Revista Brasileira de Ciência, Tecnologia e Inovação

DOI: https://doi.org/ 10.18554/rbcti.v7i1.5841

Research paper

Vegetative and reproductive development of *Bixa orellana* L. based on its propagation

Desenvolvimento vegetativo e reprodutivo de Bixa orellana L. em função da propagação

[1] Everardes Públio Júnior; [2] Tiyoko Nair Hojo Rebouças; [3] Abel Rebouças São José; *[4] Ana Paula Prado Barreto Públio; *[5] John Silva Porto

[1] Professor at the Instituto Federal de Educação, Ciência e Tecnologia da Bahia, Bahia, Brazil. Orcid: https://orcid.org/0000-0003-1626-0555 E-mail: juniorpublio@hotmail.com

* Universidade Estadual do Sudoeste da Bahia, Bahia, Brazil.

[2]Orcid: https://orcid.org/0000-0002-9341-9577 E-mail: tiyoko@uesb.edu.br

[3] Emeritus Professor from Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista, Bahia, Brazil. Orcid: https://orcid.org/0000-0002-3179-243X E-mail: abeljose3@gmail.com

[4] Orcid: https://orcid.org/0000-0003-0017-0256 E-mail: agrobarret@hotmail.com

[5] Orcid: https://orcid.org/0000-0003-4065-7210 E-mail: jsporto87@yahoo.com.br

ABSTRACT: *Bixa orellana* L., also known as achiote, is a shrub native to tropical regions. It has been arousing the interest of the industrial sector due to the film that covers its seeds, which is rich in a reddish carotenoid called bixin. Although, traditionally, it is sexually propagated through seeds, this process can also take place in a vegetative manner. Given the economic and social relevance of this species, the aim of the current study is to assess both the vegetative and reproductive development of achiote plants, based on their propagation process, to help finding the best association between plant growth and early production. The experiment was conducted in a farm, in Porto Seguro City – Bahia State. Treatments were based on two genotypes (A and B), which were multiplied through asexual (plant cutting) and sexual (seeds) reproduction. The experimental design was based on randomized blocks, at 2x2 factorial arrangement, with 5 repetitions and 7 useful plants per plot. The following parameters were assessed at 59, 154, 284, 383, 497, 656 and 840 days after planting: incidence of flowers and/or capsules, stem diameter, plant height and canopy diameter. Collected data were compared to each other through Tukey's test, at 5% probability level. Asexually propagated treatments were the first to start the reproduction stage, whereas sexually propagated treatments recorded higher vegetative development rate; no difference in vegetative development and in reproductive stage onset was observed between the investigated genotypes.

Keywords: Achiote, cuttings, genetic improvement.

RESUMO: *O urucueiro é um arbusto, originário de regiões tropicais, que vem despertando interesse da indústria devido à presença de uma película que recobre suas sementes, rica em um carotenoide avermelhado denominado bixina. Tradicionalmente é propagado por via sexual, através de sementes, entretanto, sua propagação pode ser também realizada por via vegetativa. Visando a importância econômica e social desta cultura, neste estudo objetivou-se avaliar o desenvolvimento vegetativo e reprodutivo de plantas de urucueiros em função da propagação, em busca da melhor relação entre crescimento e precocidade de produção. O experimento foi conduzido em uma fazenda no município de Porto Seguro-BA. Os tratamentos foram formados por dois genótipos A e B, os quais foram multiplicados via assexuada (estaquia) e sexuada (sementes). O delineamento foi em blocos ao acaso em esquema fatorial 2x2, com 5 repetições e 7 plantas úteis por parcela. As avaliações foram realizadas aos 59, 154, 284, 383, 497, 656 e 840 dias após plantio, onde foi avaliada a presença de flores e/ou cápsulas, diâmetro do colo, altura das plantas e diâmetro da copa. Os dados obtidos foram comparados pelo teste de Tukey a 5% de probabilidade. Os tratamentos propagados assexuadamente entraram em estádio de reprodução primeiro, enquanto os tratamentos propagados sexuadamente apresentaram maior desenvolvimento vegetativo. Não foi observado diferença no desenvolvimento vegetativo e início do estádio reprodutivo entre os genótipos trabalhados.*

Palavras-chave*: Urucum, estacas, melhoramento genético.*

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INTRODUCTION

Achiote plant (*Bixa orellana* L.) is a shrub whose mean height ranges from 2.0 m to 4.0 m, on average, although it can reach 6.0 m in height, depending on its growing conditions. It presents short woody stem measuring from 20 cm to 30 cm in diameter, as well as dark gray bark with lenticels arranged in vertical rows (REVILLA, 2001). Its seeds are coated with a reddish carotenoid called bixin, which is a natural widely used dye, mainly by the food industry (NISHA; SIRIL; NAIR, 2012).

Individuals' pursuit of healthier diets, low production cost and low toxicity level are aspects turning achiote plants into an interesting alternative to replace several synthetic dyes (FABRI; TERAMOTO, 2015). It holds the second position in the economic-importance ranking among natural dyes (SIRIL; JOSEPH, 2013).

Because achiote is a cross-pollinated plant, it does not show consistent yield in plantations; thus, vegetative propagation is a viable method to help solving this issue (JOSEPH; SIRIL; NAIR, 2011; PECH-HOIL et al., 2017). Commercial plantations can be based on the use of high-yield vegetatively-propagated plants (LOMBELLO; PINTO-MAGLIO, 2014; SIRIL; JOSEPH, 2013); however, high-yield clonal plantations are a viable alternative to those of seminal origin.

These genotypes should present satisfactory yield and pigment content results (MANTOVANI et al., 2013); however, *B. orellana* plantations are traditionally established, mostly based on seed-propagated plants (JOSEPH; SIRIL; NAIR, 2010; KALA et al., 2015), a fact that likely leads to varying yield and quality rates (SILVA et al., 2019).

Growth variables have been used to assess the behavior of seedlings deriving from forest species, since they enable describing plants' morpho-physiological conditions at different time intervals to monitor their growth (BARBIERI et al., 2011). In addition, they are quite accurate in the process to assess this species' growth and to measure the contribution of different physiological processes to plant behavior planta (BENINCASA, 2003). These variables can also provide information about plants' growth efficiency and ability to adapt to environmental conditions in places they grown in (PEIXOTO; PEIXOTO, 2009).

According to Mantovani et al. (2010), few studies available in the literature provide information about aspects involved in achiote plants' vegetative propagation. The aforementioned information plays key role in species' clonal propagation and genetic improvement processes. Thus, the aim of the current study was to assess the vegetative and reproductive development of achiote plants, based on their propagation, to help finding the best association between plant growth and early production.

MATERIAL AND METHODS

The experiment was conducted at *Sempre Viva* Farm, Porto Seguro City – Bahia State, from March 2017 to July 2019. The experimental site is located at the following geographic coordinates: latitude 16° 23' South and longitude 39° 20' West; altitude 141-m, in the Atlantic Forest biome.

Based on the updated Köppen classification system, the climate in the region is classified as AF – i.e., humid tropical climate area with non-defined dry season. The aforementioned region presents mean relative air humidity of 84.8%, mean annual temperature of 23.3°C and rainfall index of approximately 1,260 mm.

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The soil in the experimental site was classified as dystrophic red yellow argisol of sandy clay loam textural class (SANTANA, 2006). Moreover, its 0-40cm layer presented the following chemical analysis results: pH ranging from 5.5 to 5.3 and base saturation rate (V) ranging from 42% to 47% (**Table 1**).

Table 1. Chemical analysis of the soil in the experimental site, Porto Seguro, BA, 2017

Depth (cm)	pH	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+	Na^+	S.B.	t	T	V	m
	H_2O	mg/dm³	$Cmol_c/dm^3$ of soil									%	
01-20	5.5	1	0.15	2.1	1.2	0.1	4.6	-	3.5	3,6	8.2	42	3
20-40	5.8	1	0.15	2.7	1.2	0.1	3,0	-	4.0	4.1	7.1	57	2

Note: P and K was used Mehlich Extractor; for Ca, Mg and Al, it was used (KCl 1N); and for H + Al was used (0.01M $CaCl_2$ and SMP).

The adopted experimental design was based on randomized blocks, at 2 x 2 factorial arrangement, with 5 repetitions and 7 useful plants per plot. Treatments comprised 2 genotypes (A and B) and 2 propagation forms (sexual and asexual).

Plants used in the experiments were previously selected from a 7-and-a-half-year-old commercial plantation of cultivar “Embrapa 37”. Two plants showing production attributes superior to the others and bixin production higher than 220 g plant^{-1} (122.22 kg ha^{-1}) - called genotypes A and B - were herein selected, as well as asexually (cuttings) and sexually (seeds) multiplied.

Cuttings collected from mother plants were planted in 280 cm³ tubes filled with commercial substrate, on the same day. Then, they were taken to greenhouse for approximately 30 days. After this time was over, they were placed in full-sun nursery until they were ready to be planted.

Seeds were sown in 1,570-cm³ polyethylene bags filled with substrate comprising subsoil and 5 kg of N-P-K formula (4-14-8) for each m³ of soil. Then, they were taken to the nursery and covered with polyethylene screen - whose mesh allowed only 50% of light to pass through - until planting time. Seedlings, at the age of approximately 6 months, were planted in 0.40 x 0.40 x 0.40 m holes, at 6 x 3 m spacing.

Botanical assessments were performed at 59, 154, 284, 383, 497, 656 and 840 days after planting (DAP), when the following parameters were analyzed: incidence of flowers and/or fruits, collar diameter at 20 cm from the ground (with the aid of digital caliper), plant height (with the aid of graduated ruler), and canopy diameter in the North-South and East-West axes, in order to calculate the canopy area.

Collected data were subjected to variance normality and homogeneity tests. Next, analysis of variance (ANOVA) was carried out, based on using the F test; means were compared to each other through Tukey test, in AgroEstat Software, at 5% probability level (BARBOSA; MALDONADO JÚNIOR, 2010).

RESULTS AND DISCUSSION

Based on the analysis of variance results, factor “genotype” did not show difference in “plant height” in any of the performed measurements; However, this result was not observed for propagation, until 284 days after planting (DAP) (**Table 2**).

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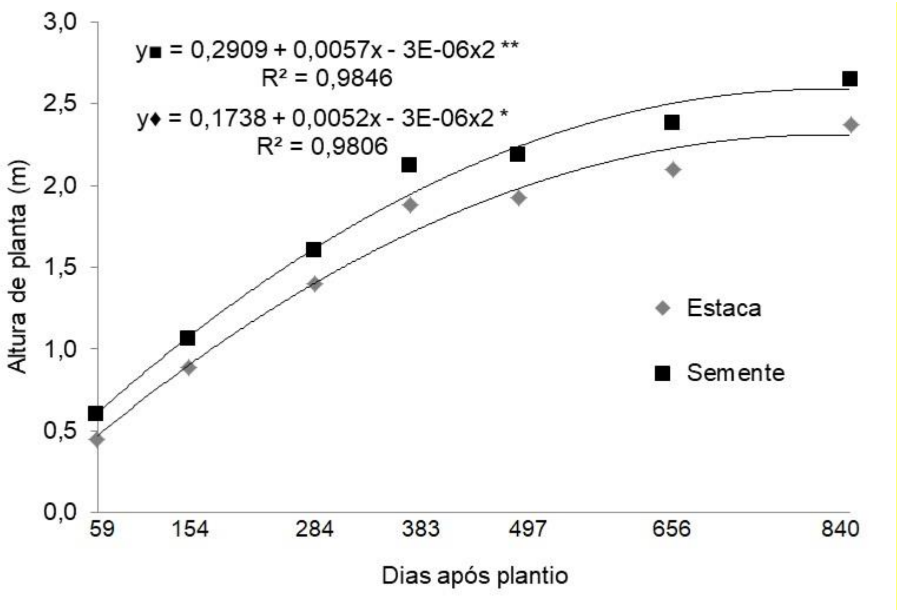
Table 2. Mean squares and coefficients of variation for plant height of annatto genotypes A and B, propagated by cuttings and seeds. Porto Seguro – BA, 2019

FV	Mean squares						
	59 DAP	154 DAP	284 DAP	383 DAP	497 DAP	656 DAP	840 DAP
Genotype (F1)	0.001 ns	0.005 ns	0.066 ns	0.156 ns	0.076 ns	0.048 ns	0.035 ns
Propagation (F2)	0.107 **	0.147 *	0.214 ns	0.267 *	0.35 *	0.395 **	0.375 *
Interaction F1 X F2	0.002 ns	0.016 ns	0 ns	0.041 ns	0.021 ns	0.001 ns	0.014 ns
CV (%)	7.39	16.97	18.1	10.5	9.62	8.67	9.73

Note: **Significance level of 1%, by the F-test. *Significance level of 5%, by the F-test. ns Non-significant.

Seedlings propagated via seed recorded higher development rate than seedlings propagated by cuttings (**Figure 1**). This condition can be attributed to the precocity of cloned plants, given the physiological maturity of cells deriving from adult tissues. It is so, because this process induces plants to activate their physiological differentiation process and to start their reproductive process early, at juvenile stage, a fact that delays plants' vegetative development.

Figure 1. Plant height of genotypes A and B of annatto plants, propagated by cuttings and seeds. Porto Seguro – BA, 2019



Note: **Significance level of 1%, *Significance level of 5%.

It is worth pinpointing that flowers and, consequently, production take place at the tip of the branches; therefore, plants lacking well-defined canopy and showing few branches may record lower yield. In addition, plant growth comprises an initial slow growth stage that, subsequently, turns itself into an exponential growth stage; Later on, plants present linear

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growth and, finally, experience a new slow growth stage that eventually stops this process (PEIXOTO; PEIXOTO, 2009).

Plant height data were adjusted to the second-degree polynomial equation, which indicated constant plant growth within the analyzed periods, although the last measurements have evidenced reduced growth rate. Smaller growth was observed between the 4th and 5th measurements, likely because plants were at production stage and allocated their reserves and photoassimilates for fruit and seed production purposes.

Plants belonging to cultivar “Embrapa 37” are often medium-sized and measure approximately 1.54 m in height (FRANCO et al., 2008). These values are lower than the ones observed in the study, according to which, plants propagated by cuttings and seeds were 2.4 m and 2.6 m in height, respectively. There was statistically significant difference in plant height between the two groups. This result is likely associated with factors such as environmental conditions and culture management. Variable “plant height” can have direct influence on increased production rates; however, higher plants can impair manual-harvesting operations.

Factor “propagation type” resulted in statistical significant difference in collar diameter between groups. However, this difference was only observed in the first three measurement periods, namely: 59, 154 and 284 DAP (**Table 3**).

Table 3. Mean squares and coefficients of variation for plant collar diameter of genotypes A and B of annatto trees, propagated by cuttings and seeds. Porto Seguro - BA, 2019

FV	Mean Squares						
	59 DAP	154 DAP	284 DAP	383 DAP	497 DAP	656 DAP	840 DAP
Genotype (F1)	0.022 ns	0.017 ns	0.046 ns	41.36 ns	3.497 ns	44.95 ns	58.59 ns
Propagation (F2)	0.243 **	0.888 *	1.38 *	58.55 ns	23.514 ns	6.684 ns	5.973 ns
Interaction F1 X F2	0.01 ns	0.33 ns	0.171 ns	15.53 ns	18.564 ns	4.603 ns	29.7 ns
CV (%)	10.70	20.05	16.98	11.67	9.00	13.66	11.02

Note: **Significance level of 1%, by the F-test. *Significance level of 5%, by the F-test. ns Non-significant.

Seed-propagated plants presented larger collar diameter than that observed for cutting-propagated plants, at early development stage (**Figure 2**). This finding suggests that seminal plants used leaf photoassimilates to grow non-photosynthetic tissues at early development stage, whereas cutting-propagated plants used these photoassimilates to produce other tissues, such as reproduction structures or even leaves.

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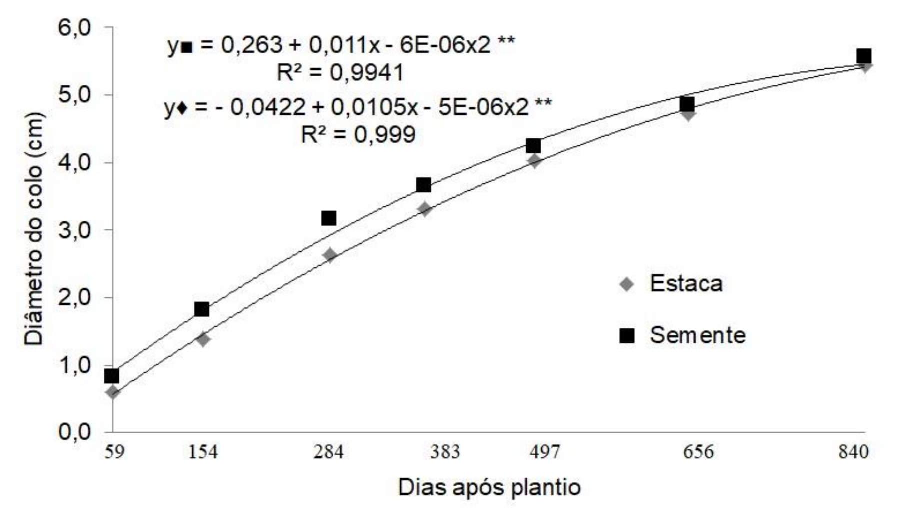
Figure 2. Collar diameter of annatto genotypes A and B, propagated by cuttings and seeds. Porto Seguro – BA, 2019.



Note: **Significance level of 1%, *Significance level of 5%.

Factor “genotype” only induced significant difference in canopy area at 383 DAP, whereas factor “propagation” induced significant different in this parameter at all measurement times, except for 284 DAP (**Table 4**), when significant interaction observed between these factors has indicated that, at this development stage, genotype “A” recorded larger canopy area in sexually-propagated plants (**Table 5**).

Table 4. Mean squares and coefficients of variation for canopy area of plants of annatto genotypes A and B, propagated by cuttings and seeds. Porto Seguro - BA, 2019

FV	Mean Squares						
	59 DAP	154 DAP	284 DAP	383 DAP	497 DAP	656 DAP	840 DAP
Genotype (F1)	0.001 ns	0.023 ns	1.052 ns	1.77 *	1.32 ns	2.388 ns	2.258 ns
Propagation (F2)	0.009 **	0.214 **	1.235 ns	1.849 *	1.957 *	3.325 *	7.565 *
Interaction F1 X F2	0.001 ns	0.072 ns	1.589 *	1.39 ns	0.38 ns	0.127 ns	1.362 ns
CV (%)	25.74	36.15	31.18	20.86	21.34	16.12	15.28

Note: **Significance level of 1%, by the F-test. *Significance level of 5%, by the F-test. ns Non-significant.

Table 5. Results of comparing averages for crown area (m^2), from the interaction between genotypes and forms of propagation of annatto trees at 284 DAP. Porto Seguro - BA, 2019

Genotype	Propagation	
	Cuttings	Seeds
A	1.5520 aB	2.6120 aA
B	1.6560 aA	1.5900 bA

Note: Values followed by the same letter, lowercase in the column and uppercase in the line, do not differ statistically from each other, according to Tukey's test, at the 5% probability level.

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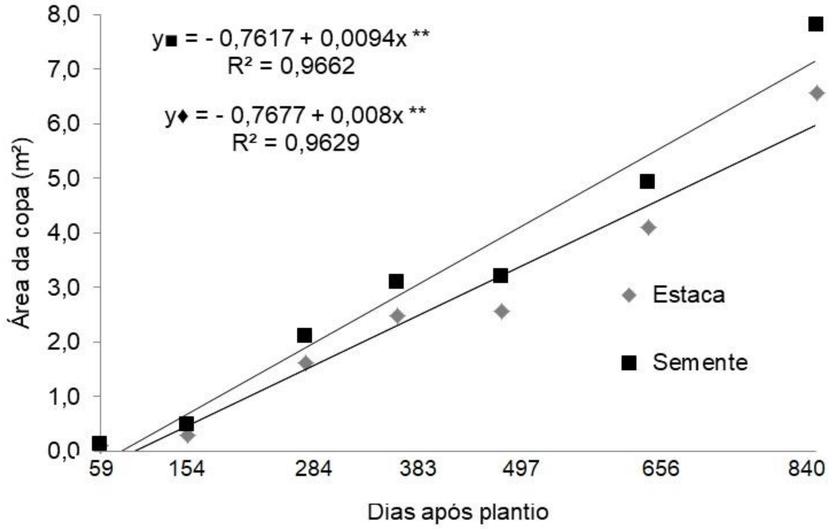
Regression analysis has evidenced linear and increasing adjustment (**Figure 3**). Seed-propagated plants recorded higher canopy growth than the one observed for asexually propagated plants.

Figure 1. Canopy area of annatto genotypes A and B, propagated by cuttings and seeds. Porto Seguro - BA, 2019



Note: **Significance level of 1%, *Significance level of 5%.

Cultivar “Embrapa 37” presents compact and hemispherical canopy tending to present lateral growth and branches close to the ground (FRANCO et al., 2008). These features were also observed in the current study.

Canopy volume is a feature of paramount importance, since larger leaf area leads to higher photoassimilate production. In addition, it can have direct influence on plant yield, since inflorescence takes place in the form of a panicle in the terminal part of branches.

The analysis applied to the incidence of flowers and/or fruits has evidenced the precocity of asexually propagated plants, since 25.72% of them presented flowers in the first measurement procedure (**Figure 4**), whereas seed-propagated plants did not present flowers at that time.

Incidence of flowers and/or fruits was not observed for any of the investigated treatments, at the second measurement time (154 DAP). It happened because the measurement was taken before flowering in the culture region. Harvests in this region take place in February and March, whereas flower blooming happens approximately 120 days before this period-of-time.

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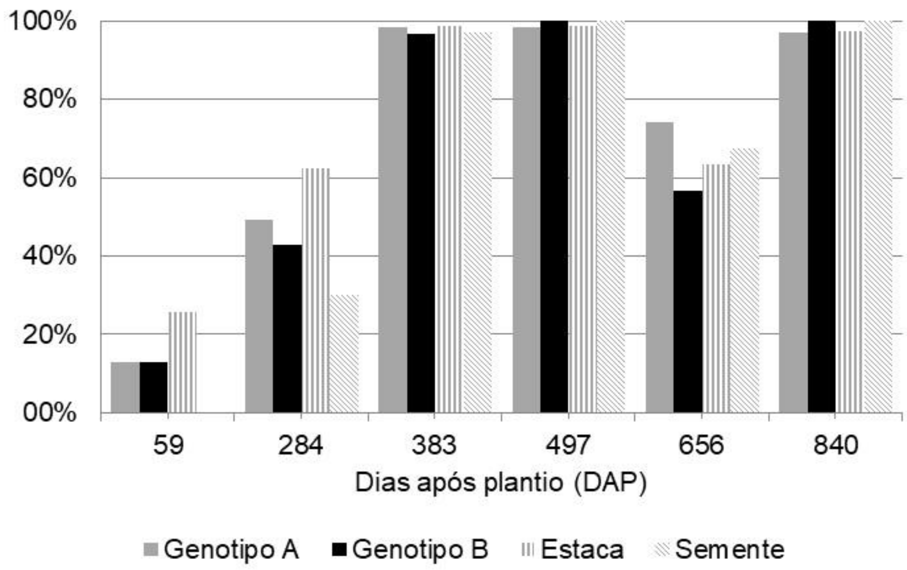
Figure 4. Percentage of flowering /fruits plants of annatto genotypes A and B, propagated by cuttings and seeds. Porto Seguro – BA, 2019



Only 30% of sexually propagated plants presented flowers and/or fruits at the 3rd measurement time (284 DAP), whereas more than 62% of asexually propagated plants presented them at this very same time. This outcome has indicated that cutting-propagated plants used their reserves to form reproduction structures and to onset the reproduction stage; consequently, they delayed their vegetative development process, as observed through variables, such as plant height, stem diameter and canopy area. The highest difference between the investigated genotypes was observed at the 6th measurement time (656 DAP), when 74% of genotype "A" plants presented flowers and/or fruits in comparison to 57% of genotype "B" plants. This difference may be associated with the combination of environmental and genetic factors.

According to Campos et al. (2012), vegetatively propagated plants have already experienced the juvenile stage. Thus, they are capable of growing shoots, leaves and flowers right after transplantation, which is one of the advantages of vegetative propagation.

São José et al. (1992) observed precocity in asexually propagated achiote seedlings, when cutting-propagated seedlings presented floral differentiation at developmental stage in plastic bags. This factor indicated early production in these plants in comparison to that of the sexually propagated ones. According to the aforementioned authors, vegetative propagation is a way to circumvent the juvenility of several cultivated plants.

The cutting-based propagation of achiote plants enables selecting high-yield and fast-growing cultivars capable of flowering early, at large quantities, and of producing their first commercial crop within 2 years (under special growing conditions), as well as of bearing fruits within 1 year after planting (KALA et al., 2015; KALA; KUMARAN, 2015). This condition was also observed in the current study. Differences observed in plant development may follow this trend until their full development; they tend to stabilize after four years, when adult plants are pruned to enable the emergence of new branches.

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CONCLUSION

Asexually propagated seedlings show precocity and anticipate their reproduction stage in comparison to seed-propagated seedlings. Seed-propagated seedlings present longer vegetative development time. There was no difference in plant growth and reproduction stage onset between the investigated genotypes.

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Received on: 2021/09/27
Approved on: 2023/02/13