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Responses of sour passion fruit (*Passiflora edulis* Sims) seeds from the third recurrent selection cycle during storage

Respuestas al periodo de almacenamiento de semillas de maracuyá-agrio (*Passiflora edulis* Sims) del tercer ciclo de selección recurrente

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Abstract

Conflicting responses have been found for the period of conservation of passion fruit seeds. Therefore, this study aimed to evaluate the response of the seeds of passion fruit progenies to a 24-month storage period. The sour passion fruit progenies from the third cycle of Universidade Estadual do Norte Fluminense Darcy Ribeiro - UENF intrapopulation recurrent selection program were grown in Itaocara- RJ and the progenies seeds were evaluated for: germination potential, germination first count, accelerated aging, first count of accelerated aging test, seedling and radicle size and germination speed index. The germination and vigor of the seeds of the full-sib progenies were assessed by analysis of variance and factorial arrangement with 3 progenies and 7 periods of storage and the averages were compared by Tukey test at 5% probability. Progenies 1 and 2 responded better to germination (92% for both progeny) and vigor test of accelerated aging (92% for both progeny), rootlet size (6.4 and 5.8 cm, respectively) and germination speed index (2.91 and 2.85, respectively) during storage. Variations were observed during the storage period in mean of progenies for germination (81-93%) and vigor for all traits, except accelerated aging (84-91%). At eight months of storage, the seeds presented increased vigor; at 16 months, they showed reduced vigor. The passion fruit seeds of the progenies under analysis can be stored for up to 24 months with high and uniform germination rate and no loss in vigor. The seeds from the passion fruit breeding program showed increases in the variables of germination and vigor during the selection process for fruit production variables, proving the efficiency of the breeding program.

Key words: Breeding program, germination, longevity, progeny, vigor, *Passiflora edulis* Sims, sour passion fruit.

Resumen

El largo del periodo de conservación de semillas ocasiona cambios variales y significativos en el desarollo de plantas de maracuya-agrio. En este trabajo se evaluó la respuesta de semillas de progenies de este cultivo a un periodo de almacenamiento de 24 meses. Progenies del tercer ciclo del Programa de selección recurrente por intrapoblación de la Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF fueron cultivadas en Itaocara-RJ y las semillas producidas fueron evaluadas en relación con la capacidad de germinación, primer recuento de prueba de germinación, envejecimiento acelerado, primer recuento del envejecimiento acelerado, tamaño de plántulas y de raíz e índice de celeridad de germinación. Para ello, las semillas de las progenies de hermanos completos fueron evaluadas por germinación y vigor en un arreglo factorial con 3 progenies y 7 periodos de almacenamiento, donde las medias fueron comparadas por la prueba Tukey (P = 0.05). Las progenies 1 y 2 presentaron las mejores respuestas para germinación (92%) y para vigor de envejecimiento acelerado (92%), tamaño de radícula (6.4 y 5.8 cm, respectivamente), índice de celeridad de germinación (2.91 y 2.85, respectivamente) durante el almacenamiento hasta 24 meses. Se encontraron variaciones en germinación (81-93%) y vigor para todas las características, pero no para envejecimiento acelerado (84-91%). El mayor vigor de plántulas se observó en semillas con 8 meses de almacenamiento y el menor, con semillas de 16 meses de almacenadas. Se concluye que, las semillas de maracuyá de las progenies evaluadas pueden ser almacenadas hasta 24 meses, con altas de germinación uniforme y sin pérdida de vigor; las semillas provenientes del programa de mejoramiento del maracuyáreo mostraron incrementos en las variables de germinación y vigor en el proceso de selección para producción de frutos, lo que confirma la eficiencia de este programa.

Palabras clave: Programa de mejoramiento, germinación, longevidad, progenie, vigor, *Passiflora edulis* Sims, maracuyá agrio.

Introduction

The genus *Passiflora* has more than 400 species, being more than 140 native to Brazil. The sour passion fruit (*Passiflora edulis* Sims) originates from the tropical regions of South America (Bernacci et al., 2008) is a multifunctional species whose fruit is of great relevance among the tropical fruit crops. Plantations of this culture help maintaining work force in rural areas, which is a strong social role of this culture (Pires et al., 2011).

The seeds of sour passion fruit are considered orthodox (Posada et al., 2014) and can be stored, since the most widely used alternative of passion fruit dispersal is sexual. However, for some authors, its seed germination is low, irregular and of fast decay. Another problem in the passion fruit production chain is the lack of quality seed commercial suppliers (Alexandre et al., 2004; Freitas, 2009; Oliveira et al.; 2012).

The conservation of seeds by storage is carried out in a place other than their natural habitat and has been performed since agriculture started to be expanded, together with the maintenance of samples of the genetic heritage, because of their importance for the conservation of cultivated crop species and their wild parents (González-Arnao and Engelman, 2013).

The maintenance of seed vigor during storage makes seed available throughout the year for producers, which allows them to produce the fruit to the market in the off season, when better prices can be obtained (de Lima et al., 2019).

The UENF passion fruit breeding program has been developed since 1998 and has assessed the genetic diversity among commercial genotypes of passion fruit and others species of genus *Passiflora* (Ribeiro et al., 2019), estimated the genetic parameters for passion fruit traits and the genetic progress for crop yield traits with the selection of full-sib families, through the recombination of the selected progenies and the registration of the new variety of passion fruit at the Ministry of Agriculture in 2015, the UENF Rio Dourado (Gonçalves et al., 2007; Gonçalves et al., 2009; Silva et al., 2009; Ferreira et al., 2016; Silva et al., 2014). This study aimed to assess how the seeds of passion fruit progenies from the UENF breeding program responded to storage, even these variables were not taken into account in the selection process in a block design with a factorial arrangement with three progenies and seven storage periods.

Material and methods

The seeds were extracted from three full-sib progenies of passion fruit (112x42, 117x19 and 46x14) of the third cycle of the intra-population recurrent selection program developed by the Universidade Estadual do Norte Fluminense Darcy Ribeiro - UENF, implemented in the Unidade Experimental Ilha Barra do Pomba, in Itaocara – RJ, in 2013.

Yellow and yellow-greenish fruits found on the ground were harvested (Negreiros et al., 2006). Each treatment was individually and the seeds were extracted by friction in steel mesh sieve under running water and dried on paper towels for 48 h, at room temperature. Seed moisture was determined by the oven with air circulation, according to Rule for Seed Analysis, with two replicates for each treatment, for 24 h, at 105 ± 3 °C (Brasil, 2009).

The seeds of the three progenies were assessed immediately after being extracted and dried. The remaining seeds were packed in paper bag, stored in the refrigerator, and assessed every four months until the storage completed 24 months. The following variables were measured: (1) Germination test (G), four replications of 50 seeds were placed in three germination paper sheets, wetted with distilled water twice the amount of the weight of the substrate and subjected to alternating temperatures of 20-30 °C in germination chamber. Normal seedlings were recorded at 28 days in percentage (Brasil, 2009); (2) First count of the germination test (FC), evaluated together with the germination test, with record of normal seedlings in percentage at 14 days after the start of the test (Krzyzanowski et al., 1999, adapted from Brasil, 2009); (3) Accelerated aging (AA), the seeds were placed on aluminum screen in mini-chambers represented by a Gerbox containing 40 mL of water and kept for 48 hours at 40 °C (Larré et al., 2007). Then, the same methodology applied for the germination test was carried out and normal seedlings in percentage were recorded at 28 days (Brasil, 2009); (4) First count of the accelerated aging testing (FCA), evaluated together with the accelerated aging test with the record of normal seedlings in percentage at 14 days after the start of the test; (5) Seedling size (SS), implemented in conjunction with the germination test where the 10 seedlings located in the top row of the paper roll were measured in centimeters at the end of test (Negreiros et al., 2008); (6) Rootlet size (RS), implemented in conjunction with the germination test where the rootlets of 10 seedlings located in the top row of the roller were measured in centimeters at the end of the test (Negreiros et al., 2008); and (7) Germination speed index (GSI), performed along with the germination test, with the record of the number of normal seedlings every four days (adapted from Maguire, 1962).

The variables were subjected to analysis of variance, arranged in a factorial design with 3 progenies (112x42, 117x19 and 46x14) and

7 periods of storage (0, 4, 8, 12, 16, 20 and 24 months after harvest), according to the following model (Equation 1):

$$Y_{ijk} = b_j + g_i + a_k + ga_{ik} + e_{ijk} \qquad \textbf{Eq. 1}$$

where, *bj* = effect of block *j*; *gi* = effect of progeny *i*; *ak* = effect of storage *k*; *gaik* = effect of the interaction between progeny and storage *ik*; *eijk* = experimental error. The degrees of freedom of the effects of the factors as well as the interaction were unfolded via regression analysis, considering a coefficient of determination of the models above 60%. When this value was not obtained, the means were compared via Tukey test at 5% probability with the aid of the Assistat program, version 7.7 Beta (Silva, 2014).

Results and discussion

The average moisture content of the three progenies assessed immediately after drying was 10%. Fonseca and Silva (2005) found that values of 7-17% of water content helped maintaining the viability of sour passion fruit seeds, regardless of the storage temperature. Catunda et al. (2003) found that the water content of 10% in passion fruit seeds was better than 8% for storing seeds in a permeable container, since they present superior vigor at ten months of storage.

We decided to present the variables of the trend of the points observed by the average test, since the values of the coefficients of determination (R^2) were too low for regression analysis, even in a 4th-order polynomial model.

The analysis of variance showed a significant difference between the means of the progenies for G, AA, RS and GSI. For the averages of the period, it was significant for G, FC, FCA, SS, RS and GSI (Table 1).

The lowest coefficients of variation were for variables G and AA, with values of 4.76 and 6.67%, respectively, comparing the best data dispersion. The variables SS, RS and GSI obtained intermediate values (14.14, 13.82 and 10.37%). The variables FC and FCA obtained the highest values (35.46 and 23.81%) showing that the data set was not as homogeneous, since these vigor tests are more variable than the average (Table 1).

In the progeny interaction with the storage period there was significant difference for FC, RS and GSI (Table 1). Table 2 shows that even though there was a significant difference by the analysis of variance, the means test showed no difference between the three progenies, proving the efficiency of the breeding program in adding favorable characteristics to the seeds of all evaluated progenies.

Table 1. Factorial analysis of variance of the results obtained from the evaluation of the physiological traits of the seeds. Germination (G), first count of germination (FC), first count of the accelerated aging testing (FCA), accelerated aging test (AA), seedling size (SS), rootlet size (RS) and speed germination index (GSI) of three sour passion fruit (*Passiflora edulis* Sims) progenies for seven periods of storage, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, 2015.

FV	DF	Mean Square						
		G	FC	FCA	AA	SS	RS	GSI
Block	1	0.9803	32.8896	770.0006	91.2779	42.9020	8.3963	0.0007
Progeny (Pr)	2	377.4621 **	481.0994 ^{ns}	493.3378 ^{ns}	673.0013 **	6.8866 ^{ns}	2.5820 *	0.6340 **
Storage (St)	6	87.9402 **	3162.7739 **	4972.5798 **	44.7037 ^{ns}	34.5226 **	11.8474 **	1.3580 **
Pr x St	12	9.7851 ^{ns}	54.3100 **	310.8619 ^{ns}	15.6643 ^{ns}	0.9546 ^{ns}	0.2158 *	0.0078 **
Error	20	17.9387	286.5955	198.6201	34.0168	2.5756	0.6660	0.0819
Total	41	—	—	—	—	—	—	—
CV%		4.76	35.46	23.81	6.67	14.14	13.82	10.37

** Significant at 1% probability; * Significant at 5% probability; ^{ns} non significant.

Table 2. Physiological traits of the seeds of three sour passion fruit (*Passiflora edulis* Sims) progenies during storage. First count of germination (FC); rootlet size (RS); and germination speed index (GSI), Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, 2015.

Storage (months)	FC (%)			RS (cm)			GSI		
	112x42	117x19	46x14	112x42	117x19	46x14	112x42	117x19	46x14
0	52aAB*	58aAB	51aAB	6.6aAB	6.6aAB	6.9aAB	2.97aAB	3.11aAB	2.90aAB
4	40aBC	42aBC	43aBC	3.9aD	4.1aC	3.8aC	2.36aBC	2.62aBC	2.71aBC
8	79aA	87aA	85aA	7.3aA	7.7aA	7.5aA	3.49aA	3.64aA	3.52aA
12	52aAB	66aAB	62aAB	7.3aA	7.3aA	7.6aA	2.83aAB	3.07aAB	2.89aAB
16	9aC	11aC	13aC	4.6aCD	4.4aC	4.3aC	1.99aC	2.09aC	2.09aC
20	28aBC	33aBC	35aBC	5.2aBCD	5.3aBC	5.3aBC	2.37aBC	2.47aBC	2.45aBC
24	48aAB	55aAB	56aAB	5.9aABC	6.3aAB	6.3aAB	2.75aB	2.85aB	2.81aB

*Means followed by the same letter do not differ statistically, lowercase in column and uppercase in row, by Tukey test at 5% probability.

Progenies 1 and 2 provided better responses for G and in vigor evaluation tests AA, RS and GSI, during storage (Table 3). Carvalho and Nakagawa (2012) refer to the longevity of the seeds, which is mainly characterized by the period in which they can live according to their genetic traits, showing the presence of genetic variability for these attributes. These traits were not included in the selection indices developed over the cycles for the selection of the best progenies, but the intrapopulation recurrent selection was effective (Silva and Viana, 2014).

The progenies assessed were obtained by the recurrent selection method, which is an intra-population breeding method with good results in the selection of superior genotypes, through the gradual increase in the frequency of favorable alleles and the maintenance of population genetic variability (Reis et al., 2011), as observed in the results of G, AA, RS and GSI (Table 3).

Finding genetic variation within a species enables the maintenance of heterosis and resistance to certain limiting problems of the culture, especially in the case of an allogamous plant, such as passion fruit, which requires population variability to facilitate cross pollination (Lobo, 2006). The presence of diversity by assessing the physiological seed quality is important for plant breeding, as it increases the number of variables to be evaluated for the identification of superior individuals.

Cardoso et al. (2009) evaluated the genetic divergence to estimate the genetic parameters of traits related to seed physiological quality in a papaya germplasm bank and found high divergence for the variables related to the physiological quality of seeds, which indicates that this can be exploited in breeding programs aimed at improving seed quality. Freitas (2009) and Alexandre et al. (2004) also found differences characterized by the genotype of plants while evaluating, among other variables, the germination of seeds from different half-sib progenies of passion fruit. Torres et al. (2019) showed differences between a segregating population of Pasiflora (*P. edulis* x *P. setacea*) by evaluating physiological and morphological quality traits.

The recurrent selection process increased the frequency of alleles favorable for the production traits under selection in the UENF passion fruit intra-population breeding program (Cavalcante et al., 2019; Silva et al., 2014; Silva et al., 2009) and affected the variables related to the physiological seed quality in progenies 1 and 2 (Table 3).

The variables FC, RS and GSI showed significant difference in progeny interaction and storage periods by analysis of variance (Table 1), but did not show different averages between progenies by Tukey test at 5% probability (Table 2), thus it was made the average of these three progeny in Table 4, for to better display the seven periods responses that were evaluated. Significance was observed for the periods for all variables, except AA, and the responses were presented in each storage period (Table 4).

Since significance was observed for the periods for all variables, except AA, they were grouped by the average value of the results of the variables assessed in the three progenies. The responses were presented in each storage period (Table 4).

At the end of 24 months of storage, the responses to the tests G, FC, SS and RS did not differ statistically from the initial period (zero) when the tests were assessed. The seeds of the progenies responded similarly to AA in all storage periods, and showed uniforme germination, which is proven with 84 to 91% germination vigor in this vigor test, being the lowest value found immediately after the seeds were harvested (Table 4).

Table 3. Physiological traits of the seeds of three sour passion fruit (*Passiflora edulis* Sims) progenies during storage. Germination (G); accelerated aging testing (AA); rootlet size (RS); and germination speed index (GSI), Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, 2015.

Progeny	G	AA	RS	GSI
	(%)		(cm)	
1	92a*	91a	6.4a	2.91a
2	92a	91a	5.8ab	2.85a
3	83b	79b	5.6b	2.52b

*Means followed by the same letter do not differ statistically by Tukey test at 5% probability.

Table 4. Physiological traits of the seeds of three sour passion fruit (*Passiflora edulis* Sims) progenies, according to the storage periods. Germination (G), first count of germination (FC), first count of germination of the accelerated aging testing (FCA), accelerated aging testing (AA), seedling size (SS), rootlet size (RS) and germination speed index (GSI), Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, 2015.

Storage (months)	G	FC	AA	FCA	SS	RS	GSI
	%				cm		
0	93a*	54ab	84a	51c	13.6a	6.7ab	2.99ab
4	81b	42bc	87a	61bc	9.7bc	3.9c	2.56bcd
8	92a	84a	91a	86ab	13.9a	7.5a	3.55a
12	90a	60ab	89a	89a	13.3a	7.3a	2.93bc
16	88ab	11c	85a	9d	7.7c	4.4c	2.05d
20	89ab	32bc	85a	39c	9.6bc	5.3bc	2.43cd
24	90a	53ab	90a	79ab	11.6ab	6.2ab	2.80bc

*Means followed by the same letter do not differ statistically by Tukey test at 5% probability.

Oliveira et al. (2012) observed in the storage of seeds of several passion fruit progenies, for 24 months, that 21% of them maintained their physiological quality for seedling emergence, when stored in multiwall paper bag at 5 °C, which corroborates the results obtained for germination and vigor and proves that they have no dormancy. Gross and Olsen (2010) reported that, in the species domestication process developed throughout selection, evolutionary traces are observed in the genotypes obtained, such as the loss of seed dormancy. This may have occurred with the passion fruit which had been reported several times for presenting low and disuniform germination (Alexandre et al, 2004; Freitas, 2009; Oliveira et al, 2012).

Seed storage for eight months proved beneficial to the variable GSI, since it reached higher average values and demonstrated high seed vigor for this test in this period (Table 4). Catunda et al. (2003) and Martins et al. (2005) also observed oscillations between the results of germination percentage during storage of passion fruit seeds for up to 10 months. Freitas (2009) found wide difference between the averages of the germination test, first germination count, accelerated aging and electrical conductivity for 26 half-sib progenies of passion fruit from the UENF first cycle of recurrent selection that were recombined by Gonçalves et al. (2007). The results obtained in this study, using seeds from the third cycle of recurrent selection, recombined by Ferreira et al. (2016), present increased uniform germination and vigor evaluation for the population, compared to that of the beginning of the breeding program.

Before storage, by comparing the results of germination and vigor of the seeds from the UENF passion fruit first cycle of recurrent selection, Freitas (2009) observed maximum and minimum germination of 96 and 75%, respectively; and those from the third cycle resulted in 97 e 88%. For FC, the seeds of the first cycle obtained the maximum and minimum values of 97 and 71%; and from the third cycle, 58 and 51%; for AA, the seeds of the first cycle obtained the maximum and minimum values of 91 and 47%; and those of the third cycle, 92 and 82%. Therefore, it is possible to verify that the range between the averages was reduced by the selection of genotypes, and the current population shows increased uniformity.

After 12 months of storage, the lowest and the highest germination percentages were respectively 76 and 91% for seeds from the UENF passion fruit first cycle of recurrent selection (Freitas, 2009) and 84 and 95% for seeds from the third cycle, which generates a range of 15 and 11 for each cycle.

In the first two months of storage, Lima et al. (2010) observed an increased germination percentage, for the first germination count and seed germination speed index for passion fruits purchased from the market. From that period on, a decreased germination was observed, until reaching the six months of storage. They concluded that the seeds presented postharvest dormancy, which was broken after 30 days of storage. In this study, no changes were observed in the germinating potential for the AA stress resistance test, which remained uniform throughout the 24 months of storage (Table 4).

Lots with higher number of normal seedlings in the first count are more vigorous and the higher the germination speed index, the higher the speed of germination of these seeds, which are also considered more vigorous (Krzyzanowski et al., 1999). Accordingly, the seeds have proven more vigorous in the FC, SS, RS and GSI tests after 8 months of storage and less vigorous at 16 months, although no statistical different was found between the average of other periods (Table 4).

Variation was observed in the results for FC, FCA, SS, RS and GSI during the storage periods, as evidenced by Martins et al. (2005). However, for AA, the results of this study disagree with those provided by the authors.

The assessment of stress resistance by accelerated aging testing showed high percentage of normal seedlings for the variable FCA, close to 90%, for the periods of 8 and 12 months of storage and, antagonistically, a very low percentage at sixteen months of storage, below 10%. However, even with reduced vigor, these seeds have reached a percentage of normal seedlings of 85%, which is equal to the averages of the other storage periods. The same happened with the vigor tests FC and GSI, which responded with low values at 16 months, but the germination rate reached 89% (Table 4).

The larger the seedling or its evaluated part, the more vigorous it is, because it is more capable of transforming the reserve supply from the storage tissues into meristematic tissues (Krzyzanowski et al., 1999). Thus, for SS and RS, seedlings of measured progenies were more vigorous during periods 0, 8, 12 and 24 months of storage.

In order to complement the information provided by the variables RS and SS, it is interesting to interpret the seedling size results together with the germination percentages, since vigorous seedlings coming from low percentage germinating seeds are useless. Table 4 shows that at periods 0, 8, 12 and 24 months of storage, the mean SS and RS obtained the same positive response as G.

The highest GSI and consequent increased seed vigor were observed at periods 0 and 8 and the lowest, at 4, 16 and 20 months (Table 4), according to most vigor tests performed in this study. In this sense, the passion fruit seeds in the third recurrent selection cycle bring with them the plant breeding benefits and even the germination and vigor variables were not considered in the selection, they followed the production variables with high values that were maintains up to 24 months of storage.

Conclusions

At eight months of storage, the three progenies seeds presented increased vigor; at 16 months, they showed reduced vigor. The sour passion fruit (*Passiflora edulis* Sims) seeds of the progenies under analysis can be stored for up to 24 months with high and uniform germination rate and no loss in vigor. The seeds of the three evaluated progenies from the passion fruit breeding program showed increase in the germination and vigor variables during the selection process of the fruit production variables, proving the efficiency of the breeding program.

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