Scientific Article

Methods of breaking seed dormancy for ornamental passion fruit species[1]

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ABSTRACT

The *Passiflora* L. genus covers a diversity of wild species with ornamental potential, especially due to the intrinsic beauty of its exotic flowers, flowering more than once a year and the lush foliage. However, *Passiflora* seeds present dormancy complicating seed germination and the establishment of commercial plant production with species with high ornamental potential. This study was conducted to determine the best pre-germination treatments to overcome seed dormancy for *Passiflora quadrangularis*, *P. nitida*, *P. foetida*, *P. eichleriana*, *P. alata*, *P. cincinnata*, *P. mucronata*, *P. micropetala*, *P. suberosa*, *P. morifolia* and *P. tenuifila*. The experimental design was completely randomized, with five treatments and four replicates, with 25 seeds per plot. Pre-germination treatments were: seeds soaked in 1,000 mg L^{-1} GA_3 (gibberellic acid) for 6 hours, seeds soaked in 0.2 % KNO_3 (potassium nitrate) for 24 hours, seeds soaked in 1 % KNO_3 for 24 hours, partial seedcoat scarification with sandpaper number 120 and control (seeds untreated). Percentage of germination, germination velocity index and radicle length were evaluated for all species. The results showed that GA_3 was effective to overcome seed dormancy in *P. suberosa* (86%), *P. morifolia* (68 %) and *P. tenuifila* (54%). KNO_3 1% had significant effect on overcoming dormancy in seeds of *P. eichleriana* (66%) and scarification with sandpaper increased seed germination of *P. micropetala* (38%).

Keywords: *Passiflora,* germination, vigor, gibberellic acid, potassium nitrate, mechanical scarification

RESUMO

Tratamentos pré-germinativos para a quebra de dormência de sementes de espécies ornamentais de maracujá

O gênero *Passiflora* L. abrange uma diversidade de espécies selvagens com potencial ornamental, especialmente devido à beleza intrínseca de suas flores exóticas, florescer mais do que uma vez por ano e a folhagem exuberante. No entanto, sementes de *Passiflora* apresentam dormência, dificultando a germinação das sementes e o estabelecimento de produção comercial de plantas com espécies com elevado potencial ornamental. Este estudo foi realizado para determinar o melhor tratamento pré-germinativo visando superar a dormência das sementes das espécies *Passiflora quadrangularis*, *P. nitida*, *P. foetida*, *P. eichleriana*, *P. alata*, *P. cincinnata*, *P. mucronata*, *P. micropetala*, *P. suberosa*, *P. morifolia* e *P. tenuifila*. O delineamento experimental foi inteiramente casualizado, com cinco tratamentos e quatro repetições, com 25 sementes por parcela. Os tratamentos foram: pré-germinação das sementes embebidas em 1.000 mg L^{-1} de GA_3 (ácido giberélico) durante 6 horas, sementes embebidas em 0,2% de KNO_3 (nitrato de potássio) durante 24 horas, sementes embebidas em 1% de KNO_3 durante 24 horas, escarificação mecânica com lixa número 120 e Controle (sementes não tratadas). Foram avaliadas a porcentagem de germinação, índice de velocidade de germinação e o comprimento radicular para todas as espécies. Os resultados mostraram que o GA_3 foi eficaz para quebrar a dormência da semente em *P. suberosa* (86%), *P. morifolia* (68%) e *P. tenuifila* (54%). O tratamento KNO_3 1% teve efeito significativo sobre a quebra de dormência em sementes de *P. eichleriana* (66%) e a escarificação com lixa aumentou a germinação das sementes de *P. micropetala* (38%).

Palavras-chave: *Passiflora*, germinação, vigor, ácido giberélico, nitrato de potássio, escarificação mecânica.

1. INTRODUCTION

Passion fruit can be propagated by asexual reproduction as cuttings, grafting, layering or tissue culture (FERREIRA, 2000). Nevertheless, passion fruit vines are usually propagated from seeds, ensuring plant health as crop diseases are not transmitted by seed (MELETTI et al., 2002). Seed propagation is also recommended for rootstock formation and plant breeding programs focused on disease-resistant and drought-tolerant hybrids with ornamental or medicinal use (JUNQUEIRA et al., 2001).

Several authors had reported that the period for passion fruit germination is from ten days to three months, with low germination rate and irregular seedling formation (DELANOY et al. 2006; FOWLER and BIANCHETTI, 2000; DOIJODE, 2001). Some species show seed dormancy, which may be physical (seed coat impermeability to water and gases), chemical (presence of inhibiting substances),

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mechanical (tegument resistance to embryo growth) or even physiological factors (mechanisms of germination inhibition) (FOWLER and BIANCHETTI, 2000). The period of seed viability for the majority of *Passiflora* species is unknown (FALEIRO et al., 2005), and the long period of natural or induced dormancy make seed germination slow and unevenness, restricting the commercial use of promising materials (DOIJODE, 2001).

Considering the number of *Passiflora* species and increasing use of these species as a resource for breeding programs of passion fruit and for ornamental, medicinal, and food purposes, this research aimed to overcome seed dormancy of 11 *Passiflora* species by pre-germination treatments as physical scarification and soaking in gibberellic acid and potassium nitrate solutions.

2. MATERIAL AND METHODS

The commercial seeds of *Passiflora alata* and ten wild species, *P. quadrangularis, P. nitida*, *P. foetida*, *P. eichleriana*, *P. cincinnata*, *P. mucronata*, *P. micropetala*, *P. suberosa*, *P. morifolia*, and *P. tenuifila* were evaluated (Figure 1).

Figure 1. Flowers of 1) *P. quadrangularis*, 2) *P. nitida*, 3) *P. foetida*, 4) *P. eichleriana*, 5) *P. alata*, 6) P. *cincinnata*, 7) *P. mucronata*, 8) *P. micropetala*, 9) *P. suberosa*, 10) *P. morifolia,* 11) *P. tenuifila*.

Passion fruit seeds resultant of natural pollination were collected from ripped fruits. After fruit harvest and pulp extraction, the mucilaginous arillus was removed by rubbing the seeds with water across a wire sieve (3-mm mesh); then they were washed and left to shade dry on paper towels for two days at room temperature. Seed moisture level was determined by the oven method at 105 °C ± 3 for 24 hours (BRASIL, 2009). Before germination test, seeds were sterilized with alcohol solution (70% v/v) for one minute, and sodium hypochlorite (2.5% active chlorine) for 5 minutes, afterwards washed in distilled water.

The experiment was performed in a completely randomized design with five treatments and four replications with 25 seeds per plot. Pre-germination treatments were seeds soaked in 1,000 mg L^{-1} GA_3 (Gibberellic Acid) for 6 hours, seeds soaked in KNO_3 0.2% for 24 hours, seeds soaked in KNO_3 1 % for 24 hours, partial seedcoat scarification with sandpaper number 120 and Control (seeds untreated).

Seeds were transferred to transparent polyethylene boxes ("Gerbox"), within three paper towel sheets ("Germitest") moistened with distilled water at a ratio of two and a half times of paper weight (BRASIL, 2009). The boxes were placed in transparent polyethylene bags and kept in germination chamber for 30 days at 20-30 °C alternate temperatures, and a 12-hour photoperiod until the end of the experiment.

Germination percentage (GERM) was daily performed and considered germinated when seed coat was broken and radicle came off, reaching at least 2 mm long. Radicle length (RL) was measure on normal seedlings and seed vigor was evaluate with Seed Germination Index (SGI) as proposed by Maguire (1962).

Normality was assessed using the Kolmogorov-Smirnov test with GENES software (CRUZ, 2013). As long as data normality was stated, means underwent variance analysis and were subsequently compared by Tukey test at 5% of probability using SISVAR software (FERREIRA, 2008).

3. RESULTS AND DISCUSSION

Seed moisture level of *Passiflora* species was (%): *Passiflora alata* - 7.70, *P. quadrangularis* - 9.30, *P. nitida* - 9.73, *P. foetida* - 7.82, *P. eichleriana* - 8.52, *P. cincinnata* - 9.44, *P. mucronata* - 9.92, *P. micropetala* - 13.01, *P. suberosa* - 8.26, *P. morifolia* - 11.47, and *P. tenuifila* - 9.30. The best germination rates for *Passiflora edulis* 'flavicarpa' seeds are 10% (MARTINS et al., 2005) and levels above 17% prejudice the maintenance of its physiological potential (FONSECA and SILVA, 2005).

Table 1 shows the results of germination test (GERM), germination speed index (GSI) and radicle length (RL) for five pre-germinated treatments applied to 11 *Passiflora* species seeds.

Table 1. Means of germination test (GERM), germination speed index (GSI) and radicle length (RL), after pre-germinated treatments to 11 *Passiflora* species.

Treatments	GERM (%)	GSI (%)	RL (cm)
	P. quadrangularis		
GA_3	0 b	0.00 c	0.00 b
KNO_3 0.2%	7 a	0.11 b	2.12 a
KNO_3 1%	8 a	0.15 a	2.18 a
Mechanical Scarification	0 b	0.00 c	0.00 b
Control	0 b	0.00 c	0.00 b
CV (%)	29.81	28.40	7.29
	P. nitida		
GA_3	26 a	0.20 a	3.00 a
KNO_3 0.2%	2 b	0.01 b	0.22 c
KNO_3 1%	24 a	0.16 a	1.52 b
Mechanical Scarification	0 b	0.00 b	0.00 c
Control	0 b	0.00 b	0.00 c
CV (%)	37.16	32.9	21.61
	P. foetida		
GA_3	15 ab	1.02 ab	1.83 a
KNO_3 0.2%	27 ab	1.90 ab	2.26 a
KNO_3 1%	32 a	2.02 ab	1.82 a
Mechanical Scarification	13 b	0.91 b	2.66 a
Control	31 a	2.30 a	2.55 a
CV (%)	34.74	36.79	23.90
	P. eichleriana		
GA_3	19 b	0.66 b	2.96 ab
KNO_3 0.2%	36 b	1.22 b	3.09 ab
KNO_3 1%	66 a	2.58 a	2.18 b
Mechanical Scarification	31 b	0.95 b	3.73 a
Control	39 b	1.36 b	3.88 a
CV (%)	24.07	28.49	19.10

Table 1. cont.

	P. alata		
GA_3	24 a	0.45 a	2.23 a
KNO_3 0.2%	9 b	0.15 b	1.75 b
KNO_3 1%	7 b	0.09 b	2.05 a
Mechanical Scarification	0 c	0.00 c	0.00 c
Control	0 c	0.00 c	0.00 c
CV (%)	24.15	31.93	9.74
	P. cincinnata		
GA_3	8 a	0.25 a	2.04 b
KNO_3 0.2%	0 c	0.00 c	0.00 c
KNO_3 1%	4 b	0.13 b	3.46 a
Mechanical Scarification	0 c	0.00 c	0.00 c
Control	0 c	0.00 c	0.00 c
CV (%)	0	13.95	14.64
	P. mucronata		
GA_3	0 b	0.00 b	0.00 b
KNO_3 0.2%	0 b	0.00 b	0.00 b
KNO_3 1%	33 a	1.06 a	2.44 a
Mechanical Scarification	0 b	0.00 b	0.00 b
Control	0 b	0.00 b	0.00 b
CV (%)	34.10	23.66	7.85
	P. micropetala		
GA_3	0 c	0.00 c	0.00 d
KNO_3 0.2%)	12 b	0.22 b	6.13 a
KNO_3 1%	13 b	0.17 b	2.99 c
Mechanical Scarification	38 a	0.61 a	5.01 b
Control	0 c	0.00 c	0.00 d
CV (%)	30.39	36.31	7.76
	P. suberosa		
GA_3	86 a	2.25 a	2.12 c
KNO_3 0.2%	79 ab	1.50 b	2.61 bc
KNO_3 1%	70 bc	1.36 b	2.85 ab
Mechanical Scarification	62 cd	1.56 ab	3.33 a
Control	49 d	1.86 ab	3.12 ab
CV (%)	9.02	19.15	9.79
	P. morifolia		
GA_3	68 a	2.49 a	1.80 a
KNO_3 0.2% (KNO_3 0.2%)	0 b	0.00 b	0.00 b
KNO_3 1%	0 b	0.00 b	0.00 b
Mechanical Scarification	0 b	0.00 b	0.00 b
Control	0 b	0.00 b	0.00 b
CV (%)	21.48	11.09	10.36
	P. tenuifila		
GA_3	54 a	1.46 a	2.09 a
KNO_3 0.2%	0 b	0.00 b	0.00 b
KNO_3 1%	0 b	0.00 b	0.00 b
Mechanical Scarification	0 b	0.00 b	0.00 b
Control	0 b	0.00 b	0.00 b
CV (%)	21.38	31.11	11.48

Means followed by the same letter in the columns do not differ from each other by the Tukey test at 5% probability.

P. quadrangularis

Seeds of *Passiflora quadrangularis* germinated only in KNO_3 1% and KNO_3 0.2% treatments, with 8% and 7% of seed germination, respectively. Radicles length were statically higher for KNO_3 0.2% and KNO_3 1% with means from 2.12 to 2.18 cm, respectively. However, KNO_3 1% presented a higher GSI (0.15), compared to KNO_3 0.2% (0.11). Few studies have been carried to describe *P. quadrangularis* seed germination; as Pereira and Dias (2000) reported that seed germination is uneven for the species with a low germination rate, delaying seedling formation and the production of plants with a desirable quality level. The species in the nature due to the reduced germination rate is rarely found (LORENZI, 2006).

P. nitida

Seed germination of *P. nitida* was statically higher for GA_3 (26%) and KNO_3 1% (24%), with GSI of 0.20 and 0.16, respectively. Radicle length of GA_3 treatment (3.0 cm) was higher than KNO_3 1% (1.52 cm). Control and mechanical scarification have not promoted germination, as well as KNO_3 0.2%, with only 2% of germinated seeds. Research for this species is still incipient; although the studies shows that newly harvested seeds have low germination (1%) regardless of arillus removal method and after four-month storage germination was increased to 25% and after nine months reached 60% and after thirteen months seeds no longer germinated (MELO, 1998). Nevertheless, Passos et al. (2004), using *P. nitida* seeds stored for two years and five months, observed a rate of 86% of seed germination receiving GA_3 treatment (1,000 mg L^{-1}) at 50-day trial.

P. foetida

KNO_3 1% and control had the highest germination rate for *P. foetida* seeds with 32% and 31%, respectively. Mechanical scarification have had the lower GSI (0.91cm) and GERM% (13%), showing seed scarification was prejudicial for *P. foetida* seed germination. Radicle length have not had statistical difference from treatments, showing 2.66 to 1.82 cm to mechanical scarification and KNO_3 1%, respectively. All pre-germination methods applied for *P. foetida* seeds had no effect, and were statically equivalent to control, thus the seeds germinated on all treatments. Santos et al. (2012) reported interspecific hybridizations have been carried with *P. foetida* because besides having an ornamental potential, it has a high germination speed; however, such fact was not observed in the current study.

P. eichleriana

To overcome *P. eichleriana* seed dormancy, KNO_3 1% shown to be effective, with germination rate of 66% and GSI of 2.58, differing statistically from others treatments. Control showed the highest radicle length (3.88 cm). There are no studies about *P. eichleriana* seed germination; such data can substantially contribute to its propagation.

P. alata

P. alata presents physiological seed dormancy as control and mechanical scarification had not promoted seed germination. GA_3, KNO_3 0.2% and KNO_3 1% treatments promoted *P. alata* seed germination. Treatment with GA_3 increased seed germination rate (24 %), GSI (0.45) and radicle length (2.23 cm). Ferreira et al. (2001) observed that GA_3 (500 mg L^{-1}) increased seed germination of *P. alata*. Additionally, Rossetto et al. (2000) showed that 150 and 300 ppm of GA_3 had higher germination rates and speed index in fresh passion fruit seeds, when compared to the control treatment.

P. cincinnata

P. cincinnata seed germination was low for all treatments and reached the higher value (8%) with GA_3 treatment. GA_3 has shown the higher value for GSI (0.25) and 1% KNO_3 the greater radicle length (3.46). The low germination rate of *P. cincinnata* might be related to its elevated dormancy level (MELETTI et al., 2002). Such dormancy is related to seed hormonal balance, since GA_3 showed the best results. The magnitude of GA_3 effect became clear when it was detected that embryos synthesize gibberellin and release them to endosperm during seed germination, accelerating the radicle and shoot development (ROBERTS and MILK, 2004).

P. mucronata

P. mucronata seeds only germinated on KNO_3 1% treatment (33%), thus the treatment had higher GSI (1.06) and RL (2.44) values. However, Santos et al., 2012 observed that fresh seeds of *P. mucronata* had a high germination potential (72%) without any pre-germination treatment, but after four months of seed storage the germination rate decay to zero, and a secondary dormancy mechanism may be involved to prevent seed germination of this species. A secondary dormancy occurs when the cotyledon is moistness and promote germination-inhibiting substances on the embryonic axis inhibiting germination and consequently maintaining dormancy (BEWLEY and BLACK, 1994).

P. micropetala

The best treatment to overcome seed dormancy of *P. micropetala* was mechanical scarification with 38% of germination. GSI for 0.61 KNO_3 0.2% was higher than all treatments for radicle length (6.13). *P. micropetala* seeds have an integumentary dormancy, so scarifying techniques can favor water entrance and subsequently faster reactivation of metabolic system, therefore increasing germination (WAGNER JÚNIOR et al., 2006).

P. suberosa

The greatest germination rate of *P. suberosa* was observed for GA_3 treatment with 86%. GA_3 also have had the best result for GSI (2.25), followed by control (1.86) and mechanical scarification (1.56). Mechanical scarification (3.33) and control (3.12) were superior to the others treatments for radicle length. Caldas et al. (2008), assessing *in vitro* germination of *P. suberosa* seeds treated with GA_3 (1,000 ppm) for 3 hours, presented 13% of seeds germinated and, 30% of germination for seeds treated with KNO_3 20% (SOUZA, 2015).

P. morifolia

Passiflora morifolia seeds presented physiological dormancy mechanism, once only GA_3 treatment promoted seed germination (68%). Gibberellic acid stimulates the synthesis of enzymes such as alpha and beta amylase, acting on seed mobilization to form sugars, amino acids and nucleic acids, that area absorbed and transported to embryo growth regions, stimulating cell elongation and, accelerating and standardizing germination (TAIZ and ZEIGER, 2009).

P. tenuifila

As observed for *P. morifolia*, in *P. tenuifila* GA_3 was the only treatment that stimulate seed germination (54%). *P. tenuifila* is very important specie for passion-flower breeding given the importance of the species due to its resistance/tolerant for soil diseases, however there is a lack of information in the literature regarding overcoming its dormancy (FALEIRO et al., 2005).

Seed dormancy is an important adaptive mechanism in many species and it is, generally, lost during plant domestication and can be classified as physiological, morphological, morphophysiological, physical and combinational dormancy (BASKIN and BASKIN, 2004). Physiological dormancy is caused by endogenous factors and responds to heat or hormonal treatment, morphological is caused by immature embryos and physical is caused by a layer that is impermeable to water (MENDIONDO and GARCÍA, 2006). Our results show that all species except *P. micropetala* were able to germinate regardless of scarification, though the tegument does not seem to represent a barrier to water penetration into the seeds, thus do not constitute a case of physical dormancy as observed for the other *Passiflora* species. *P. foetida* have not shown seed dormancy, as germination percentage to control was higher than other treatments. *P. quadrangularis*, *P. nitida*, *P. cincinnata*, *P. mucronata*, *P. micropetala* and *P. tenuifila* have not germinated without pre-germination treatment presenting seed dormancy. *Passiflora* species can be divided in two groups concerning to the dormancy type: *P. quadrangularis*, *P. nitida*, *P. alata, P. cincinnata*, *P. mucronata*, *P. morifolia* and *P. tenuifila* with chemical dormancy and responsive to chemical composts as GA_3 and/or KNO_3, and *P. foetida*, *P. eichleriana*, *P. micropetala*, and *P. suberosa* with chemical and/or mechanical dormancy. Exogenous dormancy, probably a combination of mechanical and chemical dormancy is present in *Passiflora* (DELANOY et al., 2006). Seed dormancy is one of the most important phenomena for wild species evolution to overcome adverse environmental conditions and to colonize new habitats of unsuitable growth. Our study attempt to bring information regarding *Passiflora* seed germination and provide basic study to further refinement knowledge about overcome dormancy and seed germination for wild species.

4. CONCLUSIONS

GA_3 was able to overcome seed dormancy in *P. suberosa*, *P. morifolia* and *P. tenuifila* and 1% KNO_3 was effective for *P. eichleriana*. Mechanical scarification increase germination rates for *P. micropetala*. The other species have not had germination percentage improved by the treatments.

AUTHOR CONTRIBUTION

TNM: Conception or design of the work, Data collection, Data analysis and interpretation, Drafting the article. PBL: Conception of the work, Data analysis and interpretation, Drafting and Critical revision of the article, Final approval of the version to be published. ART: Data analysis and interpretation, Drafting and Critical revision of the article. LGN: Conception or design of the work, Data analysis and interpretation. SPS: Drafting the article, Critical revision of the rticle.

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