cv. Markies, cultivated under summer conditions in the state of Minas Gerais. The treatments were 0.1, 1.0, 10 and 100 mg L^{-1} of paclobutrazol (PBZ), applied to sprouted seeds before planting and control with water. PBZ was efficient in reducing plant height. The PBZ treatments with doses of 10 and 100 mg L^{-1} delayed the emergence of shoots and the beginning of plant tuberization. PBZ at 0.1 mg L^{-1} reduced the lengh of the stem by 18% but did not differ from the control for the fresh and dry mass content, total production, specific gravity and starch content of tubers. The treatment with seed potatoes and PBZ at 0.1 mg L^{-1} resulted in smaller, more compact plants, which could be suitable for more densely planted cultivations in order to maximize plant populations and increase economic return per unit of area.

Additional key words: *Solanum tuberosum* L.; gibberellin; plant regulator; tuberization.

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RESUMEN

En condiciones de cultivo que favorezca el crecimiento de la parte aérea de la planta, la adopción de prácticas de manejo que reduzca el tamaño de las plantas puede ser una alternativa para aumentar el rendimiento de los tubérculos. El objetivo de este trabajo fue evaluar el efecto del paclobutrazol sobre el crecimiento vegetativo y la partición de la materia seca de la planta de papa cv. Markies cultivado en verano en el estado de Minas Gerais, Brasil. Los tratamientos fueron 0,1; 1,0; 10 y 100 mg L^{-1} de paclobutrazol (PBZ) aplicado a las semillas germinadas antes de plantar y um control tratado con agua. PBZ fue eficiente em la reducción de la altura de la planta. Los tratamientos con dosis de 10 y 100 mg L^{-1} de PBZ retrasaron la aparición de la parte aérea y el comienzo de la tuberización de la planta. PBZ a 0,1 mg L^{-1} redujo la longitud del tallo en un 18%, pero no difirió con el control en relación al contenido de masa fresca y seca, la producción total, la gravedad específica y el contenido de almidón de los tubérculos. El tratamiento de tubérculos de papa con PBZ a 0,1 mg L^{-1} presentan plantas más pequeñas y compactas, que podrían ser adecuadas para una plantación más densa para maximizar la población de plantas y aumentar el rendimiento económico por unidad de área.

Palabras clave adicionales: *Solanum tuberosum* L.; giberelina; regulador vegetal; tuberización.

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INTRODUCTION

With a high biological protein value, the potato is one of the most nutritious food sources for man, being the fourth most consumed staple food in the world (ABBA, 2019a). Although it is consumed fresh in most of the countries, the potato is also processed as frozen pre-fried or dehydrated products, among other derivatives.

The consumption of industrialized potatoes in Brazil has increased significantly in recent years (Evangelista *et al.*, 2011), especially frozen pre-fried French fries. Good quality tubers are of fundamental importance for the processing industry, which requires a high percentage of dry matter and starch, which favor the frying yield, providing less oil retention, guaranteeing crispness and texture in the final product (Fernandes *et al.,* 2010).

The lack of raw material in adequate quantity and quality has led to the search for new potato cultivars, with high quality for processing adapted to the Brazilian climate conditions (Muller *et al.*, 2009) and providing the characteristics required by the consumer market. Recently, the cultivar Markies, which has tubers with characteristics similar to the frying cultivar Asterix (Fernandes *et al*., 2010; Evangelista *et al.*, 2011), began to be cultivated in Brazil in significant areas and has become one of the top five cultivars of processed potatoes planted in that country (ABBA, 2019b).

The State of Minas Gerais stands out as the main national producer of potatoes, providing 32% of the total amount produced in that country (IBGE, 2019). Highland regions are preferred for potato cultivation since they present mild temperatures and a greater thermal amplitude between day and night. However, in the summer months, stem and leaf mass growth is favored by high temperatures, leading to reduced yield, increased fungal and bacterial diseases and tubers with a low dry matter content. Thus, under cultivation conditions that favor the growth of plant shoots, the adoption of management practices that reduce the size of plants can be an alternative for increasing the yield of tubers. A practice that can be used to achieve a better balance between vegetative growth and tuber growth is the use of plant growth retarding products, which inhibit some steps of the gibberellin biosynthetic pathway (Rademacher, 2000).

The application of plant regulators in propagating organs, such as seeds, bulbs, rhizomes, tubers and cuttings, has numerous benefits over conventional procedures, being simple and low cost and reducing the concentration of the chemical; the content of residues in the products is minimal or nul, with low diffusion into the environment (Magnitskiy *et al*., 2006). Therefore, the application of regulators in seed potatoes would be an alternative for avoiding the risks inherent to the use of these products in commercial potato production fields.

However, the implication of plant development for the use of these products in sprouted tubers, just before planting, is still unknown. Therefore, the objective of the present study was to evaluate the effect of paclobutrazol applied to seed tubers on growth and *dry* matter *partitioning* in cv. Markies, cultivated under summer conditions in the State of Minas Gerais.

MATERIALS AND METHODS

This experiment was carried out with cultivar Markies in Fazenda Água Santa, located in Perdizes, Minas Gerais (19°21'19" S and 47°16'58" W, with an altitude of 1,100 m), during the summer season of the southeast region, between October, 2015 and February, 2016. According to the Köppen classification, the climate of the region is high tropical (Cwb).

Thirty days before planting, the seed potatoes were removed from storage at 4°C and 85% relative humidity and placed at room temperature in the dark to induce spontaneous sprouting of the tubers. The seed potatoes were immersed for 30 min into 0.1, 1.0, 10 and 100 mg L^{-1} of paclobutrazol (PBZ).

The seed planting was done manually with spacing of 0.38 m between plants and 0.8 m between rows, at a planting depth of 0.12 m. The experiment plots were 19.2 m^2, 2.4 m wide and 8 m long, with a longitudinal distance of 4 m between the plots. The experiment was conducted with a split plot design, with the plots comprising the different concentrations of growth regulators and the subplots representing the plant evaluation times, in a completely randomized design with three replicates; each experiment unit consisted of two plants.

The fertilization used 180 kg ha^{-1} of N (MAP and ammonium nitrate), 420 kg ha^{-1} of P_2O_5 (MAP) and 270 kg ha^{-1} of K_2O (potassium chloride). Potassium chloride was applied in the pre-planting, MAP was used at planting, and potassium nitrate used at 35 (ridge) and 65 d after planting. All treatments received micronutrient applications via the central pivot throughout the cycle.

During the growth period, the total precipitation was 1,133 mm, and the average minimum and maximum monthly temperatures were 19 °C (ranging from 15.9 to 23°C) and 29°C (ranging from 19.8 a 37.1°C), respectively. The mean relative humidity was 77%, ranging from 53 to 94%. The solar radiation averaged 206 W m^{-2}.

The evaluations were carried out with 35 (Evaluation I), 50 (Evaluation II), 65 (Evaluation III), 80 (Evaluation IV) and 95 (Evaluation V) days after planting (DAP). Randomly selected plants were harvested from each treatment. The samples were divided into leaves, stems, tubers and roots/stolons. The fresh matter was determined immediately after harvest, and the dry matter of each part of the plant was determined after drying in forced air circulation oven at 70°C for 5 d.

The dry matter partitioniong was determined from the dry mass of each plant part and expressed as a percentage of total dry mass of the plant. The length of the longest stem, number and fresh mass of tubers were determined. The specific gravity was determined with the ratio between the tuber weight in air and the tuber weight in water (Maeda and Dip, 2000), and the dry matter content used the ratio between the dry and fresh mass of the tuber, expressed as a percentage. At 95 DAP, the shoot plants were desiccated with the herbicide Diquat (400 g ha^{-1} a.i.). At 110 DAP, the plants were harvested from the central rows of each plot to determine the total yield.

The quantification of total soluble sugars of leaves and tubers was carried out according to the phenol-sulfuric acid method (Dubois *et al*., 1956). The reducing sugars were quantified according to the dinitrosalicylic acid (DNS) method (Gonçalves *et al*., 2010). The non-reducing sugar content was obtained with the difference between the total soluble sugar content and the reducing sugar content. For the quantification of the starch, the method described by McCready *et al*. (1950) was used.

The data were analyzed with analysis of variance (ANOVA) using the System of Statistical Analysis and Genetics (UFV, 2008). The means were compared with the Dunnett test at 5% probability. The data for stem height and carbohydrate content of tubers were submitted to descriptive analysis.

RESULTS AND DISCUSSION

At 35 DAP, the control plants and seed potato plants treated with the two lowest doses of paclobutrazo (PBZ) had already emerged, while those treated with

10 and 100 mg L^{-1} of PBZ only showed emergence after 65 DAP (Tab. 1). In potato crops, it is desirable to break dormancy before planting the seed tubers. The process of breaking dormancy and initiation of potato sprouting is triggered by the favorable internal balance of plant growth promoting regulators, including auxins, cytokinins and gibberellins, among other factors (Sonnewald and Sonnewald, 2014).

The delay in emergence in the plants treated with 10 and 100 mg L^{-1} of PBZ may have occurred because of a drastic reduction in the content of endogenous gibberellins in the seed tubers, inhibiting sprout growth for a long period. PBZ is a triazole that blocks the biosynthesis of gibberellic acid, reducing its endogenous concentration. The response of plants to PBZ varies according to the concentration, form of application, absorption and phenological stage in which the application is performed (Santos *et al*., 2004; Mabvongwe *et al*., 2016).

Seleguini *et al*. (2013) verified a linear reduction in the germination rate with increased PBZ doses applied to tomato seeds. According to Pill and Gunter (2001), the inhibitory effect of seed germination on PBZ-containing solutions may be associated with the rate of absorption of PBZ, causing toxicity to the embryo, with a reduction in GA levels at values insufficient for germination and for seedling growth. According to Hung *et al*. (1992), the increase of concentration and exposure time of seeds to a growth regulator contributed to an increase in product penetration in seeds and a consequent reduction in germination.

The percentage of dry matter (DM) in the leaves treated with 1.0 mg L^{-1} of PBZ at 35 DAP was significantly higher than the control, accounting for 81% of the total DM of the plant (Tab. 1). At 50 DAP, there was no difference between the treatments in relation to the percentage of DM partition to the leaves. At 65 DAP, the plants treated with 1.0, 10 and 100 mg L^{-1} of PBZ had the highest DM, while the control plants and those treated with PBZ 0.1 mg L^{-1} provided the lowest percentages. In the last evaluation (95 DAP), all treatments showed a decrease in the percentage of DM partitioned to the leaves, except for the two higher rates of PBZ, where the initial growth delay was intense.

The partitioning of DM to the roots at 35 and 50 DAP showed no significant difference between the treatments in the plants that had already emerged (Tab. 1). At 50 DAP, the roots of all treatments represented,

Table 1. **Partitioning of the dry matter (%) for each part of the potato and total dry matter (g), influenced by applications of paclobutrazol (PBZ) in the seed potatoes before planting.**

PBZ doses (mg L^{-1})	Leaves (%)	Roots (%)	Stems (%)	Tubers (%)	Total DM (g/plant)
Evaluation I (35 DAP)					
Control	77.24	11.75	11.01	-	9.78
0.1	79.64^{NS}	12.34^{NS}	8.02*	-	5.71*
1.0	81.18*	12.24^{NS}	6.58*	-	3.94*
10	-	-	-	-	-
100	-	-	-	-	-
CV (%)	8.01	11.30	11.37		11.50
Evaluation II (50 DAP)					
Control	78.29	11.44	10.27	-	33.58
0.1	80.01^{NS}	12.01^{NS}	7.98*	-	18.05*
1.0	80.82^{NS}	12.49^{NS}	6.69*	-	12.60*
10	-	-	-	-	-
100	-	-	-	-	-
CV (%)	7.21	13.7	6.98	-	14.85
Evaluation III (65 DAP)					
Control	57.87	6.40	8.52	27.21	78.85
0.1	63.69^{NS}	6.55^{NS}	6.69^{NS}	23.07^{NS}	62.62*
1.0	79.91*	11.31*	8.78^{NS}	-	35.99*
10	79.38*	12.84*	7.78^{NS}	-	11.99*
100	78.60*	13.12*	8.29^{NS}	-	8.56*
CV (%)	7.94	14.7	19.6	8.97	10.23
Evaluation IV (80 DAP)					
Control	42.42	5.59	6.63	45.36	129.48
0.1	48.59*	5.21^{NS}	6.57^{NS}	39.63*	106.84*
1.0	61.36*	8.25*	6.49^{NS}	23.89*	76.38*
10	80.82*	10.35*	8.83*	-	28.95*
100	81.77*	9.74*	8.49*	-	14.28*
CV (%)	8.32	10.45	22.9	12.68	7.74
Evaluation V (95 DAP)					
Control	34.75	4.26 b	5.96	55.03	151.48
0.1	39.59*	4.54^{NS}	5.57^{NS}	50.29^{NS}	123.84*
1.0	49.03*	8.26*	5.49^{NS}	37.22*	95.38*
10	80.82*	9.69*	9.49*	-	35.61*
100	82.77*	9.07*	8.16*	-	20.28*
CV (%)	7.95	17.11	23.28	5.50	10.92

^{NS} non-significant; * significant ($P \leq 0.05$) with the control by the Dunnett test.

on average, 12% of the total DM of the whole plant. According to Tekalign and Hammes (2005), the root system represents around 4% of the total DM of the potato plant. However, this ratio may vary according to the cultivar and the growing conditions. In. the subsequent evaluations at 65, 80 and 95 DAP, the seed potato plants treated with the 1.0, 10 and 100 mg L^{-1} of PBZ showed a significant difference in relation to the control treatment, obtaining a higher percentage of DM partitioned to the roots. This effect may have been a reflection of the reduction in the growth of the plant shoot, which possibly increased the availability of carbon directed to the root system.

For the accumulation and distribution of DM to the stems, the treatments with PBZ at 0.1 and 1.0 mg L^{-1} presented significant differences in relation to the control in both evaluations at 35 and 50 DAP (Tab. 1). The treatments with 10 and 100 mg L^{-1} of PBZ had the highest indexes of partition for the stems at 80 and 95 DAP when compared to the remaining treatments.

In this experiment, the beginning of the tuberization process started after 50 DAP, except for with 1.0, 10 and 100 mg L^{-1} of PBZ, which did not show any tuber formation. At 80 DAP, the plants treated with PBZ at 1.0 mg L^{-1} already had tubers but had the lowest partition index of DM for the tubers, as compared to the control treatment. At 95 DAP, 55% of the total DM from the control plants was accumulated in the tubers, while, in the treatment with PBZ at 1.0 mg L^{-1} , the accumulation of DM was only 37.2%. The application of the growth regulator PBZ on the seed potatoes before planting did not increase the DM partition to the tubers in the bulking phase.

In the first stages of potato development, the preferential metabolic drains are the leaves and stems, followed by the tubers with fast growth during tuber bulking. Therefore, a high mobilizing capacity in the tubers caused a marked reduction in the accumulation of dry matter begining at 65 DAP for both the leaves and the stems (Tab. 1). In the treatments with 10 and 100 mg L^{-1} of PBZ, this change was not noticed until 95 DAP because of the delay in the tuberization of these plants.

Based on the accumulation of DM in the entire plant, in the first evaluation, the control treatment plants had the highest DM contents, followed by the treatment with the lowest dose of PBZ and the treatment with PBZ at 1.0 mg L^{-1} (Tab. 1). In evaluation III (65 DAP), a slow initial growth was observed in the plants treated with PBZ at 10 and 100 mg L^{-1}, presenting a lower percentage of total DM in relation to the other treatments. In the last partition evaluation, the lowest percentage of DM accumulation in the entire plant was obtained in the treatment with the highest dose of PBZ (100 mg L^{-1}).

The control plants had a longer main stem length, as compared to the PBZ treated seed potatoes, throughout the plant cycle (Fig. 1). The immersion of the sprouted seeds before planting in the solution containing 0.1; 1.0; 10 and 100 mg L^{-1} of PBZ reduced the length of the stem by about 18; 25; 51 and 55%, in comparison to the control, respectively. The treatments of seed potato with PBZ resulted in smaller, more compact plants, which could be suitable for more densely planted cultivations in order to maximize plant population and increase economic return per unit of land. Several studies, however, have indicated disadvantages of PBZ seed treatments, which include delay and reduce the shoot emergence rate (Pasian and Bennett, 2001).

In tomatoes, the application of PBZ to seedlings influenced the stems lenght up to 15 d after transplanting, showing that the effect of the regulator can be limited during the culture cycle (Ferreira *et al.*, 2017). PBZ at 0.250 L ha^{-1}, applied via spraying at 35 d after planting, was efficient at reducing the potato plant height by 20%, as compared to the control plants (Araújo *et al*., 2019).

According to Fletcher *et al*. (2000), the reduction in plant height induced by PBZ is linked to the inhibition of the conversion of ent-kaurene to ent-kaurenoic acid, resulting in a reduction in gibberellic acid levels, with a consequent decrease in the elongation rate and cell division. López *et al*. (2011) observed that spraying the growth regulators paclobutrazol and uniconazole on potato leaves was more effective in reducing the shoot growth of the potato plants that the cycocell growth regulator. Fagan *et al*. (2019) attributed the effects to the different modes of action of the compounds in gibberellin biosynthesis. Trinexapac-ethyl is an acylcyclohexanoedione, which in poaceae species, such as wheat and rye, causes a reduction in the length of internodes and, consequently, in plant height. However, in dicotyledons, this inhibition is not so strong, showing the selectivity to trinexapac-ethyl in species of the Poaceae family (Rademacher, 2000).

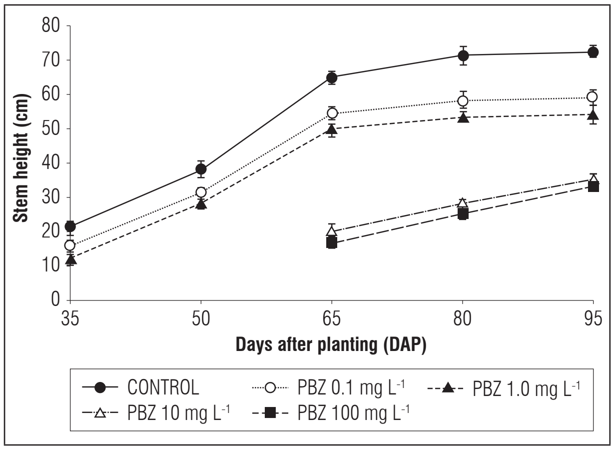

Figure 1. **Length of longest stem of plants from potato seeds treated with paclobutrazol (PBZ) in the days following planting. The vertical bars represent the standard error of the mean.**

The treatments with PBZ doses significantly decreased the number of tubers per plant when compared to the control (Tab. 2). The average of the PBZ 10 and 100 mg L^{-1} treatments was 5 tubers per plant, while, for the control, it was 7.33. Results obtained by other authors also verified that PBZ, when applied in the form of foliar spraying, reduced the number of tubers of plants (Kianmehr *et al*., 2012; Mabvongwe *et al*., 2016).

The treatments with 1.0, 10 and 100 mg L^{-1} of PBZ significantly reduced the fresh and dry mass of the tubers per plant, and the total production of the tubers was 39.2, 73.2 and 79.0% when compared to the control, respectively (Tab. 2). There was a significant reduction in the specific gravity in the tubers with 10 and 100 mg L^{-1} of PBZ, as compared to the control and the remaining treatments (Tab. 2). The PBZ treatments also delayed the emergence of plants and the beginning of tuberization. At the final harvest, at 110 DAP, the tubers with 0 and 100 mg L^{-1} of PBZ were still in the initial filling stage, which led to a reduction in the specific gravity, as compared to the other treatments.

Regardless of the treatment, the predominant soluble sugars present in the tubers was non-reducing. In all treatments, there was a decrease in the non-reducing sugars (NRS) content over time (Fig. 3C). At the end of the experiment (110 DAP), the treatments with the two lower doses of PBZ did not differ from the control.

The reducing sugars (RS) content decreased during the evaluation period for all treatments, except for PBZ 0.1 mg L^{-1}, where, at 85 DAP, an increase was observed, followed by a subsequent decrease (Figure 3B). The two higher PBZ doses presented higher levels of RS at 95 and 110 DAP, which can be explained by the delay in tuberization and consequent maturation of the tubers. In the final harvest (110 DAP), the tubers in the treatments with 10 and 100 mg L^{-1} of PBZ presented a high content of reducing sugars, with 0.2 and 0.18%, respectively (Fig. 3C).

A high accumulation of reducing sugars leads to a reaction with free amino acids, called the Maillard reaction or non-enzymatic browning (Low *et al*., 1989), resulting in products with brown coloration. After frying for 3 min at 180°C, the French fries developed an excessive brown color (data not shown) when the seed tubers were treated with 10 and 100 mg L^{-1} of PBZ.

The tuber starch content at 80 DAP decreased when treated with 0.1 and 1.0 mg L^{-1} of PBZ, followed by an increase until the end of the experiment (Fig. 3D). This decline may have occurred because of a

Table 2. **Number of tubers, fresh matter mass, dry matter mass, total yield and specific gravity of potato tubers as influenced by applications of paclobutrazol (PBZ).**

PBZ doses	Tubers number	Tuber fresh mass (g/plant)	Tuber dry mass (g/plant)	Total yield (Mg ha^{-1})	Specific gravity (g cm^{-3})
Control	7.33	654.16	104.40	23.54	1.0581
0.1	6.00*	622.93^{NS}	90.83^{NS}	22.42^{NS}	1.0564^{NS}
1.0	5.67*	397.94*	58.01*	14.32*	1.0560^{NS}
10	5.00*	175.62*	25.33*	6.32*	1.0540*
100	5.00*	137.44*	19.61*	4.95*	1.0534*
CV (%)	9.5	8.5	9.2	13.6	0.9

^{NS} non-significant; * significant ($P \leq 0.05$) with the control by the Dunnett test.

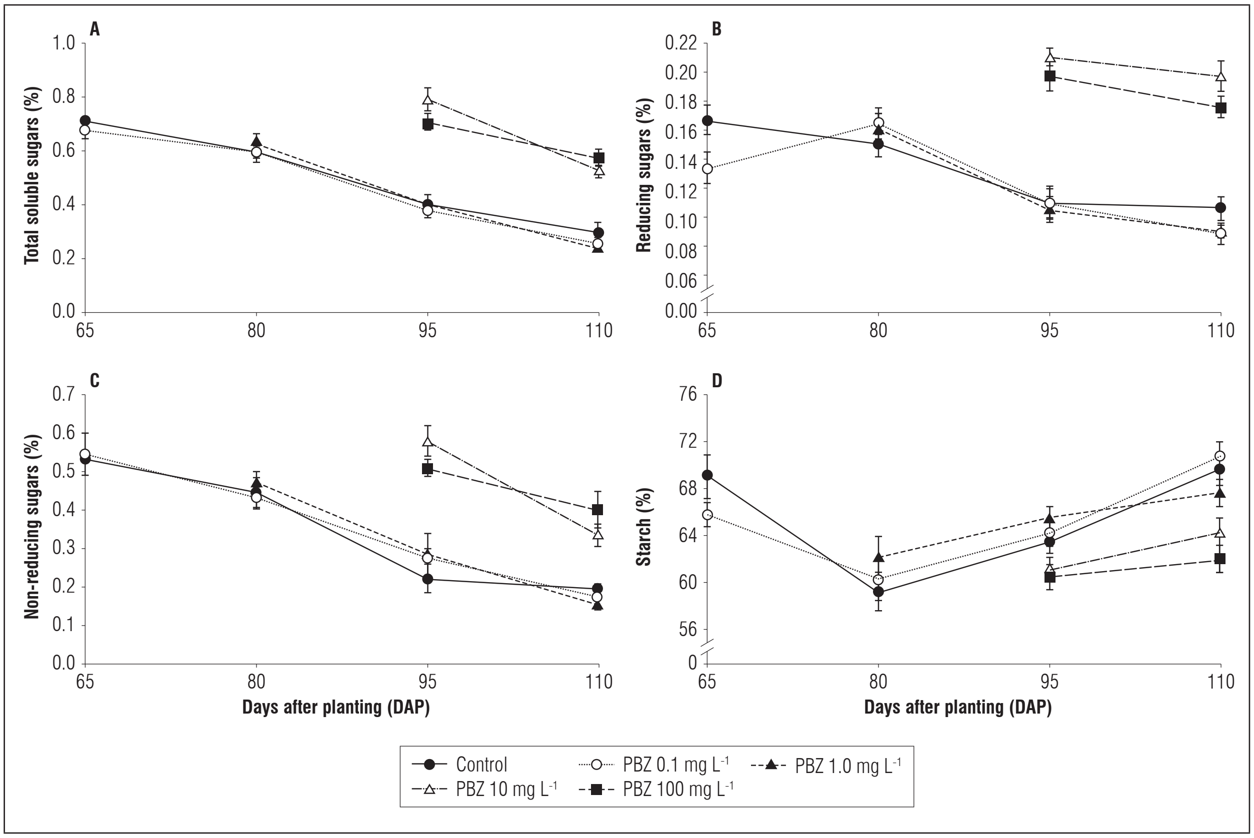

Figure 3. The content of total soluble sugars, reducing sugars, non-reducing sugars and starch in tubers of potato seed plants treated with paclobutrazol (PBZ) (A, B, C and D), respectively, according to the days after planting. The vertical bars represent the standard error of the mean.

momentary dilution of the starch content as the result of the increase in the water content of the tubers since, on the date of this evaluation, a high volume of water was reported in the soil because of heavy rains.

At the final harvest, the two higher PBZ doses had a lower starch content (Fig. 3C), indicating delayed tuber development caused by 10 and 100 mg L^{-1} of PBZ. The reduction in the total soluble carbohydrate content during the cycle coincided with the increase in the starch content, indicating the beginning of tuber maturation. Lewis *et al*. (1994) also observed reduction in total sugar levels in developing tubers, indicating an increase in the rate of starch synthesis.

CONCLUSIONS

The two higher doses (100 and 10 mg L^{-1}) of PBZ delayed shoot emergence and the beginning of tuberization. The treatments of seed potatoes with PBZ at 0.1 mg L^{-1} resulted in smaller, more compact plants, which could be suitable for more densely planted cultivation in order to maximize plant population and increase economic return per unit of land.

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Conflict of interest: this manuscript was prepared and reviewed with the participation of all authors, who declare that there exists no conflict of interest that that puts in risk the validity of the presented results.

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