Effect of fruit maturity stage and fermentation period on the germination of passion fruit (*Passiflora edulis* f. *flavicarpa* Deg.) and sweet granadilla seeds (*Passiflora ligularis* Juss.)

Efecto del estado de madurez del fruto y del período de fermentación sobre la germinación de semillas de maracuyá (*Passiflora edulis* f. *flavicarpa* Deg.) y granadilla (*Passiflora ligularis* Juss.)

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ABSTRACT

In Colombia, we have found 165 species that belong to the *Passiflora* genus and, of these, about 50% have edible fruits. Among these, passion fruit, sweet granadilla and purple passion fruit are considered to be of economic importance. This study aimed to evaluate the effect of fruit ripeness and pulp fermentation time period (mucilage) on the seed germination of passion fruits (*Passiflora edulis* f. *flavicarpa* Deg.) and sweet granadillas (*Passiflora ligularis* Juss.) under laboratory conditions (alternating temperatures (30/20°C, 12/12 hour), 60% relative humidity and in darkness for 60 days) and to establish growth parameters leaf area and plant dry mass. The seeds were extracted from fruits in three maturity stages (M4, M5 and M6) and five fermentation periods (0 days (F0);1 day (F1), 2 days (F2), 4 days (F4) and 8 days (F8)) using a completely random design with a 3x5 factorial arrangement (maturity stage x fermentation time), with four replicates of 50 seeds each. It was found that the M6F1 and M5F1 treatments were more favorable in terms of germination for the passion fruit (94.5 and 92.0%, respectively), while, for the sweet granadilla, the M6F2 (39%) and M6F1 (35%) treatments were more favorable. The growth parameters demonstrated that the highest dry mass value occurred in M6F8 for the passion fruit (136.76 mg) and in M6F1 for the sweet granadilla (104.73 mg); furthermore, these values increased as the maturity stage of the two species progressed. The leaf area of the passion fruit had maximum values in seedlings obtained from the treatment with maturity stage M5F2 and a fermentation period of 1 day, M5F2 (18.3 cm^2), while the leaf area of the sweet granadilla increased in the same fashion as the dry mass, with M6F8 having the highest value (16.8 cm^2).

Key words: tropical fruits, germinability, breeders seed, ripening, mucilages, agronomic characters.

RESUMEN

En Colombia, se ha encontrado un grupo de 165 especies pertenecientes al género *Passiflora* y de ellas cerca del 50% son de fruto comestible. Entre estas el maracuyá, la granadilla y la gulupa se consideran de importancia económica. El objetivo de este estudio fue evaluar el efecto del estado de madurez del fruto y del tiempo de fermentación de la pulpa (mucílago) sobre la germinación de semillas de maracuyá (*Passiflora edulis* f. *flavicarpa* Deg.) y granadilla (*Passiflora ligularis* Juss.) en condiciones de laboratorio (temperaturas alternas 30/20°C (12/12 horas), humedad relativa del 60% y en oscuridad durante 60 días) y establecer los parámetros de crecimiento área foliar y masa seca de la planta. Las semillas utilizadas fueron extraídas de frutos en tres estados de madurez (M4, M5 y M6) y cinco tiempos de fermentación (0 días (F0); 1 día (F1), 2 días (F2), 4 días (F4) y 8 días (F8)), utilizándose un diseño completamente al azar con arreglo factorial 3x5, (estado de madurez x tiempo de fermentación), con cuatro repeticiones de 50 semillas cada uno. Se encontró que los tratamientos M6F1 y M5F1 fueron los más favorables para maracuyá en cuanto a germinación (94,5 y 92,0%, respectivamente), mientras para granadilla fueron los tratamientos M6F2 (39%) y M6F1 (35%). Los parámetros de crecimiento que se encontraron, muestran que el valor más alto de masa seca en maracuyá se da en M6F8 (136,76 mg) y en granadilla en M6F1 (104,73 mg); además, esos valores aumentan conforme se incrementa el estado de madurez para las dos especies. El área foliar en maracuyá encuentra valores máximos en plántulas obtenidas desde el tratamiento con estado 5 de madurez y tiempo de fermentación de 1 día M5F2 (18,3 cm^2), mientras que el área foliar en granadilla crece del mismo modo que en masa seca, siendo el valor más alto M6F8 (16,8 cm^2).

Palabras clave: frutas tropicales, poder germinativo, producción de semillas, madurez, mucílagos, características agronómicas.

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Introduction

Colombia has the greatest diversity of Passifloraceae, reporting 167 species that are concentrated in the Andean region, mainly in the departments of Antioquia, Valle del Cauca and Cundinamarca (Ocampo *et al*., 2007). It is also the country with the greatest diversity of species grown commercially, with two passion fruit types, yellow (*Passiflora edulis* f. *flavicarpa* Deg.) and purple (*P. edulis* f. *edulis* Sims), the banana passion fruit (*P. tripartita* var. *mollissima* Holm-Nielsen & Jørgensen), the sweet granadilla (*P. ligularis* Juss.), and the sweet calabash (*P. maliformis* L.), among others. Brazil, with over 120 species, and Ecuador and Peru, with more than 80 species, follow Colombia although these countries mainly cultivate the yellow passionfruit (Coppens D'Eeckenbrugge, 2003).

The propagation of passion fruits and sweet granadillas can be sexual, through seeds, or asexually, through grafting, cuttings or *in vitro*. The sexual method takes precedence over the asexual methods due to a shorter waiting period for seedling growth although problems related to the physiological quality of seeds, such as uneven germination, directly affect the formation of seedlings (Wagner Júnior *et al*., 2006). Therefore, sexual reproduction is used more due to the low cost and ease of obtaining more vigorous plants with better root formation and more productivity, as compared to those propagated asexually (Rivera *et al*., 2002).

The germination process includes those events that are initiated by the absorption of water by dry seeds (imbibition) and end with the elongation of the embryonic axis. The process ends when the radicle passes through the structures surrounding the embryo, which is often referred to as "visible germination". Usually, this process is divided into three phases: in phase I, imbibition can be observed, which involves the absorption of water needed for the rehydration of proteins; in phase II, activation of the seed metabolism occurs, where the synthesis of nucleic acids and proteins is carried out; and, finally, in phase III, the emergence of the radicle (visible growth) occurs, thus concluding the germination process (Herrera *et al*., 2006).

In relation to good quality seeds, one aspect to consider is the timing of the harvest. This timing can be determined by following the development of the fruits and/or seeds, using their physical and physiological traits (Wagner Júnior *et al*., 2006). The sweet granadilla and yellow passion fruit are climacteric fruits and so can be collected in early maturity stages (Hernández and Fischer, 2009) and, in accordance with the target market, one can accelerate or retard the ripening process so that the fruits arrive with optimal ripeness at the destination. Furthermore, the ripening rate is influenced by the agro-ecological conditions and management that are found in the crop. Therefore, this timing is only a guide or reference and not an absolute value (García, 2008). The seed, in its period of development, must complete certain steps to be considered viable. If the fruit is collected before the seeds mature, there can be consequences, such as a lack of proper development for the endosperm or reserves, resulting in an embryo with delayed or arrested development, which could result in its abortion (Hartmann and Kester, 2002).

In *Passiflora* species, studies have shown that passion fruit and sweet granadilla seeds obtained from a more mature state have a higher percentage of germination and normal seedlings; in addition, the complete removal of the seed aryl promotes germination and reduces the number of hard seeds (Maciel and Bautista, 1997; Lopes *et al*., 2006; Giraldo and Aristizábal, 2008).

Plant germination is negatively influenced by the action of growth regulating substances present in the aryl, which surrounds the seeds and, considering the fact that it contributes to uneven germination, should be properly removed to obtain maximum germination with rapid seedling onset (Pereira and Dias, 2000; Irigoyen and Cruz, 2005).

In Colombia, few studies have been conducted to evaluate the effect of fruit ripeness and fermentation period for passion fruits (*P. edulis*) and sweet granadilla (*P. ligularis*); therefore, this study aimed to do just that for the germination of passion fruits (*P. edulis*) and sweet granadilla (*P. ligularis*) under laboratory conditions.

Materials and methods

Plant material

The yellow passion fruit and sweet granadilla fruits were collected and selected from farms in the municipalities of Suaza and Palestina (department of Huila - Colombia), respectively. Afterwards, the fruits were transported to the plant physiology laboratory of the Faculty of Agricultural Sciences at the Universidad Nacional de Colombia, Bogota.

The passion fruit and sweet granadilla fruits were collected from three maturity stages (M4, M5 and M6), as established for each fruit by the *Instituto Colombiano de Normas Técnicas* (Icontec Spanish abbreviation), following the NTC 4101 standard for *P. ligularis* and NTC 1267 standard for *P. edulis* (Fig. 1 and 2).

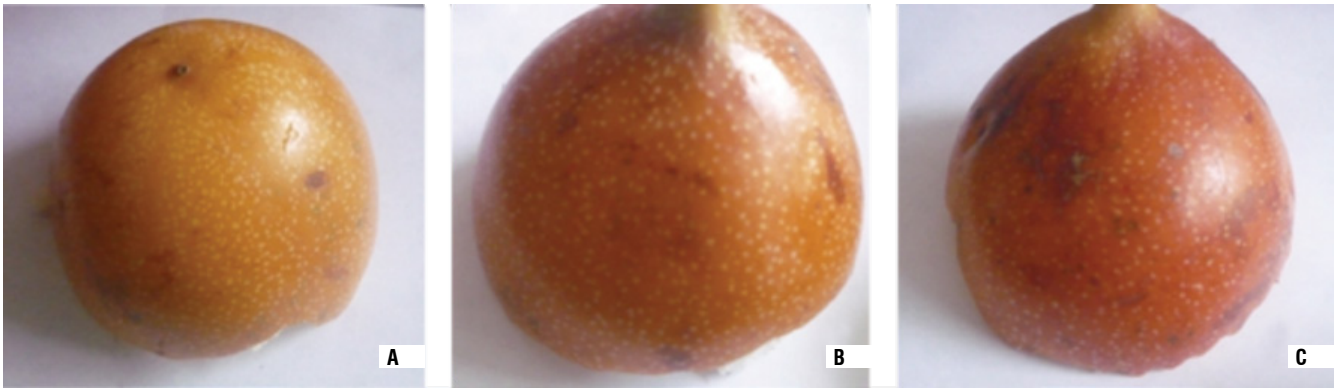

FIGURE 1. Sweet granadilla fruits in different maturity stages. A, maturity stage 4 (M4); B, maturity stage 5 (M5); C, maturity stage 6 (M6) (NTC 4101 - Icontec, 1997).

FIGURE 2. Passion fruits in different maturity stages. A, maturity stage 4 (M4); B, maturity stage 5 (M5); C, maturity stage 6 (M6) (NTC 1267 - Icontec, 1979).

Fermentation of pulp and seed extraction

The pulp was extracted from the selected fruits, poured into five different glass flasks and left to ferment (at 18-20°C) in its juice for 0 days (F0), 1 d (F1), 2 d (F2), 4 d (F4) and 8 d (F8). The flasks were placed in a well-ventilated and dark area. The F0 fermentation treatment had the aryl removed immediately after the fruits were opened. After the pulp fermentation of each treatment, the pulp was washed in a strainer with abundant water until the mucilage was completely removed and the seeds that presented buoyancy were eliminated. Subsequently, the seeds were placed on absorbent paper and left to dry (at 18-20°C) in darkness for 120 h. The initial seeds that completed the fermentation period were stored with carboxamide and dithiocarbamato fungicides (Vitavax® 300, Proficol, Bogota) in dose 1.5 g per 1 kg of seeds in order to protect them from fungal attacks so that, in the end, all of the seeds completed the fermentation period and were ready for sowing (Rivera *et al*., 2002).

Seed germination

Germination includes the emergence and development of the embryo and all of structures that are essential for the production of a normal plantlet under favorable conditions (AOSA, 2000). Selected seeds were disinfected with Vitavax® 300, they were subjected a total of fifteen treatments, four replicates each, using 50 seeds per replicate. Then they were arranged in Petri dishes on Munktell paper filters with 2 mL of distilled water and taken to a phytotron (Conviron CMP 3244, Winnipeg, Canada) under alternating temperatures of 30/20°C (12/12 h), relative humidity of 60% and in darkness for 60 d. The filter paper was changed weekly and the seed germination was evaluated three times a week, recording seed germinated if the radicle had a length of 5 mm (Delanoy *et al*., 2006). The percentage of germination (GP), mean germination time (MGT) and mean speed of germination (MSG) were calculated according to Ranal and Santana (2006), Deaquiz-Oyola *et al*. (2008) and Cárdenas (2011).

$$PG = (N / NS) \times 100 \quad (1)$$

where, N number of germinated seeds and NS total number of seeds.

$$MGT = \sum_{i=1}^{k} n_i \times t_i / \sum_{i=1}^{k} n_i \quad (2)$$

where, t_i time since the experiment started with the *i-th* observation (day or hour); n_i number of germinated seeds in time (*i-th*) and k is last germination.

$$MSG = \Sigma (n_i / t_i) \quad (3)$$

where, n_i number of seeds germinated on the *i*-th day and t_i time in days for germination on the *i-th* day.

Viability test

A tetrazolium test was used to compare the viability of the seeds. The viable seeds included those that were able to produce normal plantlets in a germination test under favorable conditions after dormancy was interrupted and, if they were affected by diseases, after the seeds were disinfected (ISTA, 1999). At the end of the treatments, the percentage of viable seeds was determined with the non-germinated seeds. One hundred seeds were used in four replications, which were first imbedded in distilled water (ISTA, 1996). The seeds were cut lengthwise at one of the extremes with a scalpel before immersion in a solution of 2,3,5-Triphenyl-2H-tetrazolium chloride (0.5% w/v) for 24 h under dark conditions following the methodology of Cárdenas (2011). The seeds were classified as viable if the embryo and cotyledons presented an intense red color and as non-viable if the embryos did not present this color.

Experimental design and statistical analysis

The evaluated treatments included seeds extracted from fruits in three maturity stages (M4, M5 and M6) and five fermentation periods, in days, (F0, F1, F2, F4 and F8) in germination tests carried out at alternating temperatures 30/20°C (12/12 h), relative humidity of 60% and dark under laboratory conditions, using a completely randomized design with a 3x5 factorial arrangement (maturity stage × fermentation period) and four replications of 50 seeds each for a total of 15 with treatments by species, where the first factor corresponded to the degree of maturity of the seed and the second one corresponded to seed fermentation time. The data that did not show normality or homogeneity were transformed according to root(x) (Molinero, 2003). Then, they were subjected to analysis of variance and a Tukey multiple comparison test of means with a significance level of 5% using the statistical package SAS® (Statistical Analysis System), v. 9.2 (SAS Institute, Cary, NC).

Results and discussion

Yellow passion fruit

Seed germination. There were significant differences between the seed germination percentages in terms of the fruit ripeness and the fermentation period. Between the maturity stages M4 and M5, the change in the fermentation period from 4 to 8 d represented decreases in germination of between 11 and 12%, respectively, while maturity stage M6 produced a germination increase of 19.5% (Tab. 1). The passion fruit seed germination started at 12 d after the start of the experiment, a result similar to that of Negreiros *et al*. (2006), who reported that passion fruit germination occurs at 10 d (during the first 2 weeks) after seeds are sown. Ocampo *et al*. (2011) found that the germination of passion began at 14 d after temporary storage. The mean speed of germination (MSG) passion fruit obtained a value of 2, indicating that the seeds should not be planted after removal of the fruit. Almeida *et al*. (1988) explained that is phenomenon could be due to the fact that seeds from fruits with a high degree of maturity can present a reduction in the production rate of autocatalytic ethylene, which could possibly promote some physiological changes in seeds, favoring the germination process.

Almeida *et al*. (1988) demonstrated that viable passion fruit seeds obtained from fruits that had less than 70 d of growth (low degree of fruit maturity) presented a low GP, as occurred in the M4F0 and M4F1 treatments. The differences between the percentages of viability and germination could be due to the presence of seed dormancy, fungal infection of the seeds and deterioration of the seeds during the germination test (Moreno, 1984) possibly due to external contamination. Other studies have demonstrated that *Passiflora* sp. seeds have an exogenous dormancy, the result of a possible combination of chemical and mechanical dormancy (Ellis *et al*., 1985; Bewley and Black, 1995; Hartmann and Kester, 2002; Schmidt, 2000; Delanoy *et al*., 2006). If fruits are harvested before seed maturation, there could be a lacking development of the endosperm (reserves); as a result, the growth and development of the embryo could be delayed or arrested (Hartmann and Kester, 2002).

The high percentage of germination was possibly associated with the effect of the alternating temperatures that the seeds were subjected to (30/20°C, 12 h each), a result similar to that obtained by Pereira and Andrade (1994) for (*P. edulis*) and by Aular *et al*. (1996) for passion fruits, which demonstrated a significant increase in the germination of the seeds subjected to alternating temperatures of 30-20°C, and to the results obtained by Severin *et al*. (2004), who, at

TABLE 1. Percentage of germination, mean germination time (MGT) mean speed of germination (MSG), and seed viability of *Passiflora edulis* f. *flavicarpa* Deg., germinated in darkness at alternating temperatures of 30/20°C, and growth parameters leaf area and plant dry mass.

Treatment	Germination (%)	MGT (d)	MSG (seed germ/d)	Viability (%)	Dry mass (mg)	Leaf area (cm^2)
M4F0	20.50 e	33.24 ab	1.28 f	93.90 a	103.50 b	13.50 b
M4F1	29.50 e	27.10 cd	1.56 ef	64.10 cd	130.67 a	14.62 b
M4F2	62.00 a	26.35 cd	4.48 a	81.60 b	103.80 b	15.75 a
M4F4	59.50 ab	25.35 cde	4.11 ab	79.20 bc	111.57 ab	14.25 b
M4F8	48.50 cd	34.36 a	3.02 cd	56.30 e	123.52 ab	15.12 a
M5F0	41.50 de	33.12 b	2.71 ef	82.30 b	111.47 b	15.15 a
M5F1	92.00 a	32.63 cd	7.20 a	95.50 a	125.00 a	16.15 a
M5F2	73.00 bc	33.98 a	5.00 bc	91.70 ab	131.10 a	18.30 a
M5F4	51.00 d	33.45 ab	3.49 d	88.40 b	125.93 a	17.00 a
M5F8	39.00 e	33.57 ab	2.10 ef	91.00 ab	123.63 a	15.37 a
M6F0	48.00 de	32.64 c	2.86 d	41.80 c	103.48 a	13.37 a
M6F1	94.50 a	32.29 c	6.07 a	95.00 a	128.63 a	16.12 a
M6F2	91.00 ab	31.43 d	5.54 ab	86.70 ab	118.00 a	13.37 b
M6F4	56.50 de	34.84 a	5.50 ab	92.10 ab	133.78 a	16.62 a
M6F8	76.00 bc	33.81 ab	4.06 c	94.00 a	136.76 a	14.12 b

MGT, mean germination time; MSG, mean speed of germination.

Means with different letters in same column indicate significant differences according to the Tukey test ($P \leq 0.05$).

the biological level, suggested that alternating temperatures greatly favor the *in vitro* germination of cut *Passiflora* seeds (*Passiflora caerulea* L.).

Although this study considered three higher maturity stages (4, 5 and 6), the fermentation period used in each treatment demonstrated favorable effects on the germination because, apparently, the presence of germination inhibitors decreased and inorganic salts and organic substances that inhibit germination were eliminated (Manica, 1981; Echeverría, 1997). Studies on the passion fruit have demonstrated the effect of the presence of aryl in the seeds, finding a higher germination percentage when the seeds are subjected to complete removal of the aryl, as compared to seeds with the aryl; furthermore, there is evidence that the presence of inhibitors and abscisic acid in the aryl of seeds interfere with the germination process (Lopes *et al*., 2006). Studies by Mendiondo and Garcia, (2008), in *P. caerulea* found that, after fermentation, germination was higher observed in seeds without the aryl, while no significant differences were observed in seeds with the aryl, and the aryl in seeds without mechanical scarification plus.

Furthermore, it was evident that the fermentation period of 8 d reduced the germination percentage of the passion fruit, but this response was associated with the fruit maturity that was used, presenting reductions in maturity stages 4 and 5, but not in 6 (Tab. 1); this fermentation period may seem excessive and unconventional due to the accumulation of substances that damage the embryo or because the change produced by the fermentation makes the seed suffer damage due to anoxia (Duarte and Alvarado, 1997). This result agrees with that found for papaya (*Carica papaya*) by Ávila (2007), who noted a decrease in the percentage of germination in treatments fermented for more than 5 d, attributable to the fact that the period to which the seeds were subjected in the fermentation process was too long and that there was a toxic effect due to the formation of alcohols or anoxia processes. According to observations, an overexposure to fermentation will decrease a seed's survival capacity due to a period of oxygen deprivation during which the carbon reserves are used as an energy source. This process has an energy yield that is less than that of respiration metabolism and is limited by the reserve quantities of the seeds (Aravena *et al*., 2010).

Mean germination time. The MGT of the passion fruit seeds presented significant differences between the evaluated treatments. For maturation stage M4, the lowest germination time corresponded to the seeds that had a fermentation period of 4 d (25.35) and the highest time (34.36) corresponded to the seeds fermented for 8 d. In stage M6, the lowest MGT (31.43) occurred in the seeds fermented for 2 d and the highest time (34.84) was seen in the seeds with

fermentation for 4 d. The passion fruit MGT responded to the fermentation in the earlier maturity stages, M4 and M5, but, in M6, there was a decrease in the MGT. In cases with a morphological-type endogenous latency, this response could be associated with rudimentary embryos, embryos that are more than a pre-embryo embedded in the endosperm, at the time of fruit maturation and the existence of chemical germination inhibitors present in the endosperm, which become particularly active at high temperatures (Varela and Arana, 2011).

Mean germination rate. The MGR presented significant differences in the evaluated treatments. For the M4 maturation stage, the lowest MGR (1.28 seeds germinated per day) was obtained when the seeds were not fermented, while the highest MGR (4.48 seeds germ/d) corresponded to the seeds that were fermented for 2 d. For the M6 maturation stage, the lowest MGR (2.86 seeds germ /d) was obtained when the seeds were not fermented and the highest MGR (6.07 seeds germ/d) corresponded to the seeds fermented for 1 d. For the M6 seeds, increasing the fermentation period from 4 to 8 d produced a slight decrease: 5.50 seeds germinated/day to 4.06 seeds germinated/day. The passion fruit seeds were more sensitive to the increase in the fermentation period, with the increase from 4 to 8 d in the fermentation process resulting in a reduction of the MGR of between 26 and 40%.

Seed viability. For the seeds obtained from the M4 fruits, the viability was reduced by 22.9% when the fermentation was increased from 4 to 8 d. For the seeds obtained from the M5 maturation stage, the lowest viability was obtained with the seeds that were not fermented (82.3%) and the highest viability (95.5%) was seen when the seeds were subjected to fermentation for 1 d. For M6, the lowest viability was seen when the seeds were not fermented (41.8%) and the highest viability (95%) occurred in the seeds fermented for 1 d. The increase from 4 to 8 d in the fermentation period increased the viability from 92.1 to 94.0%, which represented a 1.9% increase (Tab. 2).

Total dry matter of plantlets. The gain in total dry mass achieved by the passion fruit plantlets presented significant differences in the treatments in terms of the degree of maturation and fermentation period of the seeds.

Dry mass. There were significant differences in the plantlets that came from seeds subjected to treatments with a maturation stage of 4 (M4), according to the Tukey test. The lowest accumulation (103.5 mg) was reached by the plantlets whose seeds were not fermented (F0), while the highest accumulation (130.6 mg) was seen when the 1 d of fermentation; the accumulation in M5 of dry mass went from 111.5 to 123.5 mg, which represented an increase of 10.7%. For the plantlets obtained from fruits with a maturation stage of 6 (M6), the lowest accumulation of dry mass (103.48 mg) corresponded to the plantlets whose seeds were not fermented (F0) and the highest accumulation (136.76 mg) was seen in the seeds that were fermented for 8 d; the change from 4 to 8 d of fermentation increased the accumulation of dry mass by 2.9% (Tab. 1).

Leaf area. This variable presented significant differences between the treatments (Tab. 1). The smallest leaf area in the passion fruit plantlets was obtained with the seeds with a maturation stage of 4 (M4), 13.5 cm^2, and that were not fermented, while the highest, 15.75 cm^2, corresponded to the plantlets of seeds that were fermented for 2 d. For the plantlets obtained from seeds of fruits with a maturation stage of 6 (M6), the smallest leaf area, 13.37 cm^2, occurred in the plantlets whose seeds were not fermented (F0) and the largest area (16.12 cm^2) was seen in the plantlets with seeds that were fermented for 4 d (F4). The increase in the fermentation period from 4 to 8 d resulted in a reduction of the leaf area, from 16.62 to 14.12 cm^2, which represented a reduction of 2.5% (Tab. 1).

Sweet granadilla

Seed germination. There were significant differences between the percentages of germination of the seeds in terms of fruit maturation degree and the fermentation period. The percentage of germination (PG) reported for the sweet granadilla is highly variable, from 20% (Salazar, 2000) to 72.2% (Romero, 2000). None of the treatments reached 40% germination, possibly due to the presence of immature embryos with little growth (Tab. 2).

The lowest percentage of germination in the M5 maturation stage (11%) corresponded to fermentation for 2 d and the maximum percentage (31%) occurred when the seeds were fermented for 1-d. In the M6 maturation stage, the lowest germination was obtained without fermentation (10%) and the highest germination (39%) was seen with fermentation for 2 d (Tab. 2). Between the M4 and M5 stages of maturation, the change in the fermentation period from 4 to 8 d resulted in increases in the germination of between 4 and 6%, while the M6 stage of maturation produced an increase in the germination of 14% (Tab. 2). This result agrees with the findings of Salazar (2000), where the sweet granadilla with the highest degree of maturation presented the highest percentage of germination; similar results were demonstrated by Giraldo and Aristizábal (2008), where seeds

TABLE 2. Percentage of germination, mean germination time, mean germination rate, and seed viability of the sweet granadilla, germinated in darkness and alternating temperatures of 30/20°C, and plantlet growth.

Treatment	Germination (%)	MGT (d)	MGR (seed germ/d)	Viability %	Dry mass (mg)	Leaf area (cm^2)
M4F0	21.00 a	28.10 a	0.82 a	91.30 a	88.80 ab	16.00 a
M4F1	17.00 bc	27.61 ab	0.76 bc	87.50 ab	97.13 a	15.2 b
M4F2	15.00 bc	23.42 c	0.73 bc	55.00 cd	98.33 a	15.00 b
M4F4	12.00 bc	28.60 a	0.38 d	87.80 ab	91.95 a	16.40 a
M4F8	16.00 bc	20.42 c	0.83 a	56.20 c	84.30 b	14.62 bc
M5F0	13.00 bc	27.66 a	0.53d	41.50 c	87.12 b	14.80 bc
M5F1	31.00 a	27.00 ab	1.46 a	78.60 ab	95.28 a	13.20 c
M5F2	11.00 d	21.39 c	0.39 e	93.00 a	95.98 a	16.90 a
M5F4	17.00 c	27.55 ab	0.67 d	94.20 a	101.70 a	15.30 b
M5F8	23.00 b	28.38 a	1.00 c	87.40 ab	92.13 a	14.80bc
M6F0	10.00 d	13.32 d	0.53 d	84.70 b	93.10 b	15.40 b
M6F1	35.00 ab	28.52 a	1.79 b	97.30 a	104.73 a	14.90 bc
M6F2	39.00 a	29.13 a	2.33 a	96.00 ab	100.15 a	17.30 a
M6F4	20.00 c	26.56 ab	0.99 c	73.30 c	103.08 a	16.10 a
M6F8	34.00 ab	27.71 a	2.07 a	87.60 b	101.45 a	16.80 a

MGT, mean germination time; MSG, mean speed of germination.

Means with different letters in same column indicate significant differences according to the Tukey test ($P \leq 0.05$).

of the sweet granadilla (*P. ligularis*) and banana passion fruit (*Passiflora mollisima*) from fruits with 100 and 75% maturity presented superior percentages of germination. Walck (2002) indicated that recently matured seeds of some species have very small embryos (immature in terms of the size of the seed) and have a lot of endosperm and that, when these embryos still have distinguishable cotyledons and radicles, they must grow to a required length before the radicle emerges from the seed. Before a seed can germinate, it must pass through a number of phases, which include the availability of reserves in the seed. These reserves include starch, proteins, lipids, and nutrients that become available for the embryo through specific enzymatic actions and biochemical pathways of transformation (Miransari and Smith, 2014). According to Echeverría (1997), a seed that has not finished its development will lack reserves in the endosperm, resulting in an embryo with an arrested development and possible abortion, which could explain the low percentages of germination.

It is important to note that some studies suggest that *Passiflora* seeds have erratic germination and a prolonged dormancy, characteristics that make propagation difficult (Siqueira and Pereira, 2001; Pereira *et al*., 2008).

Mean germination time. The MGT of the granadilla seeds presented significant differences between the evaluated treatments. For the M4 stage of maturation, the lowest germination time corresponded to the seeds with 8 d of fermentation (20.42) and the highest time (28.6) occurred in the seeds that were fermented for 4 d. Increasing the fermentation from 4 to 8 d resulted in decreases in the MGT of 18.18%. For M6, the lowest MGT (13.32) occurred in the seeds without fermentation and the highest time (29.13) was seen in the seeds with 2 d of fermentation. The change in the fermentation from 4 to 8 d produced increases in the MGT of 1.15% (Tab. 2). For the sweet granadilla, the MGT in the M4 fruits saw reductions in the prolonged periods of fermentation and, in the M5 and M6 fruits, these periods produced slight increases (3.0-4.1%).

Mean germination rate. For the M4 stage of maturation, the lowest MGR (0.38 seeds germinated/d) was obtained when the seeds were fermented for 4 d (F4), while the highest MGR value (0.83 seeds germ/d) corresponded to the seeds that were fermented for 8 d. For the M6 stage of maturation, the lowest MGR (0.53) was obtained when the seeds were not fermented and the highest MGR (2.33 seeds germ/d) corresponded to the seeds that were fermented for 2 d. In this case, the M6 seeds produced an increase, 0.99 to 2.07 seeds germ/day, when the fermentation period was increased from 4 to 8 d (Tab. 2). The sweet granadilla responded with increases in the MGR in relation to the

stage of maturation (M4<M5<M6) and the increase in the fermentation period (F4<F8).

Seed viability. The percentage of viable seeds presented significant differences between the treatments with the following behavior: for the seeds obtained from fruits with an M4 stage of maturation, the viability decreased by 31.6% when the fermentation was increased from 4 to 8 d. For the M6 stage of maturation, the lowest viability was seen when the seeds were fermented for 4 d (73.3%) and the highest viability (97.3%) was seen in the seeds fermented for 1 d. Increasing the fermentation from 4 to 8 d increased the viability from 73.3 to 87%, which represented a 14.3% increase (Tab. 2). In the sweet granadilla, seed viability is higher when extraction occurs through fermentation (Miranda, 2009), which was not seen in the entirety of this study, which could be related to the fact that the lowest viability was seen in the M5F0 treatment. Cases that present percentages of germination below the percentage of viability (M4F0 for the sweet granadilla) also indicate the presence of latency in the seeds. In stone fruit trees, seeds lose their viability if they are left to ferment in their juice; to the point that viability is greatly reduced even if they are left to ferment in their juice for only 24 h.

Dry mass accumulated by the plantlets. The sweet granadilla presented significant differences between the treatments for this variable. In the treatment with plantlets obtained from seeds of M5 fruits, the lowest accumulation, 87.52 mg, was seen in the seeds without fermentation and the highest accumulation (101.7 mg) occurred with seeds with 4 d of fermentation. When the fermentation was increased from 4 to 8 d, the accumulation of dry mass by the plantlets was reduced by 9.1%. For the plantlets obtained from M6 fruits, the lowest accumulation of dry mass (93.1 mg) corresponded to the plantlets whose seeds were not fermented (F0) and the highest accumulation (104.7 mg) was seen when the seeds were fermented for 1 d. Increasing the fermentation from 4 to 8 d decreased the accumulation of dry mass by 1.63% (Tab. 2). According to Carvalho and Nakagawa (2000), seeds that do not fully mature can germinate, but they will not produce plantlets that are as vigorous as those collected at the appropriate maturation point; a fact that was observable in the present study.

Leaf area. The smallest leaf area in the sweet granadilla plantlets was obtained in seeds with a maturation stage of 4 (M4) and was 14.62 cm^2, occurring in seeds fermented for 8 d (F8), while the largest area, 16.4 cm^2, corresponded to the plantlets of seeds fermented for 4 d. Increasing the fermentation from 4 to 8 d generated decreases in the leaf area, from 16.4 to 14.62 cm^2; this represented a decrease of 1.78%. For the plantlets obtained from the seeds of M6 fruits, the smallest leaf area, 15.4 cm^2, was seen in the plantlets whose seeds were not fermented (F0) and the largest area (17.3 cm^2) occurred in the plantlets of seeds fermented for 2 d (F2); increasing the fermentation period from 4 to 8 d increased the leaf area by 0.7% (Tab. 2).

The leaf area of the plantlets did not present significant differences among treatments. The responses seen when increasing the fermentation from 4 to 8 d represented a reduction of 10.8 and 3.2% when the seeds were extracted from fruits with a maturation stage of 4 and 5 (M4 and M5), respectively. These results agree with those of Negreiros *et al.* (2006), where the 2 and 3 maturation stages presented higher means for the variables of total dry mass and number of leaves.

Conclusions

Conditions of darkness and alternating temperatures (30/20°C) in a laboratory can result in increases in the percentages of germination of passion with treatments involving the 5 and 6 maturation stages and a fermentation process. Aryl removal is important in this process because any germination inhibiting substances that may be present in the aryl will not interfere with the germination process.

The viability test of the non-germinated seeds showed that, despite the high viability percentages, no high germination percentages were generated, which could indicate the presence of exogenous dormancy, as seen in the seeds of some Passiflora species.

The growth parameters displayed a direct relationship with the state of maturation; a greater dry mass was found in the seedlings that came from seeds with a higher degrees of maturity.

The percentages of germination were higher for the passion fruit than for the sweet granadilla. The germination due to the effect of the fermentation of the seeds caused decreases in the germination in M4 and M5 and increases of 25% for the M6 maturation stage.

The mean germination time, MGT, of the seeds subjected to the treatments from 4 to 8 d was higher for the passion fruit than for the sweet granadilla. Similarly, the MGR was higher for the passion fruit seeds.

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