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The physiological quality of the seeds of passion fruit (*Passiflora* spp.) grown at different altitudes

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Abstract

Altitudes, ranging between 100 m and 900 m are the most suitable for growing passion fruit and the studies on the distinct geographical locations suggest the expression of different genotypes under the influence of adequate environmental conditions. The objective of this study was to evaluate the physiological quality of the seeds of *Passiflora* spp., cultivated at three different altitudes in the state of Espírito Santo, Brazil. Before conducting the physiological tests for seed quality, pre-tests were performed on the physically scarified, gibberellic acid-treated, and intact seeds of the yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Degener), the purple passion fruit (*Passiflora edulis* Sims), and the sweet passion fruit (*Passiflora alata* Curtis), collected from three different altitudes (high, medium, and low), in order to overcome dormancy. The higher values of germination and vigor were observed after the pre-germination treatments of the intact seeds of the yellow passion fruit, the scarified seeds of the purple passion fruit, and the gibberellic acid-treated seeds of the sweet passion fruit. The seeds with the highest values of germination and vigor were selected for the accelerated aging test with a saturated salt solution at 43 ºC for 72 h and the controlled deterioration test with 25% of moisture at 45 ºC for 24 h, respectively. Using these tests, it was possible to differentiate the species of passion fruit, collected from different altitudes. The seeds of the yellow passion fruit and the seeds, collected from higher altitudes presented higher physiological quality in this study.

Keywords: aging, deterioration, germination, *Passiflora* spp., vigor.
Abbreviations: A_aged seeds; A_average altitude; DM_dry mass; E_emergence; ESI_ emergence speed index; FM_fresh mass; G_ germination; GA_3_gibberellic acid; GSI_ germination speed index; H_high altitude; I_intact; L_low altitude; NA_non-aged seeds; PP_purple passion fruit; PS_physical scarification; RL_root length; SL_shoot length; SP_sweet passion fruit; YP_yellow passion fruit.

Introduction

The passion fruit tree, belonging to the family Passifloraceae and the genus *Passiflora* stands out as the most economically important plant of the family. Being the largest genetic diversity center of this genus, Brazil is considered the center of the origin for approximately 139 known species of which, 89 are endemic (Bernacci et al., 2013).

The most cultivated species, the yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Degener) accounts for 95% of the total planted area in Brazil, which presents excellent conditions for the cultivation of passion fruit (Meletti, 2011). The purple passion fruit (*P. edulis* Sims) and the sweet passion fruit (*P. alata* Curtis) are grown in smaller areas for the consumption and are present in restricted markets (Santos et al., 2011). Being classified as a crop of hot and humid climate, the plant is able to develop in tropical and subtropical regions at altitudes, ranging between 100 m and 900 m and with an average temperature, ranging between 25ºC and 26ºC (Fraife Filho et al., 2010).

The comparison between the fruits and seeds cultivated at the different geographical locations is important for the identification of phenotypic differences that are determined by environmental variations since the environment can favor the expression of certain characteristics, expressed in other places (Botezelli et al., 2000).

Passion fruit is propagated mainly by seeds and can also be realized by asexual methods. The germination is slow and non-uniform due to the different factors such as the use of seeds obtained from unselected plants, the presence of phytoregulatory substances and seeds with impermeable integuments, and the presence of fungi associated with the seeds (Lopes et al., 2007). The aryl extracts contain steroids and triterpenoids that inhibit the germination of the seeds of passion fruit (Martins et al., 2010). Scarification and plant regulators such as gibberellic acids, which is a bioactive agent, promoting germination and overcoming seed dormancy favor seedling performance and accelerate the speed of seed emergence (Aragão et al., 2006).

Being characterized by longevity, germination, and vigor, the physiological quality is related to the capacity of the seeds to perform their vital functions (Toledo et al., 2009). In order to evaluate the physiological quality of the seeds, the germination and vigor tests are employed. Of these, the accelerated aging test is an indirect method of evaluation, which simulates the stress conditions, generates a high respiration rate, and consumes reserves, thereby accelerating the metabolic processes that lead to the deterioration of the seeds by promoting greater absorption of water due to the exposure of the seeds to a humid atmosphere. Thus, the use of a saturated solution of sodium chloride (NaCl) accelerates aging and retards the absorption of water by the seeds during their aging due to the difference in osmotic pressure between the solution and the seed, which may exert a negative effect on germination (Torres et al., 2009).

Another method of evaluating the physiological quality of the seeds is the controlled deterioration test that is related to the initial events of seed deterioration such as the degradation of cell membranes and the reduction of respiratory and biosynthetic activities (Binotti et al., 2008).

Therefore, the aim of this study was to evaluate the physiological quality of the seeds *of Passiflora* spp., cultivated at different altitudes in the state of Espirito Santo, Brazil.

Results and Discussion

Pre-test overcoming dormancy

According to the results of the Analysis of Variance, followed by Tukey's test, a significant interaction was observed among the variables such as germination, germination speed index, emergence, and emergence speed index, suggesting that these variables are influenced by the altitudes and the pre-germination tests within the different species of passion fruit (Table 2).

The seeds of the scarified and intact yellow passion fruit, harvested from high altitudes presented higher values of germination (98% and 97%, respectively) and germination speed index (3.80 and 3.65, respectively), compared to those treated with gibberellic acid. However, no difference in the germination values was observed in the scarified and gibberellic acid-treated seeds of fruits, harvested from high and medium altitudes. The characteristics of germination differed considerably between the altitudinal provenances and appeared to be important in determining the ability of the species to inhabit such a wide gradient (Bauk et al., 2016).

The seeds of the purple passion fruit, collected from high and medium altitudes did not differ in the treatments for germination. However, the scarified, gibberellic acid- treated, and intact seeds of the fruits, collected from medium altitudes presented higher values of germination speed index (2.98, 2.54, and 3.23, respectively), compared to those obtained from high and low altitudes.

The gibberellic acid-treated seeds of the sweet passion fruit, obtained from medium altitudes presented higher values of germination and germination speed index (36% and 0.65, respectively), compared to those obtained from other altitudes. The balance between the promoters and the inhibitors of growth is fundamental to the process of germination. The seed hormones, especially the gibberellins influence a series of processes of plant development as a promoter of growth, including their fundamental role in seed germination, the activation of the stages of embryonic vegetative growth, mainly by promoting the elongation of the primary roots, the extension of the stem, the induction of flowering, the development of anthers and seeds, and the growth of pericarp (Taiz and Zeiger, 2017). Thus, the use of this compound is becoming evident in the germination of seeds of the genus *Passiflora* spp., according to studies with the seeds of the sweet passion fruit and the yellow passion fruit, respectively (Wagner Júnior et al., 2007; Santos et al., 2013).

At low altitudes, the intact seeds of the yellow passion fruit presented higher values of germination and germination speed index (71% and 1.79, respectively), which demonstrates the influence of the place of production on the physiological quality of the seeds, corroborating with previous studies that observed that one of the factors that may influence the physiological quality of the seeds is the place, where the parent plants are produced and the seeds are harvested (Silva and Dantas, 2013). Thus, it is verified that the tests of physiological quality of the seeds are efficient to differentiate the origin of the seeds of passion fruit, according to vigor, as verified for the seeds of Cedar (Lazarotto et al., 2013),.

The gibberellic acid-treated and intact seeds of the yellow passion fruit, collected from high altitudes showed higher values of emergence (98% and 93%, respectively) and rate of emergence (1.95 and 1.80, respectively). The pre-soaking of the seeds of the yellow passion fruit in GA_3 stimulated seed germination and promoted seedling vigor (Santos et al., 2013). The beneficial effects of GA_3 on the germination of the seeds of the yellow passion fruit were also observed in another study (Lima et al., 2009).

The scarified and intact seeds of the purple passion fruit, harvested from high and medium altitudes, presented higher values of emergence. On the other hand, the same seeds, collected from medium altitudes and treated with gibberellic acid presented higher values of emergence speed index (1.83).

The intact seeds of the sweet passion fruit, collected from low altitudes presented higher values of emergence (44%), compared to the seeds, collected from other altitudes. The same seeds, harvested from low altitudes and either treated with gibberellic acid or intact presented higher values of emergence speed index (0.43 and 0.54, respectively).

The seeds of the gibberellic acid-treated yellow passion fruit, obtained from high altitudes presented higher values of emergence and emergence speed index (98% and 1.95, respectively), compared to the seeds of the purple passion fruit and the sweet passion fruit. The scarified seeds of the purple passion fruit presented higher values of emergence (89%).

The scarified seeds of the purple passion fruit, obtained from medium altitudes showed higher values of emergence and emergence speed index (93% and 1.65, respectively), while

Table 1. The place of collecting the samples of *Passiflora* spp.

Species	Location	Latitude	Longitude	Altitude (m)
P. edulis Sims f. *flavicarpa* Degener	Santa Maria de Jetibá	19º 59' 37.68'' S	40º 40' 25.68'' W	674
	Alegre	20º 46' 13.06'' S	41º 30' 27.96'' W	365
	Marataízes	21º 09' 32.58'' S	40º 55' 07.56'' W	6
P. edulis Sims	Santa Maria de Jetibá	19º 59' 40.32'' S	40º 40' 21.66'' W	674
	Jerônimo Monteiro	20º 47' 43.86'' S	41º 30' 33.96'' W	210
	Marataízes	21º 09' 32.28'' S	40º 55' 07.62'' W	6
P. alata Curtis	Santa Maria de Jetibá	20º 02' 34.20'' S	40º 43' 34.08'' W	655
	Alegre	20º 47' 44.94'' S	41º 30' 35.52'' W	350
	Jerônimo Monteiro	20º 46' 13.06'' S	41º 25' 33.06'' W	100

Table 2. Germination (G%), germination speed index (GSI), emergence (E%), and emergence speed index (ESI) of *Passiflora* spp. cultivated at different altitudes and treated (PS - physical scarification, GA_3 - gibberellic acid, and I - intact)[(1)].

		G (%)			GSI			E (%)			ESI		
		PS	GA_3	I	PS	GA_3	I	PS	GA_3	I	PS	GA_3	I
YP	H	98 aAα	79 aBα	97 aAα	3.80 aAα	1.51 bBα	3.65 aAα	78 bBβ	98 aAα	93 aAα	1.52 aBα	1.95 aAα	1.80 aAα
	A	95 aAα	87 aABα	83 bBα	3.12 bAα	1.98 aBβ	2.11 bBβ	67 cBβ	90 aAα	91 aAα	1.24 bBβ	1.81 aAα	1.66 aAα
	L	65 bABα	59 bBα	71 cAα	1.74 cAα	1.53 bAα	1.79 bAα	89 aAα	91 aAα	90 aAα	1.63 aAα	1.76 aAα	1.69 aAα
PP	H	85 aAβ	88 aAα	84 aAβ	1.55 bAβ	1.73 bAα	1.51 bAβ	89 aABα	80 bBβ	93 aAα	1.66 aAα	1.48 bAβ	1.67 aAα
	A	87 aAα	79 aAα	87 aAα	2.98 aAα	2.54 aBα	3.23 aAα	93 aAα	93 aAα	92 aAα	1.65 aAα	1.83 aAα	1.73 aAα
	L	67 bAα	62 bABα	54 bBβ	1.57 bAα	1.52 bAα	1.27 bAβ	74 bAβ	81 bAα	63 bBβ	1.41 bAα	1.46 bAβ	1.15 bBβ
SP	H	24 aAγ	12 bBβ	20 aABγ	0.40 aAγ	0.29 bAβ	0.35 aAγ	22 aAγ	30 aAγ	31 bAβ	0.24 aABβ	0.39 abAγ	0.34 abAβ
	A	13 bBβ	36 aAβ	10 abBβ	0.25 aBβ	0.65 aAγ	0.28 aBγ	20 aAγ	13 bAβ	20 cAβ	0.20 aAγ	0.21 bAβ	0.20 bAβ
	L	33 aAβ	12 bBβ	5 bBγ	0.58 aAβ	0.21 bBβ	0.08 aBγ	12 aBγ	36 aAβ	44 aAγ	0.21 aBβ	0.43 aAγ	0.54 aAγ

[(1)]Means followed by the same letter, lowercase in the column (altitudes), uppercase in the line (treatments), and greek letters in the column (species between each altitude) do not differ by Tukey's test at the 5% probability level.
Caption: YP = yellow passion fruit; PP = purple passion fruit; SP = sweet passion fruit; H = high altitude; A = average altitude; L = low altitude.

Table 3. Germination (G), germination speed index (GSI), shoot length (SL), fresh mass (FM), and dry mass (DM) of seedlings of *Passiflora* spp. subjected to the accelerated aging with saline saturation at 43 ºC for 72 h[(1)].

		G (%)			GSI			SL (cm)			FM (mg)			DM (mg)		
		YP	PP	SP	YP	PP	SP	YP	PP	SP	YP	PP	SP	YP	PP	SP
NA	H	97 aA	85 aA	24 abB	3.65 aA*	1.56 bB	0.40 aC	6.88 aA	4.20 aB	6.13 aA*	102.92 aA	73.73 aB	59.50 aC	6.93 abA	7.53 aA	7.95 aA
	A	83 bA	87 aA	36 aB*	2.11 bB	2.98 aA	0.65 aC*	4.47 bA	4.71 aA	3.00 bB	71.10 bA	74.75 aA	57.00 aB	6.36 bA	5.96 bA	6.50 aA
	L	71 bA	67 bA	12 bB	1.79 bA	1.57 bA	0.21 aB	4.64 bA	4.41 aAB	3.50 bB	73.13 bA	81.21 aA*	60.59 aB	7.55 aA	7.13 aA	7.61 bA
A	H	93 aA	82 bA	22 aB	2.83 aA	1.84 bB*	0.23 aC	7.63 aA	5.13 abB*	3.34 aC	176.83 aA*	88.91 aC*	121.50 aB*	12.12 aB*	8.48 aC*	16.50 aA*
	A	79 bB	95 aA	20 aC	2.82 aA*	3.06 aA	0.19 aB	4.58 bB	6.06 aA*	3.32 aB	73.33 bB	85.80 aA	91.00 bA*	6.29 bB	6.68 bB	8.75 bA*
	L	64 cA	60 cA	19 aB	2.20 bA*	2.18 bA	0.38 aB	4.82 bA	4.71 bA	4.25 aA	75.84 bB	74.69 bB	122.38 aA*	6.26 bB	6.38 bB	15.55 aA*

[(1)]Means followed by the same lowercase letter in the column (altitudes), uppercase in the line (species), and asterisk (aging) do not differ among them by Tukey's test at a 5% probability level.
Caption: YP = yellow passion fruit; PP = purple passion fruit; SP = sweet passion fruit. H = high altitude; A = average altitude; L = low altitude; NA = non-aged seeds; A = aged seeds.

Table 4. Germination speed index (GSI), root length (RL) and fresh mass (FM) of seedlings of *Passiflora* spp. cultivated at different altitudes subjected to the controlled deterioration at 45 ºC with 25% humidity at 24 h[(1)].

	GSI			RL (cm)			FM (mg)		
	YP	PP	SP	YP	PP	SP	YP	PP	SP
H	3.43 aA	2.50 bB	0.77 aC	5.81 abB	5.80 aB	8.00 aA	90.35 aA	79.49 aA	58.00 aB
A	3.05 bA	3.15 aA	0.70 aB	6.05 aB	7.24 aA	8.00 aA	74.86 bA	78.50 aA	56.75 aB
L	1.83 cB	2.53 bA	0.57 aC	5.03 bA	5.31 bA	5.81 bA	66.50 bA	74.42 aA	69.71 aA

[(1)]Means followed by the same lowercase letter in the column and uppercase in the row do not differ from each other by Tukey's test at a 5% probability level.
Caption: YP = yellow passion fruit; PP = purple passion fruit; SP = sweet passion fruit. H = high altitude; A = average altitude; L = low altitude.

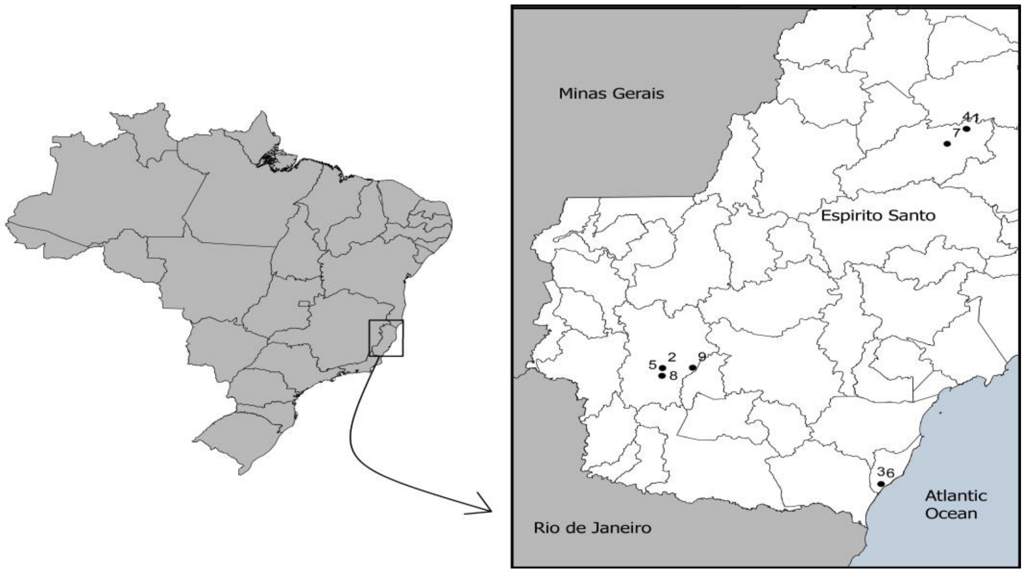

Fig 1. *Passiflora* spp sampling locations in the state of Espírito Santo, Brazil. The circles indicate the sampled localities: 1(Santa Maria de Jetibá); 2 (Alegre); 3 (Marataízes); 4 (Santa Maria de Jetibá); 5 (Jerônimo Monteiro); 6 (Marataízes); 7 (Santa Maria de Jetibá); 8 (Alegre); 9 (Jerônimo Monteiro).

the scarified and intact seeds of the yellow passion fruit, collected from low altitudes presented higher values of emergence and emergence speed index (89% and 90%, respectively). In addition, the location or origin significantly influences the germination and vigor as verified in the seeds of Pineapple (*Annona squamosa* L.) (Araújo Neto et al., 2013). In the present study, the seeds of the yellow passion fruit presented higher values of emergence and vigor in the greenhouse, compared to the other species. The vigor is determined by the speed of emergence of the seedlings under field conditions and/or in the greenhouse. Besides, the higher is the vigor of the seed, the faster is its emergence (Oliveira et al., 2009).

Physiological quality of the seeds of passion fruit

A significant interaction was observed among species, altitudes, and aging for the variables such as germination, germination speed index, shoot length, fresh mass, and dry mass (Table 3).
Our results verified that the exposure of the seeds of passion fruit to aging at 43ºC for 72 h did not influence the germination of the unripe seeds but there was a significant difference between the unaged and aged seeds of the sweet passion fruit, collected at medium altitudes. Previous studies showed that aging at 43 °C in the 48, 72, and 96 h periods promoted an increase in the percentage of germination in the seeds of Crambe (Amaro et al., 2014). A significant difference in vigor was also observed between the unaged and aged seeds in our study (Table 3).

The accelerated aging test with saturated salt solution differentiated the species of passion fruit, grown at different altitudes. The aged seeds of the purple passion fruit, collected at medium altitudes presented higher values of germination and germination speed index (95% and 3.06, respectively).The aged seeds of the yellow passion fruit, obtained at high altitudes presented higher values of aerial part length and fresh mass (7.63 cm and 176.83 mg, respectively). The aged seeds of the sweet passion fruit, harvested from high altitudes presented higher values of dry mass (16.50 mg). During seed development, individual variations were observed even within the same species due to the influence of biotic and abiotic factors and also due to genetic variability (Santos et al., 2009). In the present study, the controlled deterioration test showed that there was a significant interaction between species and altitudes for the variables such as germination speed index, root length, and fresh mass (Table 4). The seeds of the yellow passion fruit, harvested from high altitudes presented higher values of germination speed index (3.43). The seeds of the sweet passion fruit, obtained from high and medium altitudes presented higher values of root length and root width (8 cm and 8 cm respectively).The seeds of the yellow passion fruit and the purple passion fruit, obtained from high altitudes presented higher values of fresh mass (90.35 mg and 79.49 mg, respectively). In fact, earlier studies have reported that higher altitudes may favor the formation of larger and faster seeds along with a higher percentage of germination and high seed quality (Chen et al., 2017). In our study, the controlled deterioration test at 45ºC with 25% of moisture for 24 h differentiated the species of passion fruit, collected from different altitudes. However, the adjustment of the water content to 20% and 24% during the controlled deterioration test in the seeds of Sunflower, belonging to the Catissol cultivar was not sufficient to cause separation between the lots (Oliveira et al., 2013).

Materials and Methods

Plant materials

The experiments were carried out at the Seed Analysis Laboratory (SAL) and in the greenhouse on the campus of the Center of Agricultural Sciences and Engineering at the Federal University of Espirito Santo (CCAE-UFES) in Alegre-ES, Brazil. The seeds of the yellow passion fruit (*Passiflora edulis*

Sims f. *flavicarpa* Degener), the purple passion fruit (*Passiflora edulis* Sims), and the sweet passion fruit (*Passiflora alata* Curtis) were harvested from native seedlings, located at three different altitudes (between 0 and 100 m-low, >100–600 m-medium, and >600 m-high) in the state of Espirito Santo, Brazil (Fig. 1 and Table 1).

Pre-test overcoming dormancy

The seeds of the ripe and freshly harvested fruits were extracted on a sieve with a sterilized spoon after removing the pulp, using the slaked lime technique. The seeds, containing the mucilage were then washed, kept, and dried on a Germitest type paper in the shade until the moisture levels reached 10–12%.

The experiments were randomly conducted with four replicates of 25 seeds in a 3 × 3 × 3 factorial scheme [three species of passion fruit: yellow passion fruit, purple passion fruit, and sweet passion fruit; three different altitudes: 0–100 m, >100–600 m, and >600 m; and three pre-germination treatments: T1-physical scarification, made manually with sandpaper at an angle of 120º, opposite to the side of radicle emission; T2-treatment with gibberellic acid (GA_3) at a concentration of 500 mg L^{-1} with soaking for 24 h (pre-test was previously carried out and seeded immediately), and T3-intact seeds].

In order to evaluate the physiological quality of the seeds, the following tests were done in the laboratory: For each treatment, the germination test was carried out with four replicates of 25 seeds planted in Germitest type paper rolls, moistened with distilled water (equivalent to 2.5 times the mass of the dry paper), and kept in a BOD germination chamber, regulated by alternating temperatures of 20–30 °C in the dark. After 28 days of sowing, the evaluations were realized by calculating the percentage of the normal seedlings and expressing the results as a germination percentage (Brazil, 2009).

The germination speed index was determined simultaneously with the germination test and the number of seeds with primary root protrusion ≥2 mm was regularly observed up to 28 days, according to the methodology described previously (Maguire, 1962).

For the emergence test, a commercial substrate was placed in 53 cm^3 tubes with four replicates of 25 seeds. The sowing was performed with one seed per tube and daily irrigation, according to crop needs. The emergence speed index was evaluated according to the methodology described for the germination speed index.

Physiological quality of the seeds of passion fruit

After the pre-germination treatments in the laboratory and in the greenhouse, the treatments in which the seeds presented higher values of germination and vigor, following the accelerated aging test and the controlled deterioration test were selected. The accelerated aging test with the saturated salt solution was carried out in gearbox plastic boxes (11.0×11.0×3.5 cm) with an aluminum grille inside, where the seeds, after weighing (approximately 3.0 g) were distributed to form simple and uniform layers. Following the methodology described previously, 40 mL of a saturated NaCl solution (40 g of NaCl dissolved in 100 mL of distilled water) was added (Jianhua and McDonald, 1996). The boxes were then kept in a BOD germination chamber, regulated at a temperature of 43 ºC for 72 h. After aging, the four replicates of 25 seeds per treatment were placed to germinate, according to the methodology described for the germination test. The variables such as germination, germination speed index (according to the methodology described above), shoot length, fresh mass, and total dry mass of the seedlings were evaluated. For the controlled deterioration test, the water content of the seeds was adjusted to 25% and then the seeds were placed in hermetically sealed aluminized containers and maintained for 16 h in a refrigerator (5–8 °C). Subsequently, the aluminum capsules were transferred to a water bath, kept at 45 °C for 24 h and the containers were immersed in cold water, in order to reduce and standardize the temperature. The seeds were then sowed using the four replicates of 25 seeds per treatment. The variables such as germination speed index, root length, total fresh mass, and dry mass of the seedlings were evaluated.

Study design and statistical analysis

The accelerated aging test with a saturated salt solution and the controlled deterioration test were conducted in a completely randomized design with four replicates of 25 seeds in a 3 × 3 factorial scheme (the three species of passion fruit and the three different altitudes). The data were submitted to the normality test of residues without data transformation and to the Analysis of Variance test. The means were compared by Tukey's test when the F value was significant at 5%. All statistical analyses were performed using the R software and the ExpDes package (R Core Team, 2017).

Conclusion

In the present study, the intact seeds of the yellow passion fruit, the scarified seeds of the purple passion fruit, and the gibberellic acid-treated seeds of the sweet passion fruit presented higher values of germination and vigor. Again, the seeds of the yellow passion fruit presented superior physiological quality, compared to the other species. Furthermore, the seeds of the passion fruit, harvested from higher altitudes presented higher germination and vigor. Thus, the accelerated aging test with a saturated salt solution at 43 °C for 72 h and the controlled temperature deterioration test at 45 °C with 25% of moisture for 24 h can differentiate the different species of passion fruit, harvested from different altitudes.

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