Comparison of Seed Treatments on the Germination of Seven Passion Fruit Species

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ABSTRACT

Passiflora is a large genus in the family Passifloraceae Juss. ex DC. Many Passiflora species are propagated by seed. However, seeds are often slow to germinate and have low germination rates due to seed dormancy factors. This study was conducted to evaluate four different pre-germination treatments on enhancing germination potential in seven Passiflora spp. Germination was monitored every 3 days for 90 days. Germination started after two weeks and then, a gradual increase was observed in germination in most species. Passiflora laurifolia L. showed maximal germination percentage (75%) with scarification plus fermentation; thus, it is the recommended treatment for this species. The highest germination rate was obtained for Passiflora maliformis L. at 0.23 in scarification plus GA3. For P. maliformis, scarification in combination with GA3 was the most effective treatment, resulting in a germination percentage of 40%. Passiflora tripartita var. Mollissima showed highest germination percentage when soaked in water or scarified plus GA3 (30%). Scarification alone resulted in the best germination percentage in Passiflora ligularis Juss. (30%). No unique pre-germination treatment resulted in complete germination for all species. When compared to results from previous research, Passiflora edulis f. edulis Sims. and Passiflora incarnata L. did not germinate at acceptable levels, whereas similar germination percentages in P. tripartita var. mollisima, P. maliformis, and P. ligularis depended on treatment. Further research is needed to determine dormancy types present in these species and the best treatment to overcome them.

Keywords

Endogenous, Exogenous, Granadilla, Maypop, Passiflora, Seed dormancy

Introduction

Species within the Passifloraceae family are primarily native to regions with tropical climates. Passiflora is a large genus in the family Passifloraceae consisting of approximately 500 species, most of which are cultivated for edible fruits, pharmaceutical properties, and ornamental characteristics (Vanderplank, 1996). Most species are herbaceous, perennial vines with a rapid growth rate. Some of them like maypop (P. incarnata) are considered weeds due to their rampant growth (Wehtje et al., 1985).

Passiflora vines can be propagated sexually through seeds or asexually by cutting, layering, and grafting. Many Passiflora species are propagated by seed (Delanoy et al., 2006). Seeds germinate slowly and have low germination rates due to seed dormancy factors. Untreated seeds of various species
may require two weeks to several months to germinate (Osipi and Nakagawa, 2005).

Seed dormancy is a strategy that allows seeds to avoid germination under conditions that are unfavorable for seedling establishment and plant survival (Finch-Savage and Leubner-Metzger, 2006). Seed dormancy is divided into two major categories: exogenous and endogenous. Exogenous dormancy is caused by factors outside of the seed’s embryo, such as the seed coat, and it is classified into three areas: physical dormancy caused by a seed coat impermeable to oxygen and/or water, mechanical dormancy caused by a seed covering that does not allow the embryo to expand, and chemical dormancy related to inhibitors within the seed coat. Endogenous dormancy occurs due to factors in the embryo. Seeds of some species which have both exogenous and endogenous dormancy need treatments to overcome the impermeable covering first, and then for endogenous dormancy (Bewley and Black, 1994; Leadem, 1997).

In many mature, non-endospermic seeds like *Passiflora* spp., the embryo is mature and there is no endosperm (Ellis et al., 1985). The seeds have hard coats with a semi-permeable inner layer. They absorb water easily but contain chemical inhibitors that are difficult to leach possibly due to low permeability of the testa membrane located in seed coat (Delanoy et al., 2006). Embryos that are excised germinate rapidly, thus it appears that *Passiflora* spp. have exogenous dormancy which could be a combination of mechanical and chemical dormancy (Baskin et al., 2000). Dormancy can be broken by treatments including scarification, aril removal, storage for several months (Purseglove, 1979), soaking in water (McGuire 1998; Delanoy et al., 2006), various light conditions (Benvenuti et al., 2001), fire, dry heat, acid and other chemicals, hot water, mulch, cold and warm stratification, and immersing in gibberellin (Ferreira et al., 2005). However, dormancy has been reported specifically in some species, e.g.: *P. mollissima* (Delanoy et al., 2006) and *P. edulis* f. *flavicarpa* Deg. (Alexandre et al., 2004).

Several studies have evaluated different pre-germination treatments on passion fruit species, but results are inconsistent. Establishing a protocol that ensures maximum germination requires testing several methods because each species may have different seed treatment requirements.

Understanding the dormancy characteristics that inhibit seed germination may further improve germination potential, reduce propagation costs, and facilitate cultivation of these species. The objective of this study was to find the best seed treatment to overcome dormancy for each species.

**Materials and Methods**

The experiment was performed in 2018, in a greenhouse at the United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Thad Cochran Southern Horticultural Laboratory in Poplarville, MS, USA (lat. 30° 85’36” N, long. 89°49’94’’ W, elevation 97 m, USDA hardiness zone 8b). Seeds of *P. incarnate* were obtained in 2017 from physiologically ripe fruits collected from plants grown in the same location as above. Seeds of other species including *P. tripartita* var. *mollissima*, *P. maliformis*, *P. edulis* f. *edulis* ‘Frederick’, *P. ligularis*, *P. quadrangularis* L., and *P. laurifolia* were purchased from Trade Winds Fruit (Santa Rosa, CA, USA) (Table 1).

Seeds were exposed to four pre-germination treatments as explained in Table 2. Treatments in the present study were chosen based on efforts from earlier studies (Delanoy et al.,
The number of seeds per treatment varied among species from 8 to 50 (Table 1). Seeds with irregular shape or color were removed after visual inspection prior to treatment.

Seeds were sowed into 72-cell seedling flats containing a commercial substrate (SunGro Sunshine Professional Mix 3, Bellevue, WA, USA). The seeds were intermittently misted at 5 s per 10 min, under natural photoperiod with ambient sun light and 25±3°C constant daily temperature on 8th April 2018.

The day when the first seed in each treatment germinated was recorded as germination starting time ($T_0$). The number of germinated seeds was recorded for each treatment every 3 days for 90 days. Seeds were counted as germinated when the emerging seedling length was approximately 5 mm. Germination percentage (GP) was calculated as follows: $GP= (\text{Total number of seed germinated}/\text{Total number of seeds sown in all replicates}) \times 100$.

Germination rate (GR) was equal to $\Sigma n / \Sigma (tn)$, where $t$ is the time in days and $n$ is the number of seeds having completed germination on day $t$ (Delanoy et al., 2006). Mean germination time (MGT) was calculated as $\text{MGT}=\Sigma (tn)/\Sigma n$ where $n$ was number of seeds germinated at time $t$ and $t$ was days from sowing (Nichols and Heydecker, 1968).

The average germination percentage for each species from our study was compared to results from previous studies by taking averages of reported results for each species. Two species ($P. quadrangularis$ and $P. laurifolia$) were not compared, as reports of germination percentage were not found in the literature. In order to compare our results with those from other studies, Pearson’s chi-square tests ($\chi^2$) were performed in JMP 12 (SAS Institute, Cary, NC, USA) with the Distribution Procedure and the test probabilities function. $P$ values <0.05 were considered to be a rejection of the null hypothesis and therefore represent a deviation from the expected germination percentage.

Results and Discussion

After 90 days, the germination study was stopped for all species. Figure 1 shows germination percentages per treatment for each species. All the cumulative germination curves showed germination starting at least two weeks after sowing seeds in all treatments. Then, a gradual increase was observed in germination until it stabilized. Among the species, $P. maliformis$ and $P. edulis$ f. $edulis$ showed the earliest germination starting time ($T_0$) within all treatments compared to all other species (Table 3), while the latest occurred 35 d after sowing in scarification plus fermentation in $P. quadrangularis$ and $P. laurifolia$. $P. laurifolia$ showed maximal germination percentage (75%) in scarification plus fermentation, but germination percentage for that treatment was lower than 50% in all other species. Germination was observed in $P. incarnata$ and $P. quadrangularis$ only in response to soaking in water and scarification plus fermentation treatments (Table 3). Overall, in all species except $P. maliformis$ and $P. tripartite$ var. $mollissima$, scarification plus GA$_3$ resulted in poor germination. Soaking in water was effective to improve germination percentage in most species. Delanoy et al., (2006) reported no effect of water on germination on three $Passiflora$ spp., while Ellis et al., (1985) reported effectiveness of water on germination for $Passiflora$ spp. For $P. edulis$ f. $edulis$, $P. ligularis$, and $P. laurifolia$, the germination percentage was higher in scarified seed than non-scarified, suggesting that germination may be caused by physical seed coat restriction, at least in these three species. The highest germination rate (GR) was obtained in $P. maliformis$ at 0.23 in scarification plus.
GA₃, which may be due to effect of GA₃ on breaking dormancy as it has been found to be effective in increasing germination in other species (Koyuncu, 2005). Scarification removes the aril, which may act as a barrier against oxygen (Obroucheva, 1999). A higher germination level was observed when arils were removed and GA₃ was applied on *P. alata* Dryander (Ferreira *et al.*, 2005). Delanoy *et al.*, (2006) showed no single treatment improved germination on three species of *Passiflora*, which supports the results of Ellis *et al.*, (1985) that dormancy is exogenous, and a combination of pre-germination treatments may be needed to overcome both chemical and physical dormancy. In our study, soaking in water and scarification resulted in the highest germination percentages.

In a previous study (Delanoy *et al.*, 2006), germination percentages for *P. mollissima* seeds were reported as 0%, 10%, 0%, 18% for control, soaking in water for 48h, scarification, and basal point removal plus 48h 50 ppm GA₃, respectively. These results were lower than our germination percentages for similar treatments with this species. Cárdenas *et al.*, (2013) reported 84% germination for *P. ligularis* and *P. edulis* at 100 ppm GA₃ which is higher than our results for these species with scarification plus 100 ppm GA₃ (5% and 2%, respectively).

The germination percentages for each species were compared with averaged germination percentages from other studies using chi-square test (Table 4). The larger \( \chi^2 \) is, the smaller the resulting significance becomes, and the more probable it is that differences exist between germination percentages in this study and other results. Based on previous studies (La Rosa, 1984; Delanoy *et al.*, 2006; González-Benito *et al.*, 2009; Beavon and Kelly, 2012; Beavon and Kelly, 2015), the average germination percentage for *P. tripartite* var. *Mollissima* was 42.1%. Germination percentage for treatment 1 was significantly lower than the average reported, but there were no significance differences between the other treatments compared to the average. Treatment 4, soaking in distilled water for 3 d, was the easiest and most successful method for this species. For *P. maliformis*, treatment 2 resulted in significantly higher germination percentage (40%) compared to average germination (23%) reported for this species by Gutiérrez *et al.*, (2011). Based on our results, it appears that pre-soaking of the seeds in water for at least 24 h is key to better germination success. Overall, germination percentages for this species were still 40% or less, so further studies on how to improve this are needed.

The average germination percentage for *P. edulis* from other studies was significantly higher than our results. The highest percentage in our study was 16%, which is far lower than 62.79% average germination reported previously (Gutiérrez *et al.*, 2011; Mabundza *et al.*, 2010; Imliwabang and Alila, 2014; Ramírez Gil *et al.*, 2015). Reasons for this are unknown; however, as Mabundza *et al.*, (2010) noted fresh seeds germinate better and it may take much longer for germination if seeds are cleaned and stored for long periods of time. Thus, it is likely that 90 d was not long enough to observe seed germination for this species in this study. Germination percentages for *P. ligularis* were 5%, 0%, and 20% for treatments 2, 3, and 4, significantly lower than the average germination (48.25%) that was previously reported by others (Gutiérrez *et al.*, 2011; Cárdenas *et al.*, 2013; Aguacía *et al.*, 2015). Only treatment 1 resulted in a similar germination percentage. This may indicate seed coat disruption via scarification or partial removal is necessary to promote viable seed germination in this species, which Gutiérrez *et al.*, (2011) also concluded.
**Fig. 1** Germination curves for seven *Passiflora* spp. according to treatments; (1) scarification with sandpaper (2) scarification and pre-soaking for 24 h in 100 ppm GA$_3$ (3) scarification and fermentation for 7 d in 10% sucrose solution (4) pre-soaking 3 d in distilled water.
Table 1: Seven passion fruit species and number of seed per per treatment

| Species                        | Common name               | Number of seeds |
|--------------------------------|----------------------------|-----------------|
| *P. tripartitav. mollissima*   | Banana Passionfruit       | 10              |
| *P. maliformis*                | Sweet Calabash            | 50              |
| *P. edulis f. edulis*          | ‘Fredrick’ Passionfruit   | 50              |
| *P. ligularis*                 | Sweet Granadilla          | 20              |
| *P. quadrangularis*            | Giant Granadilla          | 14              |
| *P. laurifolia*                | Water Lemon               | 8               |
| *P. incarnata*                 | Maypop                    | 50              |

Table 2: Pre-germination treatments used in the seed germination test on seven passionfruit species

| Treatments                                                                 |
|---------------------------------------------------------------------------|
| 1. Scarification with sandpaper                                          |
| 2. Scarification and pre-soaking for 24 h in 100 ppm GA₃                  |
| 3. Scarification and fermentation for 7 d in 10% sucrose solution         |
| 4. Pre-soaking in distilled water for 3 d                                 |

Table 3: The effect of pre-germination treatments on germination rate (GR), mean germination time (MGT), and starting time (T₀) for seven passion fruit species

| Species                        | GR  | MGT (d) | T₀ (d) |
|--------------------------------|-----|---------|--------|
|                                | 1² | 2       | 3      | 4    | 1 | 2  | 3 | 4  |
| *P. tripartitav. mollissima*   | 0.00 | 0.03 | 0.02 | 0.03 | 0 | 53 | 54 | 49 |
| *P. maliformis*                | 0.07 | 0.23 | 0.01 | 0.17 | 47 | 50 | 89 | 56 |
| *P. edulis f. edulis*          | 0.08 | 0.01 | 0.09 | 0.03 | 52 | 46 | 64 | 56 |
| *P. ligularis*                 | 0.07 | 0.01 | 0.00 | 0.05 | 56 | 51 | 0  | 51 |
| *P. quadrangularis*            | 0.00 | 0.00 | 0.02 | 0.00 | 0  | 0  | 64 | 0  |
| *P. laurifolia*                | 0.03 | 0.00 | 0.07 | 0.05 | 63 | 0  | 69 | 58 |
| *P. incarnata*                 | 0.00 | 0.00 | 0.00 | 0.05 | 0  | 0  | 0  | 46 |
| Average                        | 0.04 | 0.04 | 0.03 | 0.05 | 31 | 29 | 49 | 45 |

²Treatments: 1, scarification; 2, scarification+GA3; 3, scarification + fermentation; 4, pre-soaking in water.
### Table 4: Germination percentage of seven passion fruit species and four seed treatments with their distributional goodness-of-fit when compared to results from other studies

| Species                 | Treatment | GP (%) | $\chi^2$ | $P$  |
|-------------------------|-----------|--------|----------|------|
| *P. tripartita var. mollissima* | 1         | 0      | 7.2712   | 0.0070* |
|                         | 2         | 30     | 0.5411   | 0.4420 |
|                         | 3         | 20     | 1.9869   | 0.1587 |
|                         | 4         | 30     | 0.5911   | 0.4420 |
| *P. maliformis*         | 1         | 12     | 3.4161   | 0.0646 |
|                         | 2         | 40     | 8.1592   | 0.0043 |
|                         | 3         | 2      | 12.4506  | 0.0004 |
|                         | 4         | 30     | 1.3834   | 0.2395 |
| *P. edulis f. edulis*   | 1         | 14     | 50.7051  | < 0.0001 |
|                         | 2         | 2      | 78.7718  | < 0.0001 |
|                         | 3         | 16     | 46.6259  | < 0.0001 |
|                         | 4         | 6      | 68.7321  | < 0.0001 |
| *P. ligularis*          | 1         | 30     | 2.6678   | 0.1024 |
|                         | 2         | 5      | 14.9829  | < 0.0001 |
|                         | 3         | 0      | 18.6473  | < 0.0001 |
|                         | 4         | 20     | 6.3923   | 0.0115 |
| *P. quadrangularis*     | 1         | 0      | NA*      | NA   |
|                         | 2         | 0      | NA       | NA   |
|                         | 3         | 14     | NA       | NA   |
|                         | 4         | 0      | NA       | NA   |
| *P. laurifolia*         | 1         | 38     | NA       | NA   |
|                         | 2         | 0      | NA       | NA   |
|                         | 3         | 75     | NA       | NA   |
|                         | 4         | 50     | NA       | NA   |
| *P. incarnata*          | 1         | 0      | 30.6452  | < 0.0001 |
|                         | 2         | 0      | 30.6452  | < 0.0001 |
|                         | 3         | 0      | 30.6452  | < 0.0001 |
|                         | 4         | 8      | 19.1002  | < 0.0001 |

Pearson’s chi-square test ($\chi^2$) test.

* $P$ values < 0.05 are significant and are a rejection of the null hypothesis that the observed distributions are the same as expected distributions.

*Not enough data available to make comparisons.
The highest germination percentage for *P. incarnate* was 8% in treatment 4, which was significantly lower than the average germination of 38.2% reported by Wehtje *et al.*, (1985) and Benvenuti *et al.*, (2001). Both authors reported that *P. incarnata* seed germination can be inhibited by light and that pre-soaking of seeds is necessary for even moderate levels of germination.

The greenhouse temperature that the seeds were kept in our study was around 25°C. Benvenuti *et al.*, (2001) found that higher temperatures of 35°C along with the absence of light were the best conditions for germination of *P. incarnata*. Even under the optimized conditions, complete germination was not achieved in their study. More effort in understanding seed dormancy factors in this species is needed.

Based on our results, scarification plus fermentation is the recommended treatment for *P. laurifolia* seeds, resulting in 75% germination. For *P. maliformis*, scarification in combination with GA$_3$ was the most effective treatment on germination percentage (40%). *P. tripartite* var. *mollissima* showed highest germination percentage when soaked in water or scarified plus GA$_3$ (30%). Scarification alone resulted in best germination percentage in *P. ligularis* (30%). Germination percentage was highest (20%) for *P. incarnata* when pre-soaked in water. Scarification plus fermentation is suggested as a treatment to improve germination in *P. edulis*. Results indicate that the seed pre-germination treatments of the seven species tested in this study may improve germination potential; however, it depended highly on species. No pre-germination treatment resulted in complete germination for all species. Dormancy is probably exogenous and combination of chemical and mechanical based on the results obtained. Further research is needed to determine dormancy types present in these species and the individual best treatments to overcome them.

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