

DIAS, NO; REBOUÇAS, TNH; SÃO JOSÉ, AR; AMARAL, CLF. 2017. Morpho-agronomic characterization and estimates of genetic parameters in annatto plant. *Horticultura Brasileira* 35: 242-246. DOI - <http://dx.doi.org/10.1590/S0102-053620170214>

Morpho-agronomic characterization and estimates of genetic parameters in annatto plant

Nilma O Dias¹; Tiyyoko NH Rebouças¹; Abel R São José¹; Cláudio LF Amaral²

¹Universidade Estadual do Sudoeste da Bahia (UESB), Vitória da Conquista-BA, Brasil; nodias@gmail.com; tiyyoko@uesb.edu.br; abeljose3@gmail.com; ²Universidade Estadual do Sudoeste da Bahia (UESB), Jequié-BA, Brasil; materdidatic@gmail.com

ABSTRACT

Originally from tropical America, annatto is an important source of natural dyes for food, pharmaceutical and textile industries, especially for the increasing demand of this pigment substituting artificial dyes. The aim of this study was to evaluate genotypic variation of annatto cultivar Embrapa 37, aiming to generate subsidies for breeding programs as well as to help out the producers using vegetative propagation of superior genotypes. Therefore, the morphological characterization was carried out and estimates of genetic and phenotypic parameters, for production and quality of the fruits, were obtained. The results showed wide rate of genetic variability among the plants with low environmental influence on the phenotype for traits bixin content, fruit width, number of fruits per bunch and mass of 100 seeds. The authors concluded that superior genotypes can be selected through the parental performance and propagated vegetatively to form more profitable crops, as well as used in breeding programs through self-fertilization with analysis of resulting progeny.

Keywords: *Bixa orellana*, natural dyes, heritability, genetic gain.

RESUMO

Caracterização morfoagronômica e estimativas de parâmetros genéticos em urucuzeiro

Arbusto originário dos trópicos da América, o urucuzeiro representa uma importante fonte de corantes naturais para as indústrias alimentícia, farmacêutica e têxtil, especialmente, pelo aumento da demanda deste pigmento na substituição dos corantes artificiais. O objetivo deste trabalho foi avaliar variação genotípica da cultivar Embrapa 37 de urucuzeiro, visando gerar subsídios para programas de melhoramento genético, bem como, para atender aos produtores por meio da propagação vegetativa de plantas superiores. Para tanto, foi realizada a caracterização morfoagronômica e obtidas as estimativas de parâmetros genéticos e fenotípicos para características de produção e qualidade dos frutos. Os resultados demonstraram haver ampla taxa de variabilidade genética entre as plantas, com baixa influência ambiental sobre o fenótipo para as características teor de bixina, largura dos frutos, número de frutos por cacho e massa de 100 sementes. Concluiu-se que genótipos superiores podem ser selecionados pelo desempenho dos parentais e propagados vegetativamente para a formação de cultivos mais lucrativos, bem como, utilizados em programas de melhoramento genético através da autofecundação com análise de progênie resultante.

Palavras-chave: *Bixa orellana*, corante natural, herdabilidade, ganho genético.

Received on January 29, 2016; accepted on November 10, 2016

Annatto (*Bixa orellana*) is a shrub from American tropics; its dissemination over several continents is due to the broad demand as a source of natural colorant for several products such as medicine, cosmetics and, mainly, food (Silva & Franco, 2000). Moreira *et al.* (2014) highlight functional properties present in *Bixa orellana* seed and suggest that its use as natural colorant benefits human health by the high antioxidant potential.

The carotenoid denominated bixin is the pigment present in greater

concentration in the pericarp of annatto seed, comprising more than 80% of the total carotenoids; it is fat soluble, so it is extracted using some organic solvents (Rebouças & São José, 1996; Santana *et al.*, 2008). Bixin contents may vary from 1.0 to 6.0% depending on the cultivar and on the environmental conditions of each region (Rebouças & São José, 1996; Franco *et al.*, 2008).

The last decade has been characterized by breeding species, aiming higher productivity and, mainly, higher pigment content. Bixin

concentration surpassed the national average of 2.5% and productivity has already reached values superior than 1500 kg/ha (Carvalho *et al.*, 2010). These days, in order to be classified as exportation type, the grain has to present minimum bixin content of 4% (Fabri & Teramoto, 2015). Market requirements tend to become increasingly stringent with the emergence of improved cultivars.

Due to this fact, in the South Region of Bahia, cultivar Bico de Pato, which presented maximum content of 2.5%,

was substituted by cultivar Embrapa 37, developed in the State of Pará, which bixin contents were described by Poltronieri *et al.* (2001) reaching among 5 and 5.5%. Thus, the authors highlight the importance of studies for making breeding programs feasible for certain regional conditions, aiming to guarantee the annatto agribusiness in the State of Bahia.

As the reproduction is predominantly by cross-pollination and propagation carried out via seeds, annatto cultivation presents high genetic variability, especially related to the seed number per capsule and to the pigment in seed teguments (Falesi, 1987). Mantovani *et al.* (2013) highlight that high productivity clonal plantations represent a feasible alternative and they can be established by vegetative propagation of superior genotypes of annatto.

Several grafting methods were tested by Bruckner *et al.* (1991), showing the viability of vegetative propagation for this crop, with the best yields obtained by the methods: budding, cleft grafting and splice graft. According to Rebouças & São José (1996) through cutting, rooting percentual, under ideal conditions, can vary between 60 to 90%. According to Mantovani *et al.* (2010), the vegetative rescue and the production of vegetative propagules of the species can be carried out successfully through layering, using total girdling of branches, treatment with IBA (4.92 mM) and branches protected with polyethylene film.

Despite the good experimental success of methods for vegetative propagation of annatto, these techniques are not usual in commercial deployment of the crop which is carried out using seeds, so evaluation on economic viability of these methods is important.

Considering the genetic variability, the authors observed that annatto cultivars show expressive variations related to morpho-agronomic traits, such as shape, color and capsule size, number of bunches per plant, capsule number per bunch and seed number per capsules (Rebouças & São José, 1996). Agronomic characteristics have quantitative inheritance; therefore, they are governed by genes which have their expression strongly influenced by

the environment (Franco *et al.*, 2002). This fact makes it difficult to identify genotypes based only on the phenotype observed.

The difficulty in studying quantitative traits lies in the existence of the large number of genes involved and the pronounced effect of the environment (Lynch & Walsh, 1998). In this sense, the estimate of genetic parameters is very important. Comprehending these estimates allows to understand the genetic structure of the population, to infer the genetic variance of this structure (Baldissera *et al.*, 2014), to redefine breeding programs to be used, to identify the nature of action of the genes involved in controlling the quantitative traits, to define efficiently different breeding strategies in order to obtain genetic gains with the maintenance of the adequate genetic base in the population (Cruz & Carneiro, 2006).

The aim of this study was to evaluate the annatto genotypes, cultivar Embrapa 37, for the conditions of the Extreme South of the State of Bahia, through morpho-agronomic characterization of the fruits and estimates of genetic parameters for production and quality traits.

MATERIAL AND METHODS

The experiment was carried out in a commercial cultivation at Fazenda Sempre Viva, located in the municipality of Eunápolis, Bahia State, Brazil, in a commercial plantation of cultivar Embrapa 37, four years old, spacing 6.0x3.0 meters.

The plantation is located in Mata Atlantica where the predominant vegetation is regenerating dirty pasture and coppice (16°40'S, 39°34'W, altitude 141 m); the region is characterized as tropical humid, without defined dry season; the local climate is AF, according to Köppen classification.

Ten genotypes of annatto plants were evaluated which constituted the main treatments (named from 1 to 10) with five replications, consisted of 10 fruits (capsules) each totalizing 50 fruits per treatment. The genotypes were visually

selected considering fruit load, vigor and sanity of the plants, resulting in 500 plants preliminarily selected. A second selection was carried out for high bixin contents resulting in 200 plants in which the material for this study was defined, resulting in a selection index at 5%.

Morphologic characterization of the fruits was carried out considering the following traits: colors of unripe fruits (red, reddish or green); length and width (using a digital caliper to measure the higher diameter, in mm); shape in relation to length:width ratio (oval = width equal or superior to 70% of length; lanceolate = width less than 70% of length) according to the methodology described by Mantovani *et al.* (2013) and dehiscence observing the capsule opening, when the bunches were at harvesting time.

Production traits were estimated by the number of fruits per bunch, the number of seeds per fruit and the mass of 100 seeds. Evaluations were carried out after harvesting, on February 2, 2014, when the bunches presented at least 2/3 of brown color capsules, considering each cardinal point (North, South, East, West). The samples were kept in polyethylene bags and sent to Biofábrica da Universidade Estadual do Sudoeste da Bahia where the laboratorial analyses were carried out.

In order to determine the bixin content, the seeds were removed from the capsules manually, put into paper envelopes and placed in a greenhouse at 40°C, for 48 h, until they reach 10% humidity. Four samples of the plants were used and analyzed using KOH method described by Yabiku & Takahashi (1991).

Statistical analysis was carried out through the analysis of variance and comparing the averages using the statistical software SISVAR v. 5.3 (Ferreira, 2011). Estimates of genetic and phenotypic parameters were determined by the methodology presented by Sunday *et al.* (2007) and Oyiga & Uguru (2010) using the following formula:

Genotypic Variability:

$$V_g = \frac{MSg - MSe}{r}$$

Phenotypic Variability:

$$V_p = \frac{MSg}{r}$$

Environmental Variability:

$$V_e = MSe$$

Where: V_g = genotypic variance; V_p = phenotypic variance; V_e = environmental variance; MSg = mean squares of the genotype; Mse = mean square error and r = number of replications.

Coefficient of Genotypic Variation:

$$GCV = \frac{\sqrt{V_g}}{\bar{X}} \times 100$$

Coefficient of Phenotypic Variation:

$$PCV = \frac{\sqrt{V_p}}{\bar{X}} \times 100$$

Coefficient of Environmental Variation:

$$ECV = \frac{\sqrt{V_e}}{\bar{X}} \times 100$$

Where: GCV , PCV and ECV = coefficient of phenotypic variation, genotypic and environmental, respectively and \bar{X} overall average of each treatment.

Heritability:

$$h^2 = \frac{V_g}{V_p}$$

Where: V_g = genotypic variability and V_p = phenotypic variability.

Genetic Gain:

$$G = i \Delta p h^2$$

Where: i = constant (which corresponds to 2.06 when the selection intensity is 5%); Δp = standard deviation of phenotypic variance and h^2 = heritability.

RESULTS AND DISCUSSION

According to the morphologic characterization, the evaluated genotypes produce simple, dry, capsuled, loculicidal, bivalve, polyspermic, unilocular and dehiscent fruits. Mantovani *et al.* (2013) describe the same attributes for fruits produced from the artificial cross between the varieties “Fruto verde piloso” and “Fruto vermelho liso”, with exception for dehiscence.

The authors observed from green to red colors for unripe fruits, becoming

uniformly brown when mature, with pile; these characteristics were also described by Poltronieri *et al.* (2001) and Franco *et al.* (2008) for cultivar Embrapa 37.

The average performance for morpho-agronomic traits for the fruits is in Table 1. The authors noticed significant difference for fruit length (CF), fruit width (LF), number of fruits per bunch (NFC) and mass of 100 seeds (MCS); no statistical difference for number of seeds per fruit (NSF) was

observed.

The data obtained for CF and LF were within the interval from 3.81 to 4.96 cm and from 3.02 to 3.52 cm respectively, which demonstrate variations among the genotypes evaluated in relation to the morphology of the fruits. Mendes *et al.* (2005), studying growth and ripening of annatto variety “Fruto Vermelho Piloso” found maximum values of 5.85 cm length and 3.46 cm width.

The genotypes 1, 3, 6, 7, 8 and 10 presented oval fruits and the other

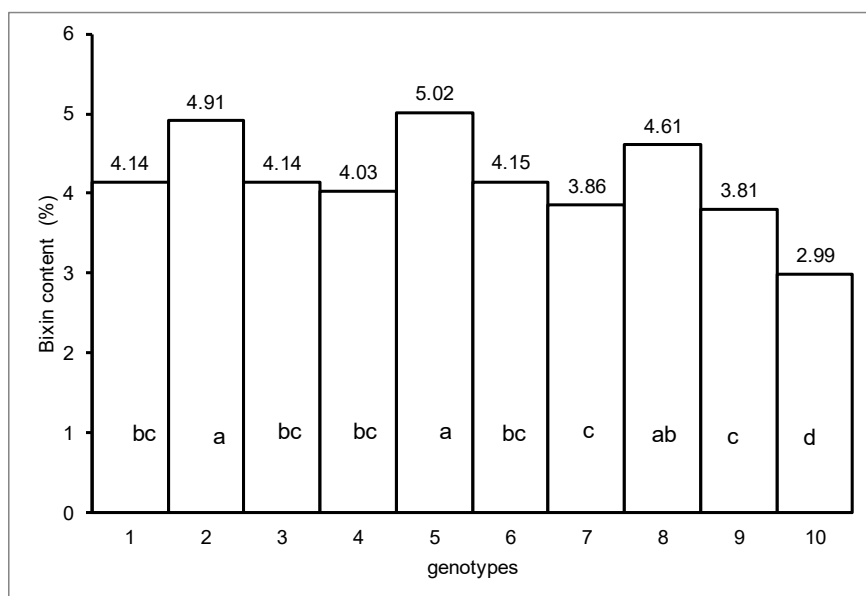


Figure 1. Bixin content in seeds of 10 annatto genotypes. Averages followed by the same letter do not differ by Tukey test at 5% of probability. Vitória da Conquista, UESB, 2014.

Table 1. CF= fruit length, LF= fruit width, NFC= number of fruits per bunch, NSF= number of seeds per fruit and MCS= mass of 100 seeds of 10 genotypes of annatto. Vitória da Conquista, UESB, 2014.

Genotype	CF (cm)	LF (cm)	NFC	NSF	MCS (g)
1	4.65 b	3.43 a	9.8 b	35.6 a	2.64 e
2	4.78 b	3.12 c	22.2 a	42.2 a	2.60 e
3	3.81 d	3.28 b	15.6 b	36.4 a	3.22 b
4	4.96 a	3.32 b	15.4 b	39.8 a	2.78 d
5	4.93 a	3.02 c	17.0 b	37.4 a	2.82 d
6	4.31 c	3.29 b	21.2 a	36.8 a	3.14 c
7	4.69 b	3.52 a	12.6 b	36.2 a	3.42 a
8	4.68 b	3.39 b	15.6 b	43.6 a	2.88 d
9	4.53 b	3.09 c	13.6 b	39.4 a	3.00 c
10	4.24 c	3.50 a	12.0 b	34.4 a	3.00 c
CV(%)	3.06	3.00	28.64	20.6	4.81

Averages followed by the same letter in the column do not differ by Scott-Knott test at 5% probability (medias seguidas de mesma letra na coluna não diferem, Scott-Knott, 5%).

Table 2. TB= estimates of genetic parameters for bixin content, CF= fruit length, LF= fruit width, NFC= number of fruits per bunch and MCS= mass of 100 seeds of 10 genotypes of annatto. Vitória da Conquista, UESB, 2014.

Parameters	TB (%)	CF (cm)	LF (cm)	NFC	MCS (g)
Vp	0.34	111.39	26.29	15.16	0.07
Vg	0.19	95.79	18.95	11.22	0.06
Ve	0.15	15.02	7.84	3.94	0.004
CVP (%)	14.69	23.15	15.70	25.12	8.39
CVG (%)	10.55	21.47	13.21	21.61	8.52
CVE (%)	18.69	8.50	8.50	12.81	2.15
h ² (%)	56.00	86.00	70.00	74.00	100.00
G	0.68	18.70	7.54	5.93	0.53
G (%)	16.26	41.02	22.88	38.28	18.10

Vp= phenotypic variance, Vg= genotypic variance, Ve= environmental variance, CVP (%)= phenotypic coefficient of variation, CVG (%)= coefficient of phenotypic variation, CVE (%)= coefficient of environmental variation, h² (%)= broad-sense heritability, G= genetic gain, G (%)= genetic gain based on the average.

genotypes presented lanceolate-shaped fruits. Mantovani *et al.* (2013) classified capsules presenting similar shapes from an artificial cross between the varieties “Fruto verde piloso” and “Fruto vermelho liso”.

The number of fruits per bunch (NFC) varied between 9.8 and 22.2. For this trait, the data found showed a wide variability forming a superior group constituted by genotypes 2 and 6. In the conditions studied, the averages were above to the ones described by Poltronieri *et al.* (2001) for cultivar Embrapa 37 which was nine fruits per bunch. Different agronomic traits for the same cultivar can be attributed to edaphoclimatic factors, cultural management and their interactions.

The values found for mass of 100 seeds (MCS) were from 2.64 to 3.42 g, being higher than the ones found by Santana (2006) for genotypes of variety Bico de Pato (2.1 to 2.7 g). Seeds showing higher mass are the most desirable for the product quality.

In Figure 1, the results for bixin contents which range from 2.99 (genotype 10) to 5.02% (genotype 5) can be seen. The averages of genotypes 2, 5 and 8 did not differ significantly among them, considering that the genotype 2 and 5 were superior to the others. Moreira *et al.* (2014) found 4.83% for contents for the same cultivar studied (Embrapa 37), in the same region

(municipality of Eunápolis). Poltronieri *et al.* (2001) report that “Embrapa 37” has contents from 5 to 5.5%. The oscillations of levels of bixin for the same cultivar can be attributed to the cultural management, environmental conditions such as humidity, luminosity and temperature and genetic factors.

According to the traits studied, the authors could observe that the commercial crops of cultivar Embrapa 37 have enough genetic variability to allow identification of superior plant-array for producing seeds and bixin contents. These plants can play an important role in crosses, aiming to contribute to breeding programs, as well as to vegetative propagation tests, in order to increase crop productivity in the short run.

The estimates of genetic parameters for traits evaluated in this study are in Table 2. An effective estimate of genetic parameters is a fundamental basis for the success of any breeding program (Farias Neto *et al.*, 2013).

Estimated values of variance related to effect of the genotype (Vg) were higher than the estimated values of the environment (Ve) for all traits evaluated. The authors noticed, as a consequence, high estimate of broad-sense heritability (h²) for the same traits. According to Stansfield (1974), heritability values higher than 50% are considered high.

The high estimate of h² can anticipate

that it is easy to select the plants, since phenotype reflects genotype, it means, environment has little influence on the desirable trait (Burton & DeVane, 1953; Allard, 1960). According to Falconer & McKay (1996), the closer to 100% for heritability, the higher the safety in selecting superior genotypes.

The authors observed low estimates of genetic gain (G) for all traits, except for fruit length (CF). G indicates the type of genetic action involved in the character expression: when it is high, G (≥ 20) indicates additive genetic action and low G (0-10) indicates non-additive genetic action (Johanson *et al.*, 1955; Singh & Narayanar, 1993). Heritability should be evaluated together with the genetic gain for a safe breeding program. Low genetic gain and high heritability indicate a non-additive genetic action (Johanson *et al.*, 1955; Eid, 2009).

For CF, the genetic gain was intermediate with a tendency to be high, in this case, since heritability value was high (86%) the authors could consider an additive genetic action for this trait, therefore, the selection could be carried out considering the F₁ generation.

The results obtained allow the authors to notice a genotypic variation rate for morpho-agronomic traits among plants of cultivar Embrapa 37, with high heritability and non-additive genetic inheritance for bixin content, fruit width (LF), number of fruits per bunch and mass of 100 seeds.

ACKNOWLEDGEMENTS

To FAPESB by Doctorate scholarship granted to the first author.

REFERENCES

- ALLARD, RW. 1960. *Principles of plant breeding*. New York: Wiley. 485p.
- BALDISSERA, JNC; VALENTINI, G; COAN, MMD; GUIDOLIN, AF; COIMBRA, JLM. 2014. Fatores genéticos relacionados com a herança em populações de plantas autógamas. *Revista de Ciências Agroveterinárias* 13: 181-189.
- BRUCKNER, CH; KHOURI, SS; MELGAÇO, AV. 1991. Propagação do urucueiro (*Bixa orellana* L.) por meio de cinco modalidades de

- enxertia. *Revista Ceres* 32: 340-344.
- BURTON, GW; DEVANE, EH. 1953. Estimating heritability in Tall Fescue (*Festuca arundinacea*) from replicated clonal material. *Agronomy Journal* 45: 487-488.
- CARVALHO, PRN; SILVA, MG; FABRI, EG; TAVARES, PER; MARTINS, ALM.; SPATTI, LR. 2010. Concentração de bixina e lipídeos em sementes de urucum da coleção do Instituto Agrônomo (IAC). *Bragantia* 69: 519-524.
- CRUZ, CD; CARNEIRO, PSC. 2006. *Modelos biométricos aplicados ao melhoramento genético*. Viçosa: UFV. 586p.
- EID, MH. 2009. Estimation of heritability and genetic advance of yield traits in wheat (*Triticum aestivum* L.) under drought conditions. *International Journal of Genetics and Molecular Biology* 1: 115-120.
- FABRI, EG; TERAMOTO, JRS. 2015. Urucum: fonte de corantes naturais. *Horticultura Brasileira* 33: 1 (artigo de capa).
- FALCONER, DS; MACKAY, TFC. 1996. *Introduction to quantitative genetics*. New York: Longman. 464p.
- FALESI, IC. 1987. *Urucuzeiro: Recomendações básicas para o cultivo*. Belém: Embrapa-UEPAE. 27p.
- FARIAS NETO, JT; CLEMENT, CR; RESENDE, MDV. 2013. Estimativas de parâmetros genéticos e ganho de seleção para produção de frutos em progênes de polinização aberta de pupunheira no Estado do Pará, Brasil. *Bragantia* 72: 122-126.
- FERREIRA, DF. 2011. Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia* 35: 1039-1042.
- FRANCO, CFO; FABRI, EG; BARREIRO NETO, M; MANFIOLLI, MH; HARDER, MNC; RUCKER, NCA. 2008. *Urucum: Sistemas de Produção para o Brasil*. João Pessoa: Emepa, APTA. 112p.
- FRANCO, CFO; SILVA, FCP; CASE FILHO, J; NETO, MB; SÃO JOSÉ, AR; REBOUÇAS, TNH; FONTINELLI, ISC. 2002. *Urucuzeiro: Agronegócio de Corantes Naturais*. João Pessoa: Emepa. 120p.
- JOHANSON, HW; ROBINSON, HF; COMSTOCK, RE. 1955. Estimates of genetic and environmental variability in Soybean. *Agronomy Journal* 47:314-318.
- LYNCH, MC; WALSH, B. 1998. *Genetics and analysis of quantitative traits*. Sunderland: Sinauer Associates Inc. 980p.
- MANTOVANI, NC; GRANDO, MF; XAVIER, A; OTONI, WC. 2010. Resgate vegetativo por alporquia de genótipos adultos de urucum (*Bixa orellana* L.). *Ciência Florestal* 20: 405-412.
- MANTOVANI, NC; GRANDO, MF; XAVIER, A; OTONI, WC. 2013. Avaliação de genótipos de urucum (*Bixa Orellana* L.) por meio da caracterização morfológica de frutos, produtividade de sementes e teor de bixina. *Ciência Florestal* 23: 355-362.
- MARMITT, S; PIROTTA, LV; STÜLP, S. 2010. Aplicação de fotólise direta e UV/H₂O₂ a efluente sintético contendo diferentes corantes alimentícios. *Química Nova* 33: 384-388.
- MENDES, AMS; FIGUEIREDO, AF; SILVA, JF. 2005. Crescimento e maturação dos frutos e sementes de urucum. *Revista Brasileira de Sementes* 27: 25-34.
- MOREIRA, VS; REBOUÇAS, TNH; MORAES, MOB; SÃO JOSÉ, AR; SILVA, MV. 2014. Atividade antioxidante de urucum (*Bixa orellana* L.) in natura e encapsulado. *Revista Iberoamericana de Tecnología Postcosecha* 15: 201-209.
- OYIGA, BC; UGURU, MI. 2010. Genetic variations and contributions of some floral traits to pod yield in bambara groundnut under two cropping seasons in the derived savanna of the South-East Nigeria. *International Journal of Plant Breeding* 5: 58-63.
- POLTRONIERI, MC; MARTINS, CS; RODRIGUES, JE; COSTA, MR; NAZARÉ, RFR. 2001. *Novas Cultivares de Urucum: Embrapa 36 e Embrapa 37*. Belém: Embrapa Amazônia Oriental, 21p. (Circular Técnica 22).
- REBOUÇAS, TNH; SÃO JOSÉ, AR. 1996. *A cultura do urucum: práticas de cultivo e comercialização*. Vitória da Conquista: UESB. 42p.
- SANTANA, KC. 2006. *Seleção de genótipos de urucueiros (Bixa orellana L.) da variedade Bico de Pato no Estado da Bahia*. Vitória da Conquista: UESB. 63p. (Dissertação mestrado).
- SANTANA, KC; GUEDES, PA; REBOUÇAS, TNH; SÃO JOSÉ, AR; LEMOS, OL; VILA, MTR; SOUZA, MJL. 2008. Teores de bixina em urucum (*Bixa orellana*) 'Piave Vermelha', em diferentes acondicionamentos e temperaturas. *Tecnologia & Ciência Agropecuária* 2: 19-22.
- SÃO JOSÉ, AR; REBOUÇAS, TNH; SOUSA, PJS; SOUZA, IVB. 1992. Seleção de urucueiros (*Bixa orellana*) superiores do tipo cultivado bico de pato na região de Vitória da Conquista-BA. *Revista Brasileira de Corantes Naturais* 1: 106-113.
- SILVA, FCP; FRANCO, CF. 2000. *Urucueiro uma alternativa de agronegócio*. João Pessoa: EMEPA. 64p.
- SINGH, P; NARAYANAN, SS. 1993. *Biometrical techniques in plant breeding*. New Delhi: Kalayani publishers. 83p.
- STANSFIELD, WD. 1974. *Genética*. São Paulo: McGraw-Hill do Brasil. 958p.
- SUNDAY, OF; AYODELE, AM; BABATUNDE, KO; OLUWOLE, AM. 2007. Genotypic and phenotypic variability for seed vigour traits and seed yield in West African Rice (*Oryza sativa* L.) genotypes. *Journal of American Science* 3: 34-41.
- YABIKU, HY; TAKAHASHI, MY. 1991. Avaliação dos métodos analíticos para determinação da bixina em grãos de urucum e suas correlações. In: SIMPÓSIO INTERNACIONAL DE URUCUM, *Anais...* Campinas: ITAL. 275-279.