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Genetic divergence for seed traits in *Passiflora* full-sib families under recurrent selection for resistance to the cowpea aphid-borne mosaic virus

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ABSTRACT: Investigating the physiological potential of seeds and genetic divergence between families is essential to identify promising materials for the generation of improved populations. This study was undertaken to examine the germination potential and estimate genetic divergence between *Passiflora* full-sib families (FSFs) based on physical and physiological seed traits. The experiment was consisted of 86 FSFs from the first cycle of recurrent selection for resistance to the cowpea aphid-borne mosaic virus. Seeds were evaluated for 19 physical and physiological traits. Dissimilarity estimates were obtained by generalized Mahalanobis distance, and the method unweighted pair group method with arithmetic mean was employed for cluster formation. The FSFs showed high germination potential and seed vigor, with mean of 92.4%, 5.68 e 5.81 cm for germination, germination speed index (GSI) and shoot length of seedlings, respectively. Cluster analysis resulted in four groups formed, being Group I composed by 68 FSF more the control *Passiflora edulis*. The trait 1,000-seed weight (TSW) was the trait that most contributed to explaining the dissimilarity between families, with 31.9%. The trait GSI (physiological), TSW (physical), as well as traits provided by the GroundEye® system (CIELab-b, hue, and circumference of seeds), were efficient in determining genetic divergence detected between the families of passion fruit. These traits can be taken as a basis in the selection of genotypes with higher physiological seed quality.

Key words: *Passiflora edulis*, digital phenotyping, germination potential, passion fruit, GroundEye® system.

INTRODUCTION

The genus *Passiflora* presents high genetic diversity, with 157 species (Bernacci et al. 2021), most originating in Brazil (Borges and Milward-de-Azevedo 2017). *Passiflora edulis* (sour passion fruit) is the most cultivated species in the world, and Brazil is the world's largest producer and consumer (IBGE 2020).

On a commercial level, passion fruit is propagated by seeds, given the ease and practicality of the process, as well as its lower production cost, when compared with other methods. It shows the importance of evaluating the contribution of its attributes in the breeding of this crop (Silva et al. 2015). Therefore, the use of genotypes with improved seed germination traits and initial seedling vigor is crucial for the formation of homogeneous and vigorous seedlings (Cremasco et al. 2021).

The occurrence of diseases, including fruit hardening virus caused by cowpea aphid-borne mosaic virus (CABMV), is one of the main factors that hinder the production and expansion of areas cultivated with the crop (Nicolini et al. 2012, Freitas et al. 2016, Lima et al. 2018).

In this scenario, the passion fruit breeding program at the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) seeks sources of resistance to CABMV to incorporate resistance genes into a passion fruit population (Santos et al. 2019, Preisigke et al. 2020a, 2020b). After crossing for the introgression of resistance genes and successive generations of backcrossing and recombinations, the program began studies with recurrent selection (Santos et al. 2015a, Santos et al. 2019, Preisigke et al. 2020a, 2020b, Vidal et al. 2021, Mendes et al. 2022). This strategy makes it possible to obtain improved materials, gradually increasing the frequency of favorable alleles without drastically reducing the genetic variability of the population (Hallauer et al. 2010).

The effect of crossing of the cv. UENF Rio Dourado with the wild species *Passiflora setacea* (with seed dormancy) and of successive generations of crossing and backcrosses on the physiological potential of the seeds was not evaluated by the breeding program developed by UENF.

Evaluating the seeds with the aim of studying the genetic divergence between genotypes and the effect of crossing and selection cycles on the germination potential of seeds is essential for the advancement of a breeding program. Thus, the present study was developed to examine physical and physiological seed traits and estimate genetic divergence between *Passiflora* full-sib families under recurrent selection for resistance to CABMV.

MATERIALS AND METHODS

Plant material and seed extraction

The seed sample from the full-sib families (FSFs) evaluated in the present study was obtained from an experiment developed by the passion fruit breeding program aimed at resistance to CABMV at UENF. The experiment was carried out at Campos dos Goytacazes (21°42’48”S and 41°20’38”W, 14 m asl), northern region of Rio de Janeiro, Brazil.

The experiment consisted of 90 FSFs of passion fruit from the first cycle of recurrent selection aiming at resistance to CABMV and two controls (*P. edulis*: cv. UENF Rio Dourado; and *P. setacea*: wild species with the CABMV-resistance allele). The initial population, which originated the 90 FSFs, derived from the crossing of different materials, namely, individuals selected from backcross population 1 (BC1) (segregating F_1 population [*P. edulis* × *P. setacea*] × *P. edulis* population [recurrent parent]) (Gonçalves et al. 2021); individuals selected from backcross population 2 (P. edulis × genotypes selected from the BC1 population) (Vidal et al. 2021); and interspecific hybrids (*P. edulis* × *P. setacea*). The 38 superior genotypes, which showed resistance to CABMV and good agronomic performance, were selected and intercrossed in pairs, originating the 90 FSFs that are being cultivated and evaluated in the first cycle of recurrent selection aiming at resistance to CABMV (Mendes et al. 2022). The 90 FSFs were evaluated in randomized blocks with three replicates and three plants per plot, totaling nine plants per family.

Physical and physiological seed quality was evaluated in a sample consisting of a mixture of seeds from the fruits of nine plants of each genotype. Ripe fruits fallen on the ground were collected and sectioned transversally. Next, the mucilaginous aril was removed manually by rubbing the seeds against a steel-mesh sieve under running water. The seeds were then placed on paper towels in trays for five days, at room temperature, in the laboratory. Subsequently, they were visually classified, separating whitish or non-uniformly colored seeds from pure seeds.

The families were evaluated for physical [1,000-seed weight, color (CIELab-a, CIELab-b, CIELab-L, hue, and brightness)]; geometric (area, maximum diameter, minimum diameter, and circumference); and physiological traits (germination percentage, abnormal seedlings, ungerminated seeds, germination speed index, seedling shoot and root lengths, seedling shoot and root dry weight, and accelerated aging).

The experiments were laid out in a completely randomized design with 88 treatments, as follows: 86 FSFs from the first cycle of recurrent selection for resistance to CABMV; and two controls, *P. edulis* and *P. setacea*.

One thousand-seed weight was determined from eight replicates of 100 seeds of each treatment, following the Rules for Seed Testing (Brasil 2009). The seeds were digitally phenotyped in the GroundEy® system, using four replicates of

50 seeds for each treatment. For this procedure, the seeds were arranged in an acrylic tray for image capture, and color and geometric data were obtained.

Physiological assessments were performed also according to the Rules for Seed Testing (Brasil 2009). Germination, germination speed index, seedling length, and seedling dry weight were evaluated in an experiment with four replicates of 50 seeds. The seeds were placed on a paper substrate moistened with water at an amount equivalent to 2.5-fold the paper weight. The rolls were then placed in transparent polyethylene bags and taken to germination chambers regulated at an alternating temperature of 30-20 °C and photoperiod adjusted to 8 a.m. and 4 p.m., respectively.

Germination was evaluated after 28 days, by counting normal and abnormal seedlings and ungerminated seeds (Brasil 2009), with results expressed in percentage terms. During the germination test period, germination speed index was determined by the formula proposed by Maguire (1962), after counting the number of seeds with 0.5 cm of root system formed every two days.

Shoot and root lengths were measured in 10 randomly selected seedlings of each replicate using a graduated ruler, with results expressed in centimeters. To determine dry matter weight, the 10 seedlings used to measure seedling length were placed (with the roots separated from the shoots) in paper envelopes and dried in a forced-air oven at a constant temperature of 65 °C for 72 hours. After the drying treatment, the samples were left to cool in desiccators and weighed, with results expressed in $mg{\cdot}seedling^{-1}$.

To evaluate accelerated aging, a new trial was conducted with four replicates of 60 seeds. The seeds were distributed on the surface of a metallic screen fixed and suspended inside a plastic germination box containing 40 mL of water, and left to stand in a germination chamber at 40 °C for 48 hours.

After this period, germination tests were set up with four replicates of 50 seeds each. Additionally, the fungicide Orthocide®500 at 0.1% concentration was applied to the seeds, which were arranged on moistened paper, to avoid possible microbial contamination. Then, the germination potential of the seeds was evaluated following the same conditions described for the germination test. The percentage of normal seedlings was determined at 28 days after the test was set up.

Statistical analysis

Data were subjected to analysis of variance with significance considered at the 5% probability level ($p \leq 0.05$) by the F test. Data analyses were performed using Genes software and the following statistical model (Eq. 1):

$$Y_{ij} = \mu + Gi + E_{ij} \tag{1}$$

in which: Y_{ij}: the observation referring to effect i in replicate j; μ: overall mean; G*i*: effect of genotype i (i = 1, 2, ..., 88); E_{ij}: experimental error.

The data matrix for cluster analysis consisted of 18 variables. The germination variable was removed from cluster analysis because most treatments showed similar results. To generate the dendrogram, generalized Mahalanobis distance (D2) (Mahalanobis 1936) was estimated. The method unweighted pair group method with arithmetic mean (UPGMA) was used to build the clusters, as it has the highest cophenetic correlation coefficient (0.84). Lastly, the Circlize package of R software was used to visualize the dendrogram.

The relative contribution of the traits was calculated using the method proposed by Singh (1981), based on the partition of the total estimates of D^2_{ii} distances, considering all possible pairs of individuals, for the part due to each trait.

RESULTS

Data analysis revealed significant differences ($p \leq 0.05$) between the FSFs for all variables analyzed (Tables 1 and 2). The experimental coefficient of variation ranged from 1.13 (1,000-seed weight) to 102.07% (abnormal seedlings)

(Tables 1 and 2). Most traits had low coefficient of variation values, especially the seed geometry variables, whose coefficient of variation was below 2% (Table 2), indicating that GroundEye® was efficient in determining the seed geometry. The high coefficient of variation values found for abnormal seedlings and ungerminated seeds (102.07 and 61.91%, respectively) can be explained by the occurrence of samples with 0% germination or absence of abnormal seedlings. Therefore, the nature of the observations is not homogeneous, which translates into a higher coefficient (Table 1).

Table 1. Estimates of mean squares of analysis of variance for nine physiological traits of seeds of *Passiflora* full-sib families under recurrent selection for resistance to cowpea aphid-borne mosaic virus. 2022.

Source of variation	DF	Mean square								
		GSI	GMN (%)	AS (%)	UG (%)	AA (%)	SL (cm)	RL (cm)	SDW (mg·seedling^{-1})	RDW (mg·seedling^{-1})
Treatments	87	3.69*	639.63*	46.17*	149.09*	813.31*	3.64*	3.29*	266.19*	93.44*
Genotypes (G)	85	2.26*	253.79*	46.69*	150.22*	547.76*	2.15*	2.11*	169.78*	80.30*
Control (C)	1	78.06*	20000*	0.00**	0.00**	11704.50*	78.38*	76.76*	4050*	364.50*
G × C	1	51.04*	14075.27*	48.18**	202.33*	12494.55*	55.91*	29.77*	4677.29*	939.51*
Residual	264	0.07	14.92	6.13	9.47	42.81	0.12	0.13	5.23	2.07
Total	351									
CV (%)		4.85	4.22	102.07	61.91	8.46	6.04	7.26	4.93	8.24
Mean Genotypes		5.68	92.43	2.48	5.08	78.23	5.81	5.05	46.96	17.71
Amplitude		3.94-7.37	59.0-100.0	0-13.5	0-32.0	33.5-98.5	4.17-7.53	3.44-6.49	32.5-64.0	8.25-30.75
Passiflora edulis		6.25	100.0	0	0	76.5	6.26	6.2	45.0	13.5
Passiflora setacea		0	0	0	0	0	0	0	0	0

DF: degrees of freedom; GSI: germination speed index; GMN: germination; AS: abnormal seedlings; UG: ungerminated seeds; AA: accelerated aging; SL: shoot length; RL: root length; SDW: shoot dry weight; RDW: root dry weight; *significant at 5% probability by the F test; **significant at 1% probability by the F test.

With respect to the physiological seed traits, the germination speed index ranged from 3.94 to 7.37 between the FSFs, averaging 5.68. The *P. edulis* control showed average index of 6.25, which was slightly higher than the average of the families (Table 1). Germination percentage ranged from 59 to 100% between the FSFs, with a mean value of 92.43%. The average percentage of abnormal seedlings and ungerminated seeds were 2.48 and 5.08%, respectively. The *P. edulis* control, i.e., the recurrent parent, showed 100% germination. After the accelerated aging test, the average germination percentage of the families was 78.23%, whereas the *P. edulis* control averaged 76.5%. Shoot and root lengths were smaller in the families (5.81 and 5.05 cm, respectively) than in *P. edulis* (6.26 and 6.20 cm, respectively). On the other hand, the families exhibited higher average shoot and root dry weights (46.96 and 17.71 mg·seedling^{-1}, respectively) than *P. edulis* (45 and 13.5 mg·seedling^{-1}, respectively) (Table 1). The *P. setacea* control did not show signs of germination.

In the analysis of physical seed traits, the FSFs differed from the *P. setacea* control, but showed similar results to those of the *P. edulis* control for color (CIELab-a, CIELab-b, CIELab-L, hue, and brightness) and geometry (area, minimum diameter, and circumference) characteristics. This was an expected finding, since *P. edulis* is the recurrent parent. The maximum diameter was the only variable whose mean of the families' (0.635 cm), ranging from 0.558 to 0.725 cm, was similar to that mean of the *P. setacea*, donor parent, which showed mean of 0,588 cm (Table 2). For 1,000-seed weight, the families averaged 23.3 g, which is close to the mean of *P. edulis* (24.6 g). This variable ranged from 16.2 to 28.3 g, whose minimum value was higher than the mean of 15.2 g of *P. setacea* control (Table 2). Genetic divergence analysis, which was based on 18 physical and physiological seed traits, resulted in the formation of four groups (Table 3, Fig. 1). Group I was composed of 69 families plus the *P. edulis* control, making it the largest group (87.5% of families). Group II was formed of two families (RS22 and RS44); group III, of 15 families; and group IV contained only the *P. setacea* control (Table 3, Fig. 1).

Table 2. Estimated mean squares of analysis of variance for ten physical traits of seeds of *Passiflora* full-sib families under recurrent selection for resistance to cowpea aphid-borne mosaic virus. 2022.

Source of variation	DF	Mean square									
		CIELab-a	CIELab-b	CIELab-L	Hue	Brightness	Area (cm^2)	Dmax (cm)	Dmin (cm)	Circ (cm)	TSW (g)
Treatments	87	2.80*	18.09*	37.81*	4307.92*	0.002*	0.001*	0.004*	0.001*	0.077*	0.278*
Genotypes (G)	85	2.86*	17.85*	37.94*	4282.90*	0.002*	0.001*	0.004*	0.001*	0.075*	0.253*
Control (C)	1	0.05**	47.73*	34.69*	9537.88*	0.001*	0.005*	0.004*	0.008*	0.123*	1.777*
G × C	1	0.25**	8.82*	29.66*	1204.15*	0.000**	0.006*	0.004*	0.014*	0.254*	0.895*
Residual	264	0.05	0.18	0.57	29.15	0.000	0.000	0.000	0.000	0.001	0.001
Total	351										
CV (%)		6.27	7.64	4.61	8.33	4.46	2.37	1.17	1.17	1.63	1.13
Mean Genotypes		3,4	5,6	16,4	64,6	0.150	0.204	0.635	0.415	1.88	23.3
Amplitude		1.8–6	0.7–11.6	10.3–25.9	23.1–168.7	0.103–0.213	0.168–0.24	0.558–0.725	0.375–0.46	1.65–2.45	16.2–28.3
Passiflora edulis		3.5	7.0	16.6	42.4	0.160	0.200	0.365	0.405	1.82	24.6
Passiflora setacea		3.7	2.1	12.4	111.5	0.135	0.150	0.588	0.340	1.57	15.2

DF: degrees of freedom; CIELab-a: shade of color in the range from green to red; CIELab-b: saturation of color in the range from blue to yellow; CIELab-L: lightness of color in the range from black to white; Hue: pure state of color; Brightness: ranges from black to white; Dmax: maximum diameter; Dmin: minimum diameter; Circ: circumference; TSW: 1,000-seed weight; *significant at 5% probability by the F test; **significant at 1% probability by the F test.

Table 3. Mean and standard deviation of physiological and physical seed variables of each of the four groups formed by unweighted pair group method with arithmetic mean of 86 *Passiflora* full-sib families under recurrent selection for resistance to cowpea aphid-borne mosaic virus and two controls (*Passiflora edulis* and *Passiflora setacea*). 2022.

Variable	Group			
	I (70)[1]	II (2)	III (15)	IV (1)
GSI	5.72 ± 0.71	5.62 ± 0.46	5.52 ± 0.94	0
AS (%)	1.56 ± 2.02	12.00 ± 0.71	5.37 ± 5.01	0
UG (%)	4.19 ± 5.56	2.25 ± 1.06	9.33 ± 7.29	0
AA (%)	79.71 ± 11.36	86.50 ± 0.00	70.07 ± 10.15	0
SL (cm)	5.90 ± 0.64	6.73 ± 0.28	5.28 ± 0.87	0
RL (cm)	5.16 ± 0.71	4.83 ± 0.65	4.63 ± 0.72	0
SDW ($mg \cdot seedling^{-1}$)	48.45 ± 6.04	46.00 ± 0.71	40.00 ± 3.87	0
RDW ($mg \cdot seedling^{-1}$)	18.36 ± 4.37	18.63 ± 0.88	14.27 ± 3.73	0
CIELab-a	3.36 ± 0.77	3.52 ± 1.07	4.15 ± 0.81	3.68
CIELab-b	5.41 ± 2.18	4.67 ± 0.15	6.73 ± 1.48	2.10
CIELab-L	16.21 ± 2.93	24.66 ± 1.76	16.38 ± 2.27	12.40
Hue	67.80 ± 34.55	81.25 ± 11.43	45.75 ± 13.12	111.50
Brightness	0.15 ± 0.02	0.18 ± 0.00	0.16 ± 0.02	0.13
Area (cm^2)	0.21 ± 0.01	0.23 ± 0.01	0.19 ± 0.02	0.15
Dmax (cm)	0.64 ± 0.03	0.66 ± 0.01	0.60 ± 0.02	0.59
Dmin (cm)	0.42 ± 0.02	0.45 ± 0.02	0.41 ± 0.02	0.34
Circumference (cm)	1.89 ± 0.13	1.90 ± 0.03	1.83 ± 0.15	1.57
TSW (g)	24.1 ± 1.8	25.4 ± 0.7	19.5 ± 1.8	15.2

[1] Number of individuals in each group; GSI: germination speed index; AS: abnormal seedlings; UG: ungerminated seeds; AA: accelerated aging; SL: shoot length; RL: root length; SDW: shoot dry weight; RDW: root dry weight; CIELab-a: shade of color in the range from green to red; CIELab-b: saturation of color in the range from blue to yellow; CIELab-L: lightness of color in the range from black to white; Hue: pure state of color; Brightness: ranges from black to white; Dmax: maximum diameter; Dmin: minimum diameter; TSW: 1,000-seed weight.

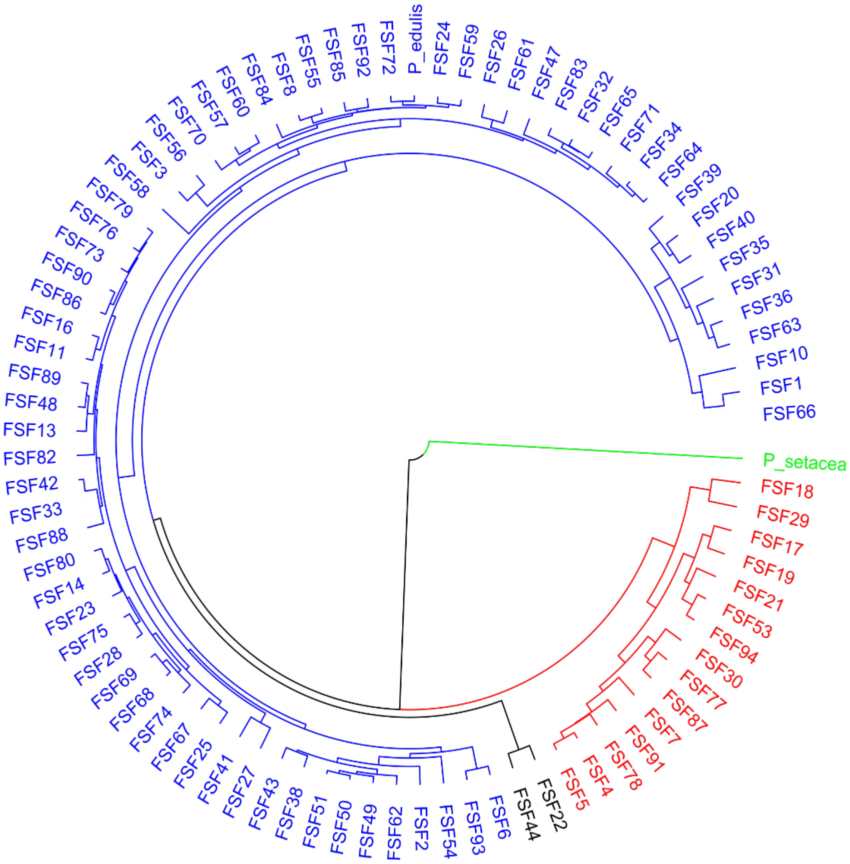


Figure 1. Clustering dendrogram for the generalized Mahalanobis distance (D2) level (unweighted pair group method with arithmetic mean clustering method) of physiological and physical quality of seeds of 86 Passiflora full-sib families and two controls (*Passiflora edulis* and *Passiflora. setacea*). Cophenetic correlation coefficient = 0.84.

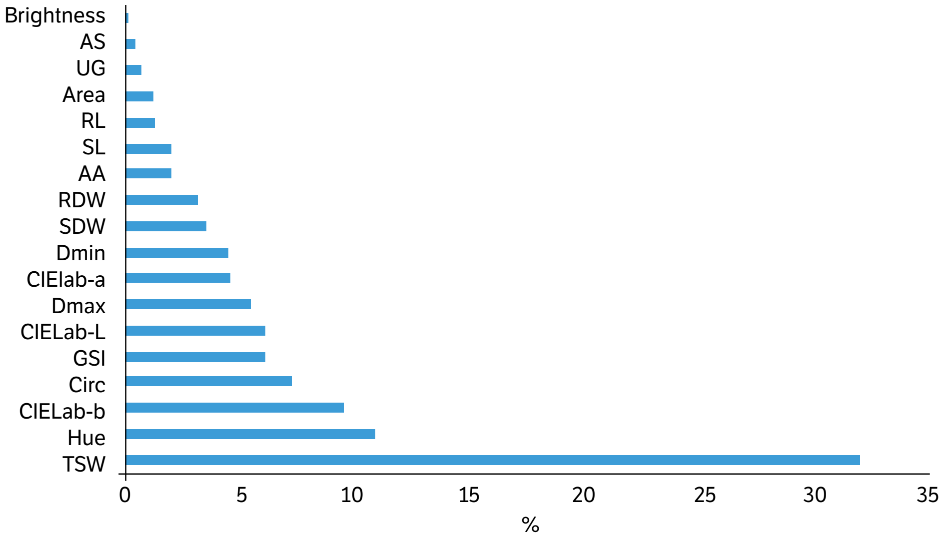
The families of Group I showed the highest means for germination speed index, root length, and shoot dry weight; and the lowest percentage of abnormal seedlings. Group II, which contained only two families, had the best means for accelerated aging, shoot length, root dry weight, and 1,000-seed weight; and the lowest percentage of ungerminated seeds. Accelerated aging was above 80% for most families in this group; therefore, they can be considered the most vigorous (Table 3).

Group III exhibited the lowest means of physiological traits related to germination and seed circumference (Table 3).

According to the seed color results, obtained using GroundEye® software, Group III had the highest means of CIELab-a (4.15) and CIELab-b (6.73), whereas Group II exhibited the highest CIELab-L mean (24.66).

Group IV, composed only of the *P. setacea* control, was the most genetically distant, as this genotype did not show signs of germination. In addition, this control exhibited the lowest means of the physical traits of area, maximum diameter, minimum diameter, circumference, and 1,000-seed weight, with values of 0.15 cm2, 0.59 cm, 0.34 cm, 1.57 cm, and 15.2 g, respectively (Table 3).

The traits with the highest relative contribution values, that is, those that most contributed to the differentiation of the families, were the physical characters of 1,000-seed weight (31.94%), hue (10.86%), and CIELab-b (9.46%) (Fig. 2). Among the physiological traits, germination speed index contributed the most, 6.12%. Ungerminated seeds (0.62%), abnormal seedlings (0.46%), and brightness (0.12%) were not very informative for the evaluation of genetic dissimilarity in passion fruit. These traits had small-magnitude contributions, so their discard was recommended (Fig. 2).



TSW: 1,000-seed weight; Hue: pure state of color; Circ.: circumference; GSI: germination speed index; Dmax: maximum diameter; Dmin: minimum diameter; SDW: shoot dry weight; RDW: root dry weight; AA: accelerated aging; SL: shoot length; RL: root length; UG: ungerminated seed; AS: abnormal seedling.

Figure 2. Estimate of the relative contribution of physical and physiological seed quality traits to genetic divergence between 86 Passiflora full-sib families and two controls (*Passiflora edulis* and *Passiflora setacea*).

DISCUSSION

The identification of genetic variability between the *Passiflora* FSFs evaluated in the present study will enable a successful selection of superior genotypes in terms of physiological and physical seed traits. Considering the aim of improving seed quality in the passion fruit population under recurrent selection, the families of Groups I and II (Table 3) stood out with high potential regarding seed vigor, especially for the variables of germination speed index, accelerated aging, seedling length, dry weight, and 1,000-seed weight. These traits can be used as basis for the selection of superior genotypes, since materials with more vigorous seeds tend to display good initial performance in the field and, consequently, better establishment of the stand, reaching high productivity levels (Abati et al. 2017).

Vigorous seeds originate seedlings with a higher growth rate, which results from their greater capacity to store reserves and to transform and incorporate them into the embryonic axis. In other words, vigorous lots are more efficient in mobilizing and transferring dry matter from the reserve tissues to the embryonic axis (Marcos-Filho 2020).

The studied genotypes correspond to FSFs from the population of the first cycle of recurrent selection for resistance to CABMV. This population is composed of genotypes derived from different generations of backcrossing, recombinations, and selection, from a segregating population of the cross between the species *P. edulis*, recurrent parent, and *P. setacea*, donor parent (Santos et al. 2015a, Gonçalves et al. 2021, Preisigke et al. 2021, Vidal et al. 2021).

Studies carried out with the population under recurrent selection in the passion fruit breeding program aimed at resistance to CABMV at UENF showed that, after successive generations of backcrossing, it was possible to recover the favorable alleles of *P. edulis*, which possesses fruit production and quality traits (Freitas et al. 2015, Santos et al. 2015b). The same was found in the present study regarding physical and physiological seed traits, for which the studied families showed greater similarity with the species *P. edulis*. The high germination rates of the evaluated FSFs demonstrate that the backcrossing and recombination generations were efficient to recover the germination potential of the recurrent parent, *P. edulis*, which has a high germination rate, unlike *P. setacea*, which presents seed dormancy.

Additionally, the *P. setacea* control, isolated in Group IV of the dendrogram, was the genotype most genetically distant from the species *P. edulis* and from the FSFs. This finding corroborates those described by Torres et al. (2019), who evaluated the diversity of *Passiflora* spp. and reported that the *P. edulis* and *P. setacea* parents were separated into different groups. In addition to not showing signs of germination in the evaluation standard for up to 28 days (Brasil 2009), the geometric values of these seeds were lower than those found in the others. Studies show that the species *P. setacea* has physical dormancy caused by the impermeability of its seed coat, which restricts the diffusion of water into the embryo (Marcos-Filho 2016).

The clustering of FSFs into four different groups based on physical and physiological seed traits indicates the existence of genetic variability between the genotypes and, consequently, their potential for selection. Similar results were described in studies evaluating seed traits in different passion fruit genotypes (Marostega et al. 2017, Rodrigues et al. 2017, Rosado et al. 2017, Torres et al. 2019).

The study of the genetic diversity between accessions of *Passiflora* spp., based on the morpho-physiological seed traits, reported the formation of four groups by the UPGMA method and seven groups by the Tocher optimization method (Marostega et al. 2017). Rosado et al. (2017) evaluated the divergence between passion fruit hybrids and reciprocals for seed emergence and vigor traits and described the formation of three groups. Torres et al. (2019), evaluating a segregating population of passion fruit from interspecific hybrids and backcrosses, identified three groups formed based on morphological and physiological seed descriptors.

In addition to the study of genetic diversity, understanding the contribution of variables to the differentiation of families is an important step in the selection of parents for a new selection cycle. This process influences the optimization of the development of new cultivars (Rodrigues et al. 2015). In the present study, the greatest contributions to genetic divergence between the studied families came from the physical variables (1,000-seed weight, hue, CIELab-b, and circumference). Among the physiological traits, only germination speed index contributed more than 5%. Torres et al. (2019) observed that physiological quality variables contributed only 3.25%, thus not being adequate to determine genetic diversity in a segregating population of *Passiflora* spp.

One thousand-seed weight was the variable that most contributed to genetic dissimilarity between the FSFs. In papaya, Cardoso et al. (2009) also found that 1,000-seed weight (37.07%) was the variable that most contributed to genetic diversity.

The amount of reserves stored is one of the factors that influence the physiological quality of seeds, as the seedling uses them until it becomes capable of performing photosynthesis (Marcos-Filho 2016). In larger seeds, the synthesis of secondary compounds, which are important for the germination process, is faster (Marcos-Filho 2016). There is also greater production of photosynthetic compounds for seedling growth, which means greater chances of survival in adverse conditions. Therefore, seed size can be a decisive factor, since larger or denser seeds have greater amounts of reserves and, consequently, the seedlings formed will show greater vigor (Henning et al. 2010).

Among the traits obtained by GroundEye®, hue, CIELab-b, and circumference were those that most contributed to explaining genetic divergence between the families. Other authors described similar results using the GroundEye® system to assess diversity based on the CIELab color system to differentiate seeds of different species (Krause et al. 2017, Fachi et al. 2019, Gentallan Junior et al. 2019, Torres et al. 2019, Buratto et al. 2021). Fachi et al. (2019) evaluated 98 FSFs of *P. edulis* and reported that the use of color and geometry descriptors, obtained by GroundEye®, contributed to quantifying genetic divergence between families.

The use of the GroundEye® system proved to be efficient in differentiating the FSFs of passion fruit based on geometric and color variables, as reported for other species. Buratto et al. (2021) concluded that the CIELab color system is efficient and practical for classifying the color of triticale (*Triticosecale wittmack*) seeds subjected to the phenol test. The same was observed by Gentallan Junior et al. (2019), who reported that the CIELab color system was efficient to distinguish different shades of brown in Pili (*Canarium ovatum* Engl.) seeds.

The study of 61 genotypes of inbred guava families proved the efficiency of digital seed image analysis to quantify genetic diversity between genotypes (Krause et al. 2017). According to the authors, the geometric variables were those which most contributed to the diversity when associated with the Ward-MLM method.

The GroundEye® system is an effective and promising tool to quantify genetic diversity from seed traits in an early, fast, non-destructive, and easy-to-reproduce manner (Venora et al. 2007). Nonetheless, aspects related to the physiological quality of the seeds under evaluation must not be neglected, as they are important criteria for the selection of superior genotypes (Silva et al. 2021). Passion fruit seeds must exhibit rapid and homogeneous germination, which allows for faster, continuous, and uniform seedling formation.

CONCLUSION

The passion fruit full-sib families under recurrent selection for resistance to the cowpea aphid-borne mosaic virus showed high germination potential and seed vigor, in addition to genetic divergence regarding physical and physiological seed quality attributes.

One thousand-seed weight and geometry and color variables, informed by GroundEye®, were efficient to explain the dissimilarity between the studied genotypes.

The families allocated to Groups I and II by cluster analysis are promising in terms of germination potential and seed vigor, as such they should be included in the selection of superior genotypes.

AUTHORS' CONTRIBUTION

Conceptualization: Rodrigues, C. A., Vieira, H. D. and Viana, A. P.; **Data curation:** Rodrigues, C. A.; **Formal analysis:** Rodrigues, C. A.; **Investigation:** Rodrigues, C. A. and Mendes, D. S.; **Methodology:** Rodrigues, C. A., Vieira, H. D., Mendes, D. S. and Viana, A. P.; **Project administration:** Rodrigues, C. A., Vieira, H. D., Mendes, D. S. and Viana, A. P.; **Visualization:** Rodrigues, C. A. and Souza, R.; **Writing – review and editing:** Rodrigues, C. A., Vieira, H. D. and Souza, R.; **Funding acquisition:** Vieira, H. D. and Viana, A. P.; **Resources:** Vieira, H. D. and Viana, A. P.; **Supervision:** Vieira, H. D.; **Validation:** Vieira, H. D., Souza, R. and Viana, A. P.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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