Article Doi: 10.33158/ASB.2018v4i2p86

Yellow passion fruit tree sexual propagation by different mucilage extraction methods and substrates

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ABSTRACT

The objective of this work was to evaluate the initial development of yellow passion fruit, as a function of the method of extracting the mucilage of the seeds (washing in water, fermentation in water and fermentation in water + sugar) and substrates (carbonized rice husk, coconut fiber and vermiculite). The completely randomized design with nine treatments and five replicates, with 50 seeds was used, in a 3 x 3 factorial arrangement (three mucilage extraction methods and three types of substrates). After 56 days of sowing, the following variables were evaluated seedling emergence percentage, number of leaves, leaf area, stem length and root largest length, dry mass of shoot and roots; and substrates characteristics such as pH, electric conductivity, density and water retention capacity. The emergence speed index was evaluated daily from sowing. It is verified that, except for the number of leaves and the shoot dry matter mass, the other variables were influenced by the study factors. In general, in the seeds without fermentation and fermentation in water mixed with sugar, the highest averages were obtained, regardless of the substrate used. Thus, both mucilage extraction methods and all tested substrates are indicated in the initial development of yellow passion fruit.

Key words: *Passiflora edulis*, initial development, carbonized rice husk, coconut fiber, vermiculite.

INTRODUCTION

The passion fruit plant (*Passiflora* sp.) from the Passifloraceae family has 630 species, 95% of them originated from South America (Lima and Cunha 2004). Brazil is considered the largest producer and consumer of passion fruit worldwide, with 703.489 tons collected in 2016 (Morgado et al., 2015; IBGE 2018).

One of the most important aspects for the success in the production areas is related to the use of quality seedlings. In the case of passion fruit, despite the cuttings (Sousa et al., 2014), grafting (Alexandre et al., 2004; Meletti 2011) and micro-propagation (Santos et al., 2010) being the options for getting seedlings, sexual propagation has been widely used (Wagner Júnior et al., 2007; Aguiar et al., 2014; Silva et al., 2015; Santos et al., 2016).

Knowledge about the seeds germination process of several *Passiflora* species is essential for propagation (Passos et al., 2004). Among the factors that can interfere with the seeds germination process are water, temperature and relative air humidity (Fachinello et al., 2005). However, passion fruit has a mucilage that involves the seeds creating a physical barrier and growth regulating substances, which can affect the process of germination (Pereira and Dias 2000) and promote the development of microorganisms.

Several authors have tested methods to extract mucilage from passion fruit seeds (Cardoso et al., 2001; Martins et al., 2006; Aguiar et al., 2014; Silva et al., 2015). However, there are no reports on the fermentation of these seeds in were mixed with sugar, technique used by Lone et al. (2014) with the same purpose in an experiment with pitaya (*Hilocereus* sp.), which reaffirms the need for studies to investigate its efficacy in passion fruit plant propagation.

Another factor to be considered is the substrate, medium in which roots of plants cultivated outside the soil develop (Kämpf 2005). Materials such vermiculite, coconut fiber and carbonized rice husk were tested in fruit species propagation (Pelizza et al., 2011; Yamamoto et al., 2013; Hussain et al., 2014). However, substrate selection or materials combination must be done according to the species at hand, in function of the specific physical and chemical characteristics of each material (Fachinello et al., 2005; Faria et al., 2010; Ristow et al., 2012). It is also necessary to check the availability and material cost since such requirements can be decisive in choosing the substrate.

Based on these aspects, the adoption of adequate procedures to separate seed from mucilage can represent a promising alternative for the propagation of passion fruit plant seeds and facilitate fruit handling during this operation. In addition, Wagner Júnior et al. (2006) emphasize that, this culture establishment success depends on several factors such as the use of an adequate substrate that may have great influence on the development of good quality seeds.

The objective of this work was to evaluate the influence of methods to extract mucilage from seeds and the use of different substrates in the sexual propagation of the yellow passion fruit tree.

MATERIALS AND METHODS

The experiment was conducted from September to November, 2012, at the Fruit Farming Sector of the Agronomy Department from Londrina State University Center for Agrarian Sciences, Paraná (latitude 23°23 S, longitude 51°11 O and altitude of 566 m).

Yellow passion fruit (*Passiflora edulis* Sims. f. *flavicarpa* Degener) seeds were used to study methods of mucilage extraction (washing with running water, water fermentation and fermentation in water + sugar) and substrates (carbonized rice husk, coconut fiber Amafibra® patter 47 and vermiculite medium granules). The experimental design was entirely randomized in a 3 x 3 factorial scheme (three mucilage extraction methods and three types pf substrates), with nine treatments and five replications of 50 seeds.

Fruit that showed complete maturation were collected from a single harvest lot. After the harvest, they were sectioned transversally and the seeds submitted to mucilage extraction by the fermentation method in water and water mixed with sugar (25 g L^{-1}). To do so, the experiment used a plastic recipient with capacity for 1.0 L, where seeds remained for four days. Next, they were washed with running water on a polyethylene sieve.

After the washing, seeds were placed immediately to dry away from the sun, at room temperature (22 °C $\pm$ 0.5), for four days, on a paper towel sheet. Then, the aryl was removed from seeds, rubbing them with a flannel towel. Next, they were stored in kraft paper bags, 0.48mm thick and kept in a refrigerator at 10 °C for 10 days (Aguiar et al., 2014). After this interval, seeding was realized on expandable polystyrene trays with 128 alveoli, with two seeds per alveolus, 1.0 cm deep in the referred substrates.

The trays remained in a nebulization chamber under a controlled intermittent regime by a timer and solenoid valve, programmed to nebulize for 10 seconds at each three-minute interval. The nebulizer nozzle used (Mist Dan Sprinklers model, Israel) has a flow of 35 liters per hour. The nebulization chamber is inserted in the agricultural greenhouse covered with a transparent polyethylene film and sombrite fabric 30%. Then, when seedlings were 50 cm high, they were thinned and the most vigorous plant remained.

During the experiment, the average temperature registered in the interior of the greenhouse was 23 °C $\pm$ 2.0 and the average air relative humidity was 57% $\pm$ 0.8. Germination was evaluated after seeding by emerged seedlings percentage. Daily counting after seeding was carried out from emergence to stabilization, to assess emergence speed index. Emergence speed index was determined according to Maguire (1962).

The following variables were evaluated 56 days after seeding (Figure 1): emergence percentage, number of leaves, leaf area (cm^2), stem and greater root length (cm) and aerial part and roots dry matter mass per plant (g). Plants with open cotyledons were considered emerged (Lima et al., 2006). Leaf area determination was done from images obtained by a scanner and later by the the SisCob images analysis program, developed by Embrapa CNPDIA.

Figure 1. Yellow passion fruit tree (*Passiflora edulis*) seedlings 56 days after experiment installation. Londrina, PR, Brazil.

Stem and greater root length were measures by a graded ruler, using the distance between the neck and the apex of the seedling stem and root, respectively, as reference. During the determination of the roots and aerial part dry matter mass, they were placed to dry in a greenhouse with forced air circulation at 60 ºC, until reaching constant weight. In addition, emergence speed index was evaluated daily from seeding.

Electrical conductivity (µS cm^{-1}), density (kg m^{-3}), pH and substrates water retention capacity (mL kg^{-1}) were determined according to Kämpf et al. (2006).

Data were submitted to analysis of variance and the means compared by the Tukey test at 5% of probability. For variables originated from the percentages, data were transformed according to the equation for arcsin $\sqrt{x/100}$; and for the variables originated from the counting, data were transformed according to equation $\sqrt{x+1}$.

RESULTS AND DISCUSSION

With the exception of number of leaves, stem length and aerial part dry matter mass, the other variables were affected by the study factors (Tables 1 and 2).

As for emergence percentage, in regards to mucilage extraction methods, there was no significant difference, regardless the substrate. However, in regards to substrates, the coconut fiber showed higher seeds water fermentation mean, differing statistically from the other materials (Table 1).

A research work on the propagation of yellow passion fruit trees, Cardoso et al. (2001) found that seeds fermentation promoted greater emergence percentage. Similarly, Lopes et al. (2007) observed greater germination percentage (84.5%) in seeds submitted to total aryl removal.

Table 1. Emergence percentage (EP %), emergency speed index (ESI), number of leaves per plant (NL) and leaf area per yellow passion plant (LA (cm^2)), in function of mucilage extraction methods (MEM) and substrates. Londrina, PR, Brazil. 2012.

	MEM	Substrates		
		CRH	CF	VE
EP (%)	NF	78,40 Aa	77,20 Aa	68,40 Aa
	FW	66,40 Ba	80,00 Aa	64,80 Ba
	FWS	77,20 Aa	84,00 Aa	76,40 Aa
	CV (%)=6,10	Value of P (M)=0,024	Value of P (S)=0,007	
ESI	NF	1,75 Aab	1,72 Aa	1,45 Ab
	FW	1,45 Bb	1,91 Aa	1,45 Bb
	FWS	1,92 Aa	2,11 Aa	1,80 Aa
	CV (%)=15,37	Value of P (M)=0,002	Value of P (S)=0,004	
NL	NF	2,79 Aa	2,79 Aa	3,14 Aa
	FW	3,20 Aa	2,91 Aa	3,33 Aa
	FWS	3,20 Aa	3,03 Aa	3,10 Aa
	CV (%)=3,75	Value of P (M)=0,079	Value of P (S)=0,060	
LA (cm^2)	NF	3,17 Aa	3,42 Aa	4,10 Aa
	FW	3,92 ABa	3,26 Ba	4,30 Aa
	FWS	3,72 Aa	3,83 Aa	3,82 Aa
	CV (%)=16,73	Value of P (M)=0,462	Value of P (S)=0,039	

Means followed by the same lowercase letters in the column do not differ statistically among them by the Tukey test (P<0.05). Legend: CRH: carbonized rice husk; CF: coconut fiber; VE: vermiculite; NF: no fermentation (water washing); FW: fermentation in water; FWS: fermentation in water + sugar; (MEM): mucilage extraction methods.

According to Wagner Júnior et al. (2006), seeds germination is influenced by substrates properties such water retention capacity, aeration, among others. In the present study, one factor that could have influenced emergence was the coconut fiber greater water retention capacity (Table 3), considering that the water plays a fundamental role in triggering germination process metabolic activities (Fachinello et al., 2005) and that, in some cases, an additional supply of substrate water for seeds germination (Martins et al., 2009).

Table 2. SL (SL (cm), aerial part dry matter mass (MSPA (g)), greater root length (CMR (cm)) and roots dry matter mass (MSR (g)) of passion fruit plant, in function of mucilage extraction methods (MEM) and substrates. Londrina, PR, Brazil. 2012.

	MEM	Substrates		
		CRH	CF	VE
SL (cm)	NF	3.21 Aa	3.31 Aa	3.24 Aa
	FW	3.42 Aa	3.60 Aa	3.53 Aa
	FWS	3.39 Aa	3.58 Aa	3.52 Aa
	CV (%)=8,85	Value of *P* (M)=0.407	Value of *P* (S)=0.374	
APMM (g)	NF	0.595 Aa	0.740 Aa	0.715 Aa
	FW	0.574 Aa	0.761 Aa	0.733 Aa
	FWS	0.740 Aa	0.893 Aa	0.787 Aa
	CV (%)=24,92	Value of *P* (M)=0.122	Value of *P* (S)=0.057	
GRL (cm)	NF	5.33 Aab	5.63 Aa	5.45 Aa
	FW	5.18 Ba	5.60 Aa	5.13 Aa
	FWS	5.97 Aa	5.96 Aa	5.40 Aa
	CV (%)=7,97	Value of *P* (M)= 0.019	Value of *P* (S)= 0.051	
RMM (g)	NF	0.225 Aab	0.291 Aa	0.245 Aa
	FW	0.189 Bb	0.347 Aa	0.267 ABa
	FWS	0.339 Aa	0.427 Aa	0.302 Aa
	CV (%)=30,63	Value of *P* (M)=0.007	Value of *P* (S)=0.007	

Means followed by the same uppercase letters in the line and lowercase in the column show no statistical difference among them by the Tukey test ($P<0,05$). Legend: CRH: carbonized rice husk; CF: coconut fiber; VE: vermiculite; NF: no fermentation (water washing); FW: fermentation in water; FWS: fermentation in water + sugar; (MEM): mucilage extraction methods.

Table 3. pH, electrical conductivity (EC μS cm^{-1}), density (kg m^{-3}) and water retention capacity (WRC mL kg^{-1}) of carbonized rice husk substrates (CRH), coconut fiber (CF) and vermiculite (VE), 56 days after seeding. Londrina, PR, Brazil. 2012.

Substrates	pH	EC μS cm^{-1}	Density kg m^{-3}	WRC mL kg^{-1}
CRH	7.43	223.40	157.06	371.00
CF	6.70	266.60	109.90	760.00
VE	7.77	257.40	282.04	170.00

As for the emergence speed index, in regards to mucilage extraction methods, the highest mean was obtained from fermentation with water and sugar, showing no difference from the seeds without fermentation by using carbonized rice husk; however, there were no significant differences between the methods in coconut fiber while fermentation in water and sugar showed the highest vermiculite mean. In regards to substrates, coconut fiber showed the highest mean compared to the other materials, only for water fermentation (Table 1).

Cardoso et al. (2001) verified that fermentation promoted yellow passion fruit tree seedlings emergence sooner when compared to those originated from seeds without fermentation; while Aguiar et al. (2014), in an experiment with the same fruit tree observed that the emergence speed was superior when seeding was realized in coconut fiber and carbonized rice husk.

Martins et al. (2009) evaluated the sexual propagation of the pupunha palm tree (*Bactris gasipaes*) mentioned that the fast and uniform germination of the seeds, followed by prompt emergence are desirable during seedlings development, since the longer the seedling remains in the initial development stadia the more vulnerable it will become to the environment's adverse conditions. In addition to the factors studied in the present experiment, it is possible to infer that the temperature also influenced the time required for germination and the IVE, since it was in agreement with the values recommended for *Passiflora edulis* seeding (Ministério da Agricultura, Pecuária e Abastecimento 2009).

In regards to number of leaves, there were no statistical differences related to mucilage removal methods and substrates. However, the largest leaf area was register for vermiculite for seeds whose fermentation done in water, differing from the other substrates (Table 1). According to Cavalcante et al. (2009), during the photosynthesis period, the largest leaf area is more efficient in transporting mineral and organic solutes to vegetal tissues.

In regards to stem length and aerial part dry matter, there was no significant difference between mucilage extraction methods and substrates (Table 2). In a study carried out by Silva et al. (2010), the highest means for passion fruit tree seedlings height were observed in recipients with soil + manure, which did not differ significantly from the mix of soil + 150 mg dm^{-3} of simple superphosphate and Plantmax®.

Great root length per plant showed significant differences in regards to mucilage extraction methods only for carbonized rice husk, with fermentation with water mixed with sugar showing the greatest length the greater length, not differing statistically from the method without fermentation (Table 2).

In regards to roots dry matter mass, the extraction methods analysis showed that plants originated from seeds with water with sugar fermentation presented higher means, showing no difference from those submitted to fermentation in carbonized rice husk. On the other hand, substrates in coconut fiber showed higher mean only when submitted to water fermentation, with no difference from vermiculite (Table 2).

Findings from this study may be the result of the coconut fiber low density when compared to vermiculite and carbonized rice husk (Table 3), considering the high-density values may represent greater resistance to roots expansion in the substrate. According to Carrijo et al. (2002), the good physical properties of the coconut fiber highlight the possible use of this substrate.

In regards to the substrates chemical properties, the pH varied from 6.70 to 7.77 and such values did not interfere in the initial development of the seedlings (Table 3). Electrical conductivity, although the highest mean was found in coconut fiber (Table 3), all values are classified as an ideal for substrates from seedbeds and tray seedlings, since, according to Ballester-Olmos (1993), values between 200-350 μS cm^{-1} are appropriate for most plants. Negreiros et al. (2004) reported that a good substrate offers adequate conditions for germination and root system development of seedlings.

For the production of passion fruit tree seedlings Costa et al. (2009) recommend the mix of vermiculite with soil and an organic compound. However, in the present study, in general, both treatment without fermentation and fermentation in water with sugar showed the highest means regardless of the substrate used. However, greater practicality was observed during the removal of the mucilage after fermentation in water + sugar. In regards to substrates, the grower can opt for the most cost-effective material available in the region, considering that this is one of the most relevant items in seedlings production. In addition, agricultural residues destination represents an environmental gain in function of their reduction in the nature.

CONCLUSIONS

Mucilage extraction without fermentation and with fermentation in water mixed with sugar, as well as coconut fiber substrates, carbonized rice husk and vermiculite are recommended for yellow passion fruit tree sexual propagation.

ACKNOWLEDGEMENTS

To CAPES (Coordination for the Improvement of Higher Education Personnel) and CNPq (National Council for Scientific and Technological Development) for the financial support.

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Received: December 22, 2017.
Accepted: February 21, 2018.
Published: May 11, 2018.