

# Acerola plant selection and breeding value prediction in second selection cycle progenies

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**ABSTRACT** - Since 1996, plants were selected and genetic parameters estimated in 64 second selection cycle progenies as part of an acerola population improvement program of Embrapa Agroindústria Tropical. The variance components, genetic parameters and genetic values were obtained by the individual REML/BLUP procedure. Results indicate that plant selection based on only one fruit harvest is not advisable. The selection encompassed 39% of the progenies (selection among progenies) and 11% of the plants (selection within progenies). This led to the selection of the best 25 progenies for the traits yield, vitamin C, total soluble solids and mean fruit size. In the end, 38 plants were selected and vegetatively propagated to establish a seed orchard to supply commercial seed.

Key words: Malpighia emarginata, genetic parameters, selection index, seed orchard.

# INTRODUCTION

In spite of having been introduced into Brazil in the mid 50ies, acerola only became a crop of commercial status in the beginning of the 80ies, based on the demand in countries of Europe, Japan and United States, and more recently, of the growing domestic market. The euphoria provoked an unbalanced surge of orchards where the most varied planting techniques possible were used. The planting of seedlings obtained through seeds led to a strong segregation in the plant architecture, size, shape and vitamin C content of the fruits and in the yield (Lopes and Paiva 2002, São José and Batista 1995). Mean yields on such plantations attain between 20 and 50 kg fruits per plant:year<sup>-1</sup> (Alves and Menezes, 1995).

To meet the demands of increased productivity and fruit quality on commercial plantations implanted along the northeastern coastline, in 1996 the Embrapa Agroindústria Tropical (Tropical Agroindustry Research Center) developed actions along two lines of research: the population improvement and clonal improvement. In the first phase, plants from seed-based commercial plantations were selected, targeting the establishment of open-pollinated progenies and cloning.

An evaluation of the progenies in the first selection cycle consisted of the measurement of the yield and physical-chemical fruit evaluation to determine the vitamin C content, soluble solids content (°Brix) and mean fruit weight (MFW). The proportional gain estimated for the monthly yield, with selection among and within progenies varied from 3.68 to 44.98, respectively, for the months of June and May 1999, while for the other traits the variation in total tritratable acidity

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and pH was 28.3 to 327.2, respectively. It was expected that the following population would present an over 30% higher vitamin C content than in the plants of the first selection cycle (Paiva et al. 2002).

Acerola clone selection is the most efficient way of satisfying the immediate demand of varieties, with visible results in the short term (Paiva et al. 2003a). In 2003 Embrapa Agroindústria Tropical released four acerola clones with a view to commercial planting in the region and recommended the joint planting of the clones BRS 235 (Apodi), BRS 236 (Cereja), BRS 237 (Roxinha) and BRS 238 (Frutacor), to avoid genetic uniformity and reduce the vulnerability to pests and diseases in the orchard (Paiva et al. 2003b).

The present study had the objectives of selecting plants and estimating the magnitude of the genetic population parameters obtained by the REML/BLUP procedure in the second selection cycle of the acerola improvement program.

# MATERIAL AND METHODS

#### **Experimental area**

The experiment was installed on an area of the Experimental Field of Pacajus (Embrapa Agroindústria Tropical), in the county of Pacajus, state of Ceará (lat 4° 10' S, long 38° 27' W, alt 60 m asl). The climate type is Aw and the region belongs to the tropical rainy climate group (Aguiar et al. 2002). The soil is a sandy red-yellow podzolic TB eutrophic.

## **Plant material**

Acerola seeds were used to establish the second selection cycle progenies derived from plants selected in the first cycle, after natural plant recombination. The experiment was carried out in August 2000, in a simple 8 x 8 lattice design with 64 progenies, 2 replications and 9 plants per plot, in a 4 x 3 m spacing. The cropping practices used in the experiment consisted of foundation fertilizers, daily irrigation by means of microsprinklers with a discharge of 28 L h<sup>-1</sup>, monthly fertigation, pest control, and weeding under the canopies.

The progenies were evaluated by assessing the fruit yield (evaluated in ten harvests; eight in 2002 and two in 2003); the plant morphology (plant height and stem and canopy diameter); and physical-chemical aspects of the fruits of each progeny. The vitamin C content was determined by titrameter with a 0.02% 2,6-diclorophenolindophenol solution according to Strohecker and Henning (1967); the soluble solids content (°Brix) of the juice, using a digital Atago PR-101 refractometer, according to AOAC (1992); and the mean fruit weight (MFW).

#### **Genetic parameters**

The components of variance, genetic parameters and genetic values were estimated by the individual REML/BLUP procedure (Restricted Maximum Likelihood/Best Linear Unbiased Prediction), using software SELEGEN – REML/BLUP (Resende 2002a). The univariate model in a lattice design was used for each individual harvest and the same model and design for the repeated measurements (yield in several harvests), which considered the heritability and repeatability of the trait yield simultaneously.

The effective size was estimated by the expression

$$N_{e} = \frac{4N_{f}\overline{k}_{f}}{\overline{k}_{f} + 3 + (\sigma_{kf}^{2}/\overline{k}_{f})}$$

where:

 $\overline{k}_{f}$  = mean number of selected plants per family  $\sigma_{\nu r}^{2}$  = variance of the number of selected plants per family

The predicted genetic gain is equal to the mean of the genetic effects of selected plants (Resende 2002b).

## Selection index

The progenies were selected based on an index (Mulamba and Mock 1978), and on the total fruit yield, vitamin C content, total soluble solids content (<sup>o</sup>Brix), and mean fruit weight as reference. Firstly, the progenies were classified for each trait separately and then the sum of ranks. In the selection the sum of ranks of each progeny performance were considered for the evaluated traits. This methodology classifies the progenies in increasing order of the sum of ranks obtained in the individual evaluation, to select those that present the lowest values.

The individual genetic gains for each trait were obtained by comparing the progeny means in the first cycle with those obtained in the second cycle, as recommended by Vencovsky (1987). The genetic progress in percentage was calculated, based on the progeny mean of the first cycle.

# **RESULTS AND DISCUSSION**

#### Genetic and phenotypic parameters

Table 1 presents the genetic parameters for plant height (PH), stem diameter (SD1) and canopy diameter (CD2 and CD3) in the first, second and third year of the plants. The greatest additive variance and highest genetic variation coefficient were estimated for trait CD3. The coefficient of narrow-sense heritability varied from 19% (SD1) to 44% (PH3), indicating a higher genetic variation that can be exploited by selection at more advanced plant ages.

The genetic parameters for fruit yield in the second (PROD1 to 8) and third (PROD9 and 10) plant year and for the yield of ten harvests simultaneously, in the model of heritability and repeatability, which presents the results in mean terms per individual harvests (PROD-Combined), are displayed in Table 2. The coefficient of narrow-sense heritability varied from 1.2 to 21.7% for the fourth and sixth harvest and the individual genetic variation coefficient from 14.5 to 67.9%, respectively.

In an overall analysis involving all harvests, the individual narrow-sense heritability per harvest, free of any interaction with harvests, was 2.1%. The individual repeatability per harvest was 7.50%. These results

indicate that the plant selection based on a single fruit harvest is not recommendable. The individual heritability based on the mean of the ten harvests was 13.74%. The individual repeatability of a monthly harvest was 7.5%, so 30 monthly harvests would be needed to achieve a determination of 71%, i.e., more than two years of evaluation.

The determination coefficient of the plot effects  $(c_{plot}^2)$  indicates the proportion of the total phenotypic variation due to the variation among plots within blocks. Therefore, conclusions can be drawn on the environmental heterogeneity within blocks. In the present study, this coefficient varied from 3 to 7%, indicating a small environmental heterogeneity within blocks and efficacy of the experimental design.

# Selection and predicted genetic values

For the traits with highest genetic additive variances the estimates of the predicted values of the effective size  $\hat{N}e$  and the predicted genetic gain  $\hat{G}s$  are presented in Table 3. For canopy diameter in the second year the selection of 21 progenies, involving 65 plants, is necessary, considering the maintenance of the effective size (Ne) around 30, while for canopy diameter in the third year 26 progenies with 61 plants are necessary and, for the ninth harvest, 23 progenies with 58 plants are selected. Although the parameter means for the three traits

Genetic parameters*	PH1	PH2	PH3	SD1	CD2	CD3
$\hat{\sigma}_a^2$	0.0179	0.0236	0.0304	0.0206	0.0709	0.1178
$\hat{\sigma}_{plot}^2$	0.0029	0.0025	0.0030	0.0082	0.0117	0.0204
$\hat{\sigma}_{e}^{2}$	0.0460	0.0461	0.0322	0.0750	0.1494	0.1409
$\hat{\sigma}_{ m f}^2$	0.0692	0.0757	0.0685	0.1101	0.2394	0.2968
$h_a^2$	0.2585	0.3120	0.4445	0.1874	0.2960	0.3969
$c_{plot}^2$	0.0415	0.0327	0.0436	0.0744	0.0489	0.0686
CVig(%)	10.20	9.32	9.16	6.94	9.98	10.32
CVe (%)	7.41	5.92	5.00	6.52	6.93	6.44
General mean	1.3119	1.6503	1.9047	2.0701	2.6681	3.3248

 Table 1. Genetic parameters for plant height (PH in m), stem diameter (SD1 in millimeter) and canopy diameter (CD2 and CD3 in m) in the first, second and third year of age of the plants, in an acerola population in the second selection cycle

 $* \hat{\sigma}_a^2$  - additive genetic variance;  $\hat{\sigma}_{plot}^2$  - environmental variance among plots;  $\hat{\sigma}_e^2$  - residual variance within plots (environmental + non-

additive;  $\hat{\sigma}_{f}^2$ - individual phenotypic variance;  $h_a^2$ - individual narrow-sense heritability in the block, that is, of the additive effects;  $c_{plot}^2$ - determination coefficient of the effects of plot; CVig - individual genetic variation coefficient; CVe – experimental variation coefficient

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n the second (PROD1 to 8 in kg plant <sup>-1</sup> harvest <sup>-1</sup> ) and third (PROD9 and 10 in kg plant <sup>-1</sup> harvest <sup>-1</sup> ) year and for yield in ten harv	ty and repeatability (PROD-Comb) of plants in an acerola population of second selection cycle
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Genetic parameters*	PROD1	PROD2	PROD3	PROD4	PROD5	PROD6	PROD6 PROD7	PROD8	PROD9	PROD10	<b>PROD-Comb</b>
$\hat{\sigma}_a^2$	0.0336	0.0051	0.0035	0.0003	0.0353	0.0059	0.0027	0.0006	0.4047	0.0373	0.0088
$\hat{\sigma}^2_{plot}$	0.0159	0.0023	0600.0	0.0018	0.0062	0.0009	0.0042	0.0002	0.1437	0.0399	0.0035
$\hat{\sigma}_e^2$	0.2741	0.0330	0.1313	0.0215	0.1534	0.0199	0.0637	0.0077	1.8266	0.5662	0.3836
$\hat{\sigma}_f^2$	0.3330	0.0414	0.1450	0.0237	0.1954	0.0274	0.0714	0.0087	2.5979	0.7467	0.4147
$h_{a}^{2}$	0.1010	0.1229	0.0245	0.0116	0.1806	0.2166	0.0373	0.0721	0.1558	0.0499	0.0211
$c_{ m plot}^2$	0.0479	0.0538	0.0620	0.0756	0.0317	0.0346	0.0583	0.0183	0.0553	0.0535	0.0084
CVig(%)	48.02	49.48	24.15	14.47	63.70	67.89	32.51	46.62	37.79	45.99	25.90
CVe (%)	58.09	55.16	62.64	56.62	54.87	53.30	67.47	60.93	36.64	77.55	59.90
Repeatability	ı	ı	ı	I	I	ı	ı	ı	ı	ı	0.0750
General mean	0.3818	0.1441	0.2467	0.1145	0.2949	0.1135	0.1587	0.0537	1.6831	0.4197	0.3613

Traits	Nr. of selected progenies	Nr. of selected plants	Ne	Gs	Gs%	
CD2	21	65	29.16	0.33	12.36	
CD3	26	61	30.23	0.49	14.76	
PROD9	23	58	29.07	1.07	58.79	

did not present great differences, the estimate of the genetic gain of the new population based on the ninth harvest was somewhat superior to the others, with about 66% gain in relation to the population mean.

The genetic gain obtained with the selection of 63 plants, based on the yield of the ninth harvest only, was estimated at 44.83%, on a yield mean of 1.6831 kg plant<sup>-1</sup> harvest<sup>-1</sup> in this harvest. However, the genetic correlation of the yield in the ninth harvest with the total yield in the 10 harvests was 0.7060. Since the heritability in the ninth harvest was 15.58%, this value multiplied by 0.7060 results in 11.00%, which is lower than the individual heritability at the level of harvest means (13.74%). The selection based on all harvests simultaneously is therefore more precise and safer. This result shows that the selection based on a single measurement would be subjected to problems related to interaction effects with harvests and is therefore not recommended.

#### Plant selection based on ranking

The progeny classification for fruit yield, vitamin C content, total soluble solids and the mean fruit weight is shown in Table 4. The selection of the progenies was based on the lowest values of the sum of r, considering, simultaneously, the classification each progeny had obtained in the ranking of the evaluated traits, in other words, the progenies with best performance for the individual traits. Initially, 50 plants were selected based on the selection criterion of the 25 progenies with lowest values of the sum of r and, amongst these, the two most productive plants. With this criterion 39% of the progenies (selection among) and 11% of the plants (selection within) were selected.

Among the 25 progenies selected by the sum of r, the values of the individual r for those with superior

Table 4. Classification (r) of 64 acerola progenies for fruit yield (PROD) (sum of 10 harvests), vitamin C (Vit. C) content of green fruits, total soluble solids (TSS), and mean fruit weight (MFW)

PROGENY	PROD (kg)	r	<b>Vit</b> C (mg 100g <sup>-1</sup> )	r	TSS (°Brix)	r	MFW (g)	r	Sum of ranks
66/7	99.0	2	3,068.8	9	9.2	16	6.4	32	59
47/5	48.1	51	3,239.6	4	10.7	1	7.3	6	62
38/6	55.8	40	2,920.9	21	9.9	5	7.0	7	73
26/8	60.7	34	2,882.4	23	9.9	4	6.8	14	75
87/11	65.8	27	2,999.5	10	9.1	21	6.7	18	76
56/3	59.5	30	2,971.1	13	9.2	15	6.6	22	80
51/3	45.6	56	3,421.1	1	10.5	2	6.6	23	82
26/2	94.6	4	2,387.3	52	9.5	8	6.7	19	83
23/7	81.7	15	2,464.6	49	9.4	11	6.9	10	85
56/6	57.9	36	2,963.0	15	9.1	23	6.9	12	86
12/7	90.6	9	2,929.7	19	9.9	3	5.1	61	92
72/7	80.4	16	3,311.5	2	9.2	17	5.5	57	92
26/5	71.3	22	2,845.9	25	8.4	45	7.5	2	94
79/10	76.6	20	2,928.3	20	8.2	52	7.4	3	95
26/6	83.0	13	2,490.7	48	9.6	6	6.4	31	98
91/7	55.3	41	2,984.9	12	9.4	12	6.2	38	103
20/6	76.6	19	2,420.9	50	8.7	36	7.6	1	106
38/7	54.0	44	2,571.7	44	9.5	9	7.0	9	106
51/7	94.8	3	2,836.6	26	8.5	43	6.3	34	106
47/6	42.3	60	2,963.7	14	9.5	10	6.6	24	108
68/1	80.2	12	3,126.6	6	8.8	35	5.5	56	109
72/3	66.6	26	2,945.8	17	9.1	26	6.2	40	109
20/4	107.9	1	2,292.1	57	9.0	28	6.6	25	111
56/7	46.5	54	3,121.6	7	9.1	24	6.5	28	113
8/4	46.7	53	3,238.6	5	9.5	7	5.6	55	120
20/8	68.3	25	2,726.6	31	8.8	31	6.3	33	120
23/6	86.1	8	2,084.6	64	8.8	32	6.7	17	121
8/11	63.2	29	2,736.9	30	9.2	14	5.8	49	122
23/3	82.8	14	2,344.3	55	9.0	29	6.6	26	124
51/1	62.4	31	3,295.0	3	8.7	37	5.7	53	124
63/7	92.5	6	2,724.9	33	8.0	57	6.4	30	126
72/9	93.0	5	2,725.9	32	8.5	44	5.8	47	128
63/8	56.5	38	3,091.1	8	9.1	25	5.3	59	130
20/2	55.2	42	2,309.8	56	9.0	27	7.0	8	133
51/4	57.3	37	2,723.3	34	9.1	20	6.1	43	134
56/2	46.5	55	2,560.0	45	9.1	22	6.9	13	135
47/2	76.3	21	2,710.1	35	8.8	33	5.8	48	137
38/8	50.2	48	2,391.9	51	9.1	19	6.6	21	139
68/4	51.8	45	2,985.2	11	8.6	41	6.1	42	139
66/4	36.4	64	2,812.4	28	8.6	39	6.9	11	142
12/10	61.6	32	2,699.6	36	9.3	13	5.1	62	143
8/2	87.3	11	2,510.2	47	8.2	49	6.2	37	144
12/5	89.8	10	2,806.7	29	8.5	42	5.0	64	145
66/3	49.0	49	2,935.5	18	8.7	38	6.1	41	146
47/4	56.1	39	2,630.5	40	8.2	50	6.7	20	149
54/12	61.2	33	2,846.0	24	8.8	34	5.2	60	151

To be continued

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PROGENY	PROD (kg)	r	<b>Vit</b> C (mg 100g <sup>-1</sup> )	r	TSS (°Brix)	r	MFW (g)	r S	um of ranks
75/3	50.5	47	2,374.0	54	8.4	46	7.4	4	151
63/2	60.0	35	2,960.7	16	8.3	47	5.6	54	152
23/2	77.8	18	2,143.8	62	7.8	59	6.8	15	154
68/6	44.8	57	2,884.4	22	9.0	30	5.9	45	154
75/2	65.7	28	2,217.6	61	8.1	56	6.7	16	161
79/3	48.5	50	2,220.6	60	8.3	48	7.3	5	163
63/3	71.2	23	2,629.5	41	8.1	55	5.9	46	165
66/5	40.9	61	2,676.6	37	8.6	40	6.5	29	167
72/5	92.0	7	2,608.0	42	7.6	62	5.5	58	169
8/10	44.6	58	2,541.2	46	9.1	18	5.7	51	173
75/11	54.1	43	2,590.2	43	7.8	60	6.5	27	173
12/9	75.7	17	2,382.8	53	8.1	53	5.1	63	186
79/9	69.3	24	2,125.7	63	7.2	64	6.2	39	190
54/6	35.8	62	2,815.2	27	7.6	61	6.0	44	194
75/9	51.2	46	2,643.3	39	8.0	58	5.7	52	195
68/7	42.6	59	2,289.3	58	8.2	51	6.3	35	203
54/11	37.3	63	2,663.4	38	8.1	54	5.7	50	205
79/5	47.7	52	2,242.4	59	7.3	63	6.2	36	210

and inferior performance were 1 and 60 for fruit yield, 1 and 57 for vitamin C, 1 and 52 for total soluble solids and 1 and 61 for fruit size. The progenies that stood out in the individual trait evaluation (r = 1) were 20/4 in fruit yield, with 108 kg; 51/3, with vitamin C content of 3.421 mg/100g of pulp (evaluation in green fruits), 47/5, which obtained a soluble solids content of 10.7 °Brix, and 20/ 6 that presented a mean fruit weight of 7.6g. It is noteworthy that the use of this methodology allowed the selection of the progenies with best individual performances. Therefore, it is expected that the next generation will have a better performance than the current, considering that the concentration of genes with additive inheritance is expected to increases as well.

Thereafter, one more phenotypic selection was carried out on the field, discarding the plants that did not meet the previously established standards of vigor and canopy architecture. In the end, 38 plants were selected, corresponding to a selection intensity of 3.3% in relation to the initial population. Such a strong selection intensity in this second cycle was used to maximize the genetic gain, with a view to bringing the project to an end, due to the reduced demand for acerola seeds. The use of a higher selection intensity leads to a greater genetic progress and, consequently, to a lower level of variability in the population, which is not recommendable for selection over successive

generations (Paterniani and Miranda Filho 1987).

This methodology was preferred over the traditional selection method among and within, owing to its ease and handiness of use. Besides the efficacy of the result presented by Paiva et al. (2002), the number of selected progenies compared with the two procedures showed a level of 84% coincidence, in other words, by the index there was a coincidence of 16 progenies of the 19 selected by the selection method among and within practiced in the first cycle. Silva et al. (2003) also used the methodology of Mulamba and Mock (1978) for the selection of superior clones in a backcross program of potato with favorable results.

The 38 selected plants were then multiplied vegetatively. Later, five grafted plantlets of each plant were planted on an isolated area, arranged randomly on the field, for the formation of a garden that produces commercial seed. The plant positions on the field were determined by lot so that each genotype would have the opportunity to be pollinated by different genotypes. The implantation of the seed garden, besides providing acerola seeds for commercial plantations, had the further objective of maintaining the selected progenies, derived from the second selection recurrent cycle, which could serve as germplasm for the future continuation of acerola population improvement.

The means of the progenies in the original population and those selected in the selection cycles I

 $(PO_I \text{ and } PS_I)$  and II  $(PO_{II} \text{ and } PS_{II})$  and, the genetic gains obtained (GS%) for the traits canopy diameter, plant height, fruit yield, vitamin C content, pH, total soluble solids content and mean fruit weight are presented in Table 5. In general, the selection was favorable for lower plants, with a higher vitamin content and larger fruits. The lower productivity detected in the second cycle can be ascribed to the period of yield evaluation which, in the first cycle, included 54 harvests in 12 months, while in the second, only 10 harvests were realized distributed in the periods of high and low yield.

#### CONCLUSIONS

1. Selection based on all harvests simultaneously is more precise and safer than selection based on individual harvests.

2. Plant selection in second cycle progenies based on estimates of genetic parameters, obtained with the individual REML/BLUP procedure is easier, owing to a better discrimination of the individual genetic values of the plants.

3. The methodology of the ranking index for the selection among progenies allowed a selection of the progenies with best individual performances.

**Table 5**. Means of acerola progenies, of the original population and selected in the cycles I ( $PO_i$  and  $PS_i$ ) and II ( $PO_{ii}$  and  $PS_{ii}$ ) of selection and genetic gain performed (GS%) for the traits canopy diameter (CD in m), plant height (PH in m), fruit yield (PROD in kg plant<sup>-1</sup> harvest<sup>-1</sup>), vitamin C content (Vit. C in mg 100g<sup>-1</sup> pulp), pH, total soluble solids content (TSS in Brix) and mean fruit weight (MFW)

Progenies	CD	<b>GS</b> %	РН	<b>GS</b> %	PROD	<b>GS</b> %	Vit. C	<b>GS</b> %	pН	<b>GS</b> %	TSS	<b>GS</b> %	MFW	<b>GS</b> %
PO	3.30	100.0	2.20	100.0	0.3978	100.0	1,337.6	100.0	3.3	100.0	9.1	100.0	5.7	100.0
PSI	3.35	101.5	2.25	102.3	0.4671	117.4	1,479.4	110.6	3.3	100.0	10.6	116.5	5.5	96.5
PO	3.32	100.6	1.90	86.4	0.3613	90.8	2,718.5*	203.2	3.2	97.0	8.8	96.7	6.3	110.5
PS <sub>II</sub>	3.36	101.8	1.90	86.4	0.3869	97.3	2,893.1	216.3	3.2	97.0	9.3	102.2	6.6	115.8

\* analysis of fruits in the nearly ripe stage, before complete maturation. The vitamin C content in these fruits is around 41% higher than in mature fruits, according to Moura et al. (2003)

# Seleção de plantas e predição de valores genéticos em progênies de aceroleira de segundo ciclo

**RESUMO** – No desenvolvimento do programa de melhoramento populacional da aceroleira na Embrapa Agroindústria Tropical, iniciado em 1996, foram realizadas seleção de plantas e estimação de parâmetros genéticos em 64 progênies de segundo ciclo. Os componentes de variância, parâmetros genéticos e valores genéticos foram estimados pelo procedimento *REML/BLUP* individual. Os resultados indicam que a seleção de plantas com base em uma única colheita de frutos não é aconselhável. Adotando-se o critério de selecionar 25 progênies que se destacaram em produção, vitamina C, sólidos solúveis totais e tamanho médio de fruto, foram selecionadas 39% das progênies (seleção entre) e 11% das plantas (seleção dentro). No final, foram selecionadas 38 plantas que, a seguir, foram multiplicadas vegetativamente, visando a formação de um jardim para o fornecimento de sementes comerciais.

Palavras-chave: Malpighia emarginata, parâmetros genéticos, índice de seleção; jardim de semente.

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