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Overcoming seed dormancy in *Passiflora* species

Superar el dormance en semillas de especies de Passiflora

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ABSTRACT

The knowledge about aspects related to seed germination are extremely important to the propagation of *Passiflora* species. Considering the diversity of *Passiflora* species and the need of studies that can elucidate the germination process of *Passiflora* seeds this study aimed was to evaluate methods to overcome seed dormancy in two *Passiflora* species (*P. morifolia* and *P. cincinnata*) by pre-germinative treatments as physical scarification, immersion in gibberellic acid (GA_3) and Promalin® (GA_{4+7} + N- (phenylmethyl) -aminopurine). To dormancy overcoming, the pre-germinative treatments were: scarification in sandpaper; immersion in water at 50 ºC for 5 minutes; imbibition in 400, 1000, 2000 and 3000 mg L^{-1} GA_3 for 5 hours; scarification in sandpaper + imbibition in 400, 1000, 2000 and 3000 mg L^{-1} GA_3 for 5 hours; and imbibition in 0.03, 0.45, 0.90 and 2% Promalin® for 6 and 12 hours. The treatments were distributed in a completely randomized design, with four repetitions of 25 seeds. The data were submitted to variance analysis and the averages compared by Tukey test and Scott-Knott, respectively, at 5% of probability using the software SISVAR. Germinability, germination time, germination velocity, uncertainty and germination synchrony were evaluated. The results showed that 0.45, 0.90 and 2% Promalin® for 6 and 12 hours were effective to overcome the seed dormancy in *P. cincinnata*. The treatment 1000 mg L-1 GA_3 achieved the highest result to overcome the dormancy in *P. morifolia* seeds.

Keywords: *Passiflora cincinnata*, *Passiflora morifolia*, Promalin®, seed germination.

RESUMEN

El conocimiento sobre aspectos relacionados con la germinación de semillas es de fundamental importancia para la propagación y mantenimiento de especies de Passiflora *silvestres. Hay varios factores que pueden interferir con la germinación de estas especies. La latencia de las semillas puede ser regulada por mecanismos de origen embrionario, del tegumento, por la presencia de inhibidores químicos o por el comportamiento fisiológico causado por el equilibrio hormonal. Teniendo en cuenta la gran diversidad de especies del género Passiflora y la necesidad de estudios que puedan dilucidar el proceso de germinación de las semillas de maracuyá, esta investigación tuvo como objetivo evaluar los métodos para superar la latencia de las semillas de dos especies de* Passiflora *(*Passiflora morifolia *y* Passiflora cincinnata*). Los tratamentos prévios a la germinación pueden ser la escarificación física, la inmersión en ácido giberélico (GA_3) y Promalin® (GA_{4+7} + N- (fenilmetil) –aminopurina). Para superar la latencia, los tratamientos pregerminativos fueron: escarificación de papel de lija; inmersión en agua a 50 ºC durante 5 minutos; remojo en GA_3 a 400, 1000, 2000 y 3000 mg L^{-1} durante 5 horas; escarificación en papel de lija + imbibición en GA_3 a 400, 1000, 2000 y 3000 mg L^{-1} durante 5 horas; y remojo en Promalin® a 0.03, 0.45, 0.90 y 2% por 6 y 12 horas. Los tratamientos se distribuyeron en un diseño completamente al azar, con cuatro repeticiones de 25 semillas. Los datos obtenidos para* P. morifolia *y* P. cincinnata *fueron sometidos a análisis de varianza y la comparación de medias por la prueba de Tukey y Scott-Knott, respectivamente, con un 5% de probabilidad utilizando el programa informático SISVAR. Se evaluaron la germinación, tiempo promedio, velocidad promedio, incertidumbre y sincronía de la germinación. Los resultados revelaron que Promalin® a 0.45, 0.90 y 2% durante 6 y 12 horas fueron efectivos para superar la latencia de las semillas de* P. cincinnata*. Para las semillas de* P. morifolia*, el tratamiento con GA_3 a 1000 mg L^{-1} tuvo un resultado superior para superar la latencia.*

Palabras clave: *Passiflora cincinnata.* Passiflora morifolia*. Promalin®.*

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Introduction

Passiflora vines can be propagated sexually through seeds or asexually through cutting and grafting. Seed propagation is used in commercial *Passiflora* crops since the decade of 70 and is the most important method used in Brazil (Lima *et al*., 2011). Plant propagation through seeds ensure that interested phenotype characteristics are potentially heritable to the next generation, and, simultaneously, that genetic variations of each species enable advantages when passing from one generation to another (Farias and Hoppe, 2004).

Seed propagation is also recommended because it is a simple method and don't need nurseries with high technology. On the other hand, seeds of *Passiflora* wild species, as *P. setacea*, *P. cincinnata*, *P. nitida* and *P. morifolia* have a long dormancy period, which requires a storage period equal or higher than two years to obtained satisfactory germination rates, which makes the commercial production of these species problematic (Meletti *et al*., 2002).

The knowledge about the physiology of seed germination is fundamental to *Passifora* wild species propagation and germplasm conservation (Marostega *et al*., 2015). Dormancy in seeds can be regulated by mechanisms from embryonic origin, integument, presence of chemical inhibitors, or physiological comportment caused by hormonal disbalance (Marcos Filho, 2015).

Treatments overcoming seed dormancy of *Passiflora* involve chemical and mechanical scarification, thermal treatments and seed storage under different environments and periods. The association of drying seed in the shade and scarification with sandpaper or immersion in water at 50 ºC are efficient methods to overcome dormancy in *P. cincinnata* seeds (Oliveira Júnior *et al*., 2010). Seeds of *P. mucronata* freshly harvested germinate utilizing water bath pre-treatments at 50 ºC and scarification with sandpaper; however, as the storage period increase (4 to 12 months), the germination percentage decreases (Santos *et al*., 2012). Germination of *P. edulis* seeds increase when 500 and 1250 mg L^{-1} gibberellic acid is applied (Simonetti *et al*., 2017).

Considering the variety of species belonging to *Passiflora* gender and the necessity of studies that can elucidate the germination process of Passion Fruit seeds, the present research aims to evaluate methods to overcome seed dormancy in two *Passiflora* species (*P. morifolia* and *P. cincinnata*) by physical scarification, immersion in gibberellic acid (GA_3) and Promalin® (GA_{4+7} + N- (phenylmethyl) -aminopurine) as pre-germinative treatments.

Material and methods

P. cincinnata and *P. morifolia* seeds were obtained of fruits from Mato Grosso State University Germplasm Active Bank, Campus Caceres, Caceres, Mato Grosso State. Ripe fruits were selected in 2017 from healthy plants, free of pests and diseases.

The fruit mucilage was removed by rubbing the seeds with hydrated whitewash on wire sieve (3 mm), following Marostega *et al.* (2015) recommendations. The seeds were dried for 2 days on shade with ambient temperature around 25 ºC, and posteriorly, stored in glass recipients, hermetically closed, and conditioned in a cold room at 7 ºC for 60 days. The humidity degree was determined by drying the seeds (n = 20) in oven (105 ± 3 ºC for 24 hours), according to Brasil (2009). The results were expressed in the average percentage of the seed lots on a wet basis.

The experiment to overcome dormancy of the seeds of *P. cincinnata* and *P. morifolia* was composed of the treatments:

- T1 - Mechanical scarification of seeds with sandpaper;
- T2 - Water immersion at 50 ºC for 5 minutes;
- T3 and T4 - Seeds were embedded in 400 or 1000 mg L^{-1} gibberellic acid (GA_3), at 30 ºC for 5 hours, in the dark;
- T5 and T6 - Mechanical scarification of seeds with sandpaper and seeds were embedded in 400 or 1000 mg L^{-1} gibberellic acid (GA_3), at 30 ºC for 5 hours, in the dark;
- T7 - Seeds without treatment (Control).

Additionally, only for the *P. cincinnata* seeds, were realized the treatments:

- T8 and T9 - Seeds were embedded in 2,000 and 3,000 mg L^{-1} gibberellic acid (GA_3), at 30 ºC for 12 hours in the dark;
- T10 and T11 - Mechanical scarification of seeds with sandpaper and seeds were embedded in 2,000 and 3,000 mg L^{-1} gibberellic acid (GA_3), at 30 ºC for 12 hours in the dark;
- T12 and T13 - Seeds embedded in 0.03% Promalin® (GA_4 + GA_7 + N-(phenylmethyl)

-aminopurine) solution, during 6 and 12 hours at 30 ºC, in the dark;

- T14 and T15 - Seeds embedded in 0.45% Promalin® (GA_4 + GA_7 + N-(phenylmethyl) -aminopurine) solution, during 6 and 12 hours at 30 ºC, in the dark;
- T16 and T17 - Seeds embedded in 0.90% Promalin® (GA_4 + GA_7 + N-(phenylmethyl) -aminopurine) solution, during 6 and 12 hours at 30 ºC, in the dark;
- T18 and T19 - Seeds embedded in 2% Promalin® (GA_4 + GA_7 + N-(phenylmethyl) -aminopurine) solution, during 6 and 12 hours at 30 ºC, in the dark.

The mechanical scarification treatments with sandpaper were prepared by scarifying the seeds on the region near the embryo, enabling the exit point of the embryonic axis free from impediment.

The germination test was achieved in a germination chamber with alternated temperature between 20 and 30 ºC, in the dark for 30 days (Brasil, 2009). Four replicates of 25 seeds were sown in Gerbox-type plastic boxes containing 2 blotting papers, moistened with distilled water in the proportion of two and a half times the mass of the dry paper, and continuous watering to maintain moisture. Seeds with a primary root length bigger than 2 mm were considered germinated. The germination data was daily recorded.

The germination parameters germinability (%) (transformed in % sin arc), ATG (TMG) - average time of germination, ASG (VMG) - average speed of germination (Maguire, 1962), U - germination uncertainty, indicates if the process occurred or not (Labouriau and Valadares, 1976), and Z - germination synchrony, indicates when at least two seeds germinate together (Primack, 1980) were calculated utilizing the software GerminaQuant 1.0 (Marques *et al*., 2015).

A completely randomized design was implemented, each treatment were constituted of 4 replications of 25 seeds, with 7 treatments for dormancy overcoming in *P. morifolia* seeds and 19 treatments for seeds dormancy overcoming in *P. cincinnata*.

The data for *P. morifolia* and *P. cincinnata* were submitted to variance analysis and the averages compared by Tukey and Scott-Knott test, respectively, at 5% of probability using the software SISVAR.

Results and discussions

The level of moisture in *P. morifolia* seeds at the beginning of the experiment was 11.99% and 1.04% in *P. cincinnata*. The values are similar to the values obtained by Marostega *et al.* (2017), where they found 9.44 and 11.47% for *P. cincinnata* and *P. morifolia*, respectively.

Germinability (GERM%) of *Passiflora morifolia* seeds, statistic differences between the treatments were observed (Table 1).

Treatments with imbibition in 1000 mg L^{-1} GA_3 (T_4) and scarification associated with imbibition in 400 and 1000 mg L^{-1} GA3 (T_5 and T_6) were the

Table 1. Germinability (GERM%), Average Time of Germination (ATG), Average Speed of Germination (ASG), Germination Uncertainty (U) and Germination Synchrony (Z) treated focusing on overcoming dormancy of Passiflora morifolia seeds, Caceres - MT - Brazil. UNEMAT, 2018.

Treatments	GERM (%)	ATG (days)	ASG	U (bits)	Z
T_1	6 c	9.58 b	0.1070 c	0.3962 a	–
T_2	0 d	–	0.0000 d	0.0000 a	–
T_3	22 b	8.23 ab	0.1225 bc	1.7519 b	0.1500 b
T_4	57 a	6.19 a	0.1615 a	0.4226 a	0.8321 a
T_5	60 a	7.08 a	0.1414 ab	1.7317 b	0.3463 b
T_6	60 a	7.49 ab	0.1346 b	1.5478 b	0.4093 b
T_7	4 c	8.00 ab	0.1250 bc	0.0000 a	–
CV (%)	15.79	12.19	9.59	53.13	33.52

Averages followed by the same letter in the columns do not differ from each other by the Tukey test at 5% probability. T1 = Sandpaper; T2 = Water 50 ºC - 5 min; T3 = GA_3 400 mg L^{-1} - 5 hours); T4 = GA_3 1000 mg L^{-1} - 5 hours; T5 = Sandpaper + GA_3 400 mg L^{-1} - 5 hours; T6 = Sandpaper + GA_3 1000 mg L^{-1} - 5 hours; T7 - Seeds without treatment - Control.

treatments with highest germination percentages, 57, 60 and 60%, respectively. Corroborating with our results, Rezazadeh and Stafne (2018) evaluating four different pre-germination treatments to increase the germination potential of seven *Passiflora* species, concluded that the scarification associated with imbibition in 1000 mg L^{-1} GA_3 for 24 hours were efficient to increase the germination percentage of *P. maliformis* and *P. tripartita* var. *mollissima*. Mechanical and chemical scarification can produce fissures on seed teguments (Franke and Baseggio, 1998). The tegument rupture caused by mechanical scarification is capable of allowing increased permeability to water and gases, accelerating the germination process (Carvalho and Nakagawa, 2012).

The treatments that applied 400 and 1000 mg L^{-1} GA3 differed from each other, with germination values of 22 and 57%, respectively. Marostega *et al*. (2017) observed that the *Passiflora* seeds immersion in 1000 mg L^{-1} GA_3 for 6 hours resulted in a percentage of germination of 26% for *P. nitida,* 15% for *P. foetida*, 19% for *P. eichleriana*, 24% for *P. alata*, 8% for *P. cincinnata*, 86% for *P. suberosa*, 68% for *P. morifolia* and 54% for *P. tenuifila*.

The effects on germination increase on the treatments with gibberellic acid but did not replicate on time of germination (ATG) of *P. morifolia* seeds. The effects of gibberellic acid in all concentrations, with or without scarification, was the same as the seeds without any treatment. The reduced TMG for untreated seeds (T_7) does not show the success of the treatment, and in this case, the few seeds germinated in the treatment happened quickly, that is, in the first days of the test. The higher time of germination was observed when the seeds were simply sanded with seed germination in approximately 9 days. The highest results for ASG were observed on treatments 1000 mg L^{-1} GA_3 for 5 hours (T_4) and sandpaper + 400 mg L^{-1} GA_3 for 5 hours (T_5).

Cadorin *et al*. (2017), investigating pre-germinative treatments with temperature and gibberellic acid in *Passiflora ligularis* seeds, observed a reduction on time of germination by imbibing the seeds in 100 mg L^{-1} GA_3 for 15 minutes when compared to seeds without treatment. Gibberellins are efficient in overcoming seed dormancy, because they are involved in modulating the development of the plant cycle, increasing the growth of the embryo and controlling the growth of the embryonic axis (Taiz and Zeiger, 2013; Cavusoglu and Sulusoglu, 2015).

The results revealed a statistic difference between the treatments for the variables of Uncertainty (U) and Synchrony (Z) of germination. Seeds without treatment, embedded in water at 50 ºC for 5 minutes, scarified in sandpaper or embedded in 1000 mg L^{-1} GA_3 for 5 hours showed the lowest uncertainty values (Uncertainty, U) 0.0000; 0.0000; 0.3962 and 0.4226, respectively. The low uncertainty value validates the rapid germination of the seeds of a determined treatment to overcome dormancy. The uncertainty is related to the distribution of the relative frequency of germination; thus, low uncertainty values indicate that germination is more concentrated in a certain time (Carvalho *et al*., 2015). The treatment 1000 mg L^{-1} GA_3 for 5 hours presented the highest synchrony (Z) index. The synchrony index is calculated if 2 or more seeds germinated at the same time and will have value equal 1 when all seeds germinate at the same time or, Z approaches zero when at least two seeds germinate one at a time (Ranal and Santana, 2006).

Table 2 shows the results of the Germinability (GERM%), Time of Germination (ATG), Speed of Germination (ASG), Uncertainty of Germination (U) and Synchrony of Germination (Z) of *P. cincinnata* seeds under treatments to overcome the dormancy. The treatments T_{14}, T_{15}, T_{16}, T_{17}, T_{18} and T_{19} were statistically superior to the others treatments, with seed germination higher than 43%. The result is discordant with Moura *et al*. (2018), which observed a higher percentage of *P. cincinnata* emergence seedlings with 0.03% Promalin® promoting an increase of 2 to 65% of seedling emergency rate. The combination of gibberellins with cytokinin promotes germination. The gibberellins effect is on the activation of the vegetative growth of the embryo, on the weakening of the endosperm layer, as well as the mobilization of energy reserves; besides acting on the protein synthesis and specific RNA in germination, in both dormancy overcoming and control of hydrolysis of reserves (Amaro *et al*., 2009). The cytokinin is responsible to promote the radicle growth and act on the division control and cell stretching, regulating the active inhibitors contained in the seed, which allows the gibberellins action (Taiz and Zeiger, 2013).

The seed dormancy overcoming treatments reduced the mean germination time (ATG), the results confirm the effectiveness of treatments with Promalin® and gibberellic acid in decreasing the mean germination time of seeds of this

Table 2. Germinability (GERM%), Average Time of Germination (ATG), Average Speed of Germination (ASG), Germination Uncertainty (U) and Germination Synchrony (Z) treated focusing on overcoming dormancy of *Passiflora cincinnata* seeds, Caceres - MT - Brazil. UNEMAT, 2018.

Treatments	GERM (%)	ATG (days)	ASG	U (bits)	Z
T_1	4 c	18.50 b	0.0564 b	0.0000 a	–
T_2	0 d	–	0.0000 c	0.0000 a	–
T_3	0 d	–	0.00 00c	0.0000 a	–
T_4	0 d	–	0.0000 c	0.0000 a	–
T_5	7 c	11.45 a	0.0955 a	0.4795 a	0.1666 b
T_6	12 b	12.67 a	0.0764 b	1.2304 b	0.0250 b
T_7	0 d	–	0.0000 c	0.0000 a	–
T_8	4 c	11.00 a	0.0940 a	0.0000 a	–
T_9	22 b	9.88 a	0.1055 a	1.8014 b	0.0964 b
T_{10}	13 b	9.03 a	0.1133 a	0.7177 a	0.6166 a
T_{11}	14 b	10.66 a	0.0986 a	1.2508 b	0.1083 b
T_{12}	6 c	11.12 a	0.0912 a	0.5000 a	0.0000 b
T_{13}	22 b	13.10 a	0.0791 b	1.8205 b	0.0928 b
T_{14} (Promalin® 0,45%; 6 hou\s)	22 b	13.10 a	0.07 b	1.82 b	0.09 b
T_{14}	43 a	10.32 a	0.0972 a	2.1646 c	0.1690 b
T_{15}	55 a	9.62 a	0.1044 a	2.6220 c	0.1083 b
T_{16}	49 a	10.26 a	0.0984 a	2.3852 c	0.1361 b
T_{17}	63 a	9.21 a	0.1086 a	2.6896 c	0.1174 b
T_{18}	49 a	9.41 a	0.1066 a	2.4911 c	0.1194 b
T_{19}	52 a	8.76 a	0.1145 a	1.8350 b	0.1570 b
CV (%)	25.44	21.56	18.83	44.78	97.78

Averages followed by the same letter in the columns do not differ from each other by the Tukey test at 5% probability. T1 = Sandpaper; T2 = Water 50 ºC - 5 min; T3 = GA_3 400 mg L^{-1} - 5 hours; T4 = GA_3 1000 mg L^{-1} - 5 hours; T5 = Sandpaper + GA_3 400 mg L^{-1} - 5 hours; T6 = Sandpaper + GA_3 1000 mg L^{-1} - 5 hours; T7 - Seeds without treatment – Control; T8 = GA_3 2000 mg L^{-1} - 12 hours; T9 = GA_3 3000 mg L^{-1} - 12 hours; T10 = Sandpaper+ GA_3 2000 mg L^{-1} - 12 hours; T11 = Sandpaper+ GA_3 3000 mg L^{-1} - 12 hours; T12 = Promalin® 0,03% - 6 hours; T13 = Promalin® 0,03% - 12 hours; T14 = Promalin® 0.45% - 6 hours; T15 = Promalin® 0.45% - 12 hours; T16 = Promalin® 0.90% - 6 hours; T17 = Promalin® 0.90% - 12 hours; T18 = Promalin® 2% - 6 hours; T19 = Promalin® 2% - 12 hours.

species, which is in agreement with the results de Amaro *et al.* (2009), who observed a consequent decrease in the mean germination time (ATG) of *P. cincinnata* seeds, as the concentrations of Promalin® were increased (0, 100, 200, 300, 400, 500 mg L^{-1}).

The ASG showed different average indexes for the several treatments to overcome dormancy. The treatments T_{19}, T_{20}, T_{28}, T_{30}, T_{31} and T_{32} presented better results to the average speed of germination. The values of the uncertainty of germination (U) above zero found in this study reveal the germination process of *P. cincinnata* scattered over the average time, with a high uncertainty degree. It is noticeable that the treatments, which the germination is null or with low percentage, the uncertainty value tends to be zero, explaining that when there is germination, it takes place in a short period, concentrated. Santana *et al.* (2010) found similar uncertainty results for *Kielmeyera coriácea*, both in the germination process and in the emergence revealed a high degree of uncertainty.

For T_{20} treatment (Sandpaper + GA_3 2000 mg L^{-1} 12 hours), the germination synchrony, which measures the germination uniformity, was higher than the others. Considering the synchrony results, it is possible to observe that, although the treatments to overcome dormancy increased the germination percentage of *P. cincinnata* seeds, there was no germination synchrony. Lopes and Franke (2011) explain that the distribution asymmetry of the synchrony shows that the germination heterogeneity is because of a majority of the seeds that take long to germinate, or because of a majority of the seeds that germinate fast (or because of both cases).

Similar results were related by Junghans and Junghans (2017) that evaluating the vigor of the seeds and the seedling emergence of two access of *P. cincinnata* during three storage periods, noticed that the emergence synchrony was not uniform,

with values close to zero. Ranal and Santana (2006) explain that the closer to one is the synchrony values, the greater will be the germination of the seeds occurring at the same time. Otherwise, when the values approach zero, they will indicate that at least two seeds can germinate one at a time. Because of the uniformity lack and low germination percentage, it is suggested that the uncertainty and synchrony characteristics be included in the selection of superior genotypes in the genetic improvement of *P. cincinnata*, for later use in agriculture.

Conclusions

To overcome the dormancy of *P. morifolia* seeds, the gibberellic acid (GA_3) is effective at the concentration of 1000 mg L^{-1} for 5 hours, because it ensures better germinability results, uncertainty results, and synchrony results. The overcoming dormancy of *P. cincinnata* seeds is obtained with Promalin® solution, at the concentrations of 0.45, 0.90 and 2% for 6 and 12 hours of imbibition.

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