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Original Article

Methods of aril removal and lightness conditions on seeds physiological quality of sour passion fruits

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Abstract

Passion fruits species presents a larger economic importance for *in natura* consumption and industry. The main way to propagate these species is by seeds; however, the aril presence and environmental conditions may inhibit the seeds germination. In this sense, the objective of this study was to evaluate the effect of aril removal methods and light conditions on *P. edulis* var. *flavicarpa* seeds quality and germination. There were used five methods to remove the seeds aril: (1) friction on steel mesh sieve for 5 minutes; (2) friction with coarse sand on steel mesh sieve for 5 minutes; (3) immersion in lime solution at 10% (w/v), for 30 minutes; (4) fermentation in sugar solution at 10% (w/v), during 48 hours; and (5) natural pulp fermentation, during five days. The seeds were submitted to germination test at darkness and lightness, as well as, to length seedlings and emergence test. Regardless the aril removal method, the germination percentage was lower at lightness than darkness highlighting the natural pulp fermentation treatment, which presented 86% of seeds germinated. On the other hand, the aril removal methods did not affect the seedlings emergence, with emergence percentages ranged from 88 to 94%.

Keywords: Passiflora edulis, seed germination, seedlings length, darkness, dormancy.

Métodos de remoção do arilo e condições de luminosidade sobre a qualidade fisiológica das sementes de maracujá azedo

Resumo

As espécies de maracujá apresentam grande importância econômica, tanto para o consumo *in natura*, quanto para a indústria. A principal maneira de propagar essas espécies é por sementes; contudo, a presença de arilo e condições ambientais pode inibir a germinação. Nesse sentido, objetivou-se estudar o efeito de métodos de remoção do arilo e luminosidade na qualidade e germinação das sementes de *Passiflora edulis* var. *flavicarpa*. Cinco métodos foram empregados para a remoção do arilo: (1) fricção em peneira de malha de aço por cinco minutos; (2) fricção com areia grossa em peneira de malha de aço por cinco minutos; (3) imersão em solução de cal a 10% (m/v), por 30 minutos; (4) fermentação da polpa em solução de açúcar a 10% (m/v), por 48 horas; e (5) fermentação natural da polpa, por cinco dias. As sementes foram submetidas ao teste de germinação no escuro e no claro, teste de comprimento de plântulas e teste de emergência. Independente do método de remoção do arilo, a porcentagem de germinação no claro apresentou-se menor em comparação ao escuro, com destaque para o tratamento de fermentação natural da polpa, o qual apresentou 86% de sementes germinadas. Por outro lado, os métodos de remoção do arilo não afetaram a emergência das plântulas, com porcentagem de emergência variando entre 88 a 94%.

Palavras-chave: Passiflora edulis, germinação de sementes, comprimento de plântulas, escuro, dormência.

1. Introduction

The Passifloraceae family is represented in South America by four genera, which the *Passiflora* is constituted by over 500 species, approximately 150 originated from Brazil (Campos et al., 2007; Hansen et al., 2006) where the largest center of geographic distribution of this genus occurs in the northern central region (Faleiro et al., 2005). Among these species, the yellow passion fruit (*Passiflora edulis* Sims) is considered the most important specie in Brazil, representing 95% of commercial yields in passion fruit (Campos et al., 2007).

Passiflora species can be propagated by seeds or asexually by grafting, cuttings or *in vitro* propagation; however, the seeds propagation is the most used method (Araújo et al., 2007; Pantano, 2007; Zucareli et al., 2015). According to Delanoy et al. (2006) the *Passiflora* seeds germination may occurs from ten days to three months,

showing a low germination percentage and irregularity in seedlings development. However, Tozzi and Takaki (2011) reported that the primary root protrusion occurred just from sixth day after sowing for *P. edulis*.

The irregularity of seeds germination can be caused by dormancy mechanisms, which control the water absorption by seeds, considered as physical or physiological dormancy (Alexandre et al., 2004; Marcos-Filho, 2015; Dalling et al., 2011). The *P. edulis* var. *flavicarpa* is a specie that presents seeds involved by aril and hard seed coat with a semi-permeable inner layer (Delanoy et al., 2006), which confers a combination of physical and physiological dormancy (Alexandre et al., 2004).

The physical dormancy plays a key role protecting seeds against microbial and predator attacks, as well as, the seed banks maintenance in the soil. In the other hand, compounds that inhibit seed imbibition or regulate the hormones balance during the germination process, as abscisic acid and gibberellic acid, cause physiological dormancy (Bewley et al., 2013).

For seedling growers, the irregularity of seed germination, caused by dormancy is not desired; then, it is important to study methods to overcome seed dormancy and reach homogeneous seedlings. Aril removal has been used as promisor method to increase seeds germination with several results in *Passiflora* species (Pereira and Dias, 2000; Martins et al., 2006; Osipi et al., 2011; Silva et al., 2015).

Additionally, passion fruit seeds are usually negative photoblastic, which means they are affected by light incidence; according to Brasil (2009), the germination test for *P. edulis* may be performed at darkness conditions to overcome seed dormancy. These results are in agreement with other authors, which were reported the light inhibitory effect in others *Passiflora* plants, such as *P. cincinnata* (Zucareli et al., 2009) and *P. incarnata* (Benvenuti et al., 2001; Zucareli et al., 2015). In this sense, the objective of this study was to evaluate the effect of aril removal methods and light conditions on *P. edulis* var. *flavicarpa* seeds quality and germination.

2. Material and Methods

2.1. Seeds obtaining

Sour passion fruits (*Passiflora edulis* var. *flavicarpa*) were bought at CEASA-Londrina, PR-Brazil. At the Fruits Analysis Laboratory of the Londrina State University, a sample with 15 fruits was collected to perform the physical characterization, as follow in the Table 1. Sixty-seven uniform fruits were chosen and cut to remove the pulp and seeds, which were homogenized in glass beaker (4 liters). Three pulp samples were collected to carry out the chemical analysis, according to IAL (2008) (Table 1). Then, the remaining pulp was divided into five similar amounts to test five methods of aril removal.

2.2. Aril removal methods

There were used five methods (treatments) to remove the seeds aril: (1) friction on steel mesh sieve for 5 minutes; (2) friction with coarse sand on steel mesh sieve for 5 minutes; (3) immersion in lime solution at 10% (w/v), for 30 minutes; (4) fermentation in sugar solution at 10% (w/v), during 48 hours; and (5) natural pulp fermentation, during five days.

The treatments regarding fermentation were performed in glass beaker (2 liters) and kept at 22 ± 2 °C. The beakers were covered with a plastic film to prevent insects and the pulp was homogenized twice a day. After the fermentation periods, the pulps with seeds were washed under running water on steel mesh sieve. All seeds obtained in the treatments were kept to dry at 22 ± 2 °C during five days and stored in paper bags at 3 ± 1 °C until to the experimental installation.

2.3. Analysis performed

The seed water content (%) was determined by the incubator method at 105 °C (Brasil, 2009), using two samples of 20 seeds for each treatment. The thousand seed mass was determined from eight samples of 100 seeds, according to Brasil (2009).

Seedling length test was performed in dark chamber at 25 °C, using four replications of 20 seeds per treatment on paper rolls, moistened with distilled water equivalent to 2.5 times the weight of the dry paper (Brasil, 2009). The seedling length was measured through the ruler and seedling dry matter weighted in a precision balance (0.0001 g), after drying at 70 °C during five days in an incubator.

For the germination test, four replications of 50 seeds were placed on paper rolls, previously moistened with distilled water equivalent to 2.5 times the weight of the dry paper (Brasil, 2009). The paper rolls were kept in germination chamber at 25 °C, under constant darkness and

Table 1. Physicochemical properties of fruits and pulp of sour passion fruits (*Passiflora edulis* var. *flavicarpa*). Color attributes of lightness (L^*), saturation (C^*) and hue angle (h^o); fruit mass (FM); pulp mass (PM); fruit diameter (FD), soluble solids content (SS), pulp pH; titratable acidity (TA, % of citric acid) and SS/TA.

	L^*	C^*	h^o	FM (g)	PM (g)
Average	62.30	46.82	88.81	296.93	105.56
SD	2.83	4.00	1.51	48.68	17.36
CV (%)	4.55	8.53	1.70	16.40	16.45
	FD (mm)	**SS (ºBrix)**	**pH**	**TA (%)**	**SS/TA**
Average	92.40	11.37	3.89	3.88	2.93
SD	5.26	0.06	0.16	0.11	0.10
CV (%)	5.70	0.51	4.02	2.91	3.25

SD = standard deviation; CV = coefficient of variation.

lightness. From eighth day after experimental installation, the numbers of germinated seeds were recorded daily. The germination speed index (GSI) (Maguire, 1962) and the total germination were calculated, considering as normal seedlings those with proportional shoot and root (Brasil, 2009). For the germination test, performed at darkness and lightness conditions, the percentages were established from the 28th and 25th days after the test installation, respectively.

Seedling emergence test was carried out in a greenhouse applying a completely randomized design with four replications of 25 seeds per treatment. The seeds were sown in Styrofoam trays with 128 cells, one seed per cell, filled with semi-composted pine bark substrate and watered twice a day. Seedling emergence was recorded at 9th, 17th and 26th days after the test installation evaluating normal seedlings with healthy shoot.

2.4. Statistical analysis

All data recorded were tested for normality and variance homogeneity; then, submitted to analysis of variance (ANOVA). The data recorded at germination test were subjected to analysis of join variances to compare the environments (darkness and lightness conditions), and the treatments means compared by Tukey's test at 5% probability level.

3. Results

The *Passiflora edulis* var. *flavicarpa* fruits showed intense bright yellow skin (L^* = 62.30 and h^o 88.81) and the pulp mass corresponded about 30% of the total mass (Table 1). The soluble solids content recorded was 11.37 ºBrix, followed by maturation index of 2.93 (SS/TA ratio), which indicates that the seeds had already reached the physiological maturity.

Regarding the thousand seeds mass (Table 2), the values ranged from 22.30 to 23.03 g with non-significant alteration independently of the treatment used. Also, it was observed that seeds water content was similar between the treatments (max variation 1.77 percent point) showing that seeds from all treatment had the same metabolic level.

In relation to the seed germination test, performed in the darkness and lightness conditions, there were not significant interaction between aril removal methods and light conditions (Table 3); however, the results showed that in the darkness occurred the highest germination percentage (59%) compared to lightness condition (47%). Among the aril removal methods, the natural pulp fermentation during five days was the most appropriated method to overcome physiological seed dormancy caused through the aril presence, due to the highest germination percentage (81%) followed by lime solution for 30 minutes. On the other hand, the pulp fermentation in sugar solution during 48 hours showed 37% of seeds germination, followed by friction on steel mesh sieve for 5 minutes (45%).

The seed vigor evaluated by germination speed, at darkness, higher germination speed index (2.25) than lightness conditions (1.85), indicating that at darkness the germination occurs uniform (Table 3). Agreeing with the germination results for aril extraction methods, the natural pulp fermentation during five days showed superior germination speed index compared to other methods, being just equal to lime solution for 30 minutes that was the second high germination value.

Table 2. A thousand seeds mass (TSM), coefficient of variation (CV) and water content (WC) of *Passiflora edulis* var. *flavicarpa* seeds submitted to different aril removal methods.

Treatment	TSM (g)	CV (%)	WC (%)
Friction on sieve	22.30	1.88	8.42
Friction with coarse sand on sieve	22.76	1.59	8.38
Immersion in lime solution	22.72	1.54	7.96
Pulp fermentation in sugar	23.03	1.29	7.43
Natural pulp fermentation	22.86	1.22	9.20

Table 3. Germination percentage and germination speed index (GSI) of *Passiflora edulis* var. *flavicarpa* seeds submitted to different aril removal methods and germination conditions.

Treatment	Germination (%)		Mean	GSI		Mean
	Dark	Light		Dark	Light	
Friction on sieve	50	40	45 cd*	1.86	1.43	1.65 b
Friction with coarse sand on sieve	55	44	49 bc	2.10	1.78	1.94 b
Immersion in lime solution	59	51	55 b	2.35	2.40	2.38 a
Pulp fermentation in sugar	48	26	37 d	1.91	1.20	1.56 b
Natural pulp fermentation	86	76	81 a	3.05	2.45	2.75 a
Mean	59 A	47 B		2.25 A	1.85 B	
CV (%)	12.36			14.08		

*Equal letters, lowercase in the column and uppercase in the row for each variable, do not differ by Tukey's test at 0.05 probability level; CV = coefficient of variation.

Table 4. Seedlings length test, dry matter and emergence recorded at 9, 17 and 26-day after sowing (DAS) of *Passiflora edulis* var. *flavicarpa* seedlings submitted to different seed aril removal methods.

Treatment	Length (cm)	Dry matter (mg)	Emergence (%)		
			9DAS	17DAS	26DAS
Friction on sieve	11.67 ab*	5.0 ns	43 ab	77 ns	88 ns
Friction with coarse sand on sieve	8.39 b	4.0	41 ab	82	88
Immersion in lime solution	9.97 ab	5.0	38 ab	90	94
Pulp fermentation in sugar	14.48 a	6.0	26 b	79	88
Natural pulp fermentation	11.35 ab	4.0	46 a	84	89
CV (%)	18.84	18.55	23.28	12.38	7.64

*Means followed by the same letters in the column do not differ statistically by Tukey's test at 0.05 probability level; nsnon-significant; CV = coefficient of variation.

For the length plants test, it was observed significant differences between the treatments friction with coarse sand on steel mesh sieve, for 5 minutes (8.39 cm), and fermentation in sugar solution at 10% (w/v), during 48 hours (14.48 cm), caused mainly due to the mechanical embryo damage that the coarse sand promoted during the friction. Seedlings dry matter did not differ between the treatments (Table 4), which revels that even small seedlings had similar matter compensated by the lateral roots growth.

The Table 4 presents the data recorded according to emergence test. There were performed three emergence evaluations: at 9th, 17th and 26th days after sowing (DAS). At 9th DAS, significant differences were observed between the treatments T4 and T5. On the other hand, there were not noticed differences between the treatments in the other evaluations (at 17th and 26th DAS). The values observed for seedlings emergence ranged from 88 to 94%, which can be considerate satisfactory for this species.

4. Discussion

The fruits ripening stage is a very important characteristic to determine the harvest moment and predict the seeds maturation; thus, it is important to determine and consider some ripening parameters to harvest these fruits (Table 1). Seeds collected from ripe and withered fruits presented higher germination percentage than seeds collected from ripe fruits, but not withered, 82 and 71%, respectively (Lopes et al., 2007). However, in the present study the fruits were harvested before wrinkling and the seedlings emergence were higher than 88% (Table 4), it supposes that the seeds had already reached the physiological maturity. About the chemical fruits properties, similar results were reported by Pongener et al. (2014), which were observed soluble solids content ranging from 11.45 to 13.24 and titratable acidity from 3.04 to 2.53% of citric acid, in ripe *Passiflora edulis* fruits at harvest period.

Regarding the germination and emergence tests, it is possible to observe discrepant differences between them. The germination percentages recorded at darkness and lightness conditions were lower than the results observed in emergence test, except for seeds submitted at natural pulp fermentation during five days (81%). There were observed exudates in some seeds during the germination final evaluation, which may have inhibited the germination process, whereas in the emergence test the water from irrigation probably leached the exudates, allowing more seeds to germinate. Similar result for low germination on paper substrate was reported by Ferrari et al. (2008), whose observed for *P. alata* 50% of normal seedlings even at alternating temperature (20/30 °C); however, these authors did not describe the aril removal method applied in their study.

For aril remove, there was observed that the natural pulp fermentation during five days may have reduced or degraded inhibitory substances even on paper substrate. This indicates that this method promoted the total aril removal and allowed seeds to germinate (Table 3).

Osipi et al. (2011) testing aril removal methods in *P. alata* seeds observed higher germination for friction on sieve method (45%), followed by natural fermentation during five days (23.5%) and friction on sieve using sand (9%), when the test was performed in the dark at alternating temperature (20/30 °C). On the other hand, for *P. edulis* var. *flavicarpa*, the natural pulp fermentation for aril removal did not affect the seeds germination (Martins et al., 2006; Pereira and Dias, 2000). Therefore, according to the literature and the results obtained in this study, there was observed that the effect of the method to remove the aril might vary from specie to specie.

Concerning to the light influence on seeds germination, some authors have recommended performing *Passiflora* species germination test in darkness because the constant light may inhibit the seeds germination (Benvenuti et al., 2001; Brasil, 2009; Zucareli et al., 2009, 2015). In this study was reported that constant light did not affect the seeds germination, although in light was observed the lowest germination percentages. Similar results were reported by Benvenuti et al. (2001) for *P. incarnata* in the germination test carried out at lightness and darkness; the germination percentage observed at darkness was superior to those recorded at lightness, independent the temperature used for the germination test.

Since the sour passion fruits have presented a considerably economic importance, this species has gone through the breeding programs and selections; thus, some

characteristics may have been lost, among them, the seeds darkness responses.

The seedlings emergence was observed from the 9th day after sowing, with seedlings emergence ranged from 26 to 46%. Ferreira et al. (2007) using plant growth regulator (Stimulate®) on *P. edulis* seeds, observed similar values for seedlings emergence using Rendmax as substrate. In contrast, the seedlings emergence shows to be depend of the different substrates as observed for *P. edulis*, which started at 17th days after sowing (Aguiar et al., 2014).

Regarding the seedlings final emergence results, the aril removal methods did not affect the emergence percentage. Similar results were observed by Aguiar et al. (2014) for *P. edulis* with non-significant differences between the methods used to remove the aril and the seedlings emergence, except for the mechanical method (blender), which promoted lower seedlings emergence.

These results are in disagreement to Osipi et al. (2011), whom observed a significant influence for the aril removal method on *P. alata* seedlings emergence. The high seedlings emergence was observed for the friction on sieve using sand method to remove the aril, followed by the lime, HCl and natural fermentation methods.

The differences observed between germination and emergence tests are interesting, and suppose that the effect of the aril removal methods on seeds germination are superadded by the emergence test conditions, developing higher percentages of normal seedlings (Osipi et al., 2011).

The emergence test results observed in this study (90%) and those observed by Aguiar et al. (2014), also 90%, and Santos et al. (2015), more than 90%, for the same specie are higher than the results recorded in the germination test for that and other studies e. g. Martins et al. (2006), Pereira and Dias (2000) and Santos et al. (2013). Thus, it is possible that recommend the seedlings emergence test like the most appropriate test to analyze *P. edulis* seeds quality. Although the germination test on paper is usually recommended as germination test standard for a lot of species; however, for *P. edulis* the best results were recorded in the emergence test. The aril removal methods did not affect the seedlings emergence in *Passiflora edulis* var. *flavicarpa* seeds.

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