REVISTA DELOS DESARROLLO LOCAL SOSTENIBLE

Effect of pre-germination treatments on seed germination of *Passiflora setacea* DC (Passifloraceae)

Efeito de tratamentos pré-germinativos na germinação de sementes de *Passiflora setacea* DC (Passifloraceae)

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ABSTRACT
P. setacea seeds without aril, were submitted to pre-germination treatments: T1(control); T2 (pre-soaking in Promalin® solution [gibberellin (GA_{4+7}) + N-(phenylmethyl)-1H-purine 6-amine (6-benzyladenine) both at a concentration of 18.8 $g.L^{-1}$] in bainMarie, 45 °C/20 min.; T3 (osmopriming with KNO_3 at a concentration of 2.6$g.L^{-1}$ H_2O, 25 °C/1 day, with aeration); T4 (osmopriming with KNO_3 at a concentration of 2.6$g.L^{-1}$ H_2O, 25 °C/2 days, with aeration); T5 (T3 followed by T2) and T6 (T4 followed by T2). The initial moisture contents, ranging from 6.7% to 8% did not differ from each other (α 0.05). The highest fresh mass increment at 168h and the highest moisture content at the end of the germination test were achieved by T2, 40.9% and 44.3%, respectively. The lowest fresh mass increment at 168h was T1 (13.1%). Osmopriming with KNO_3 and aeration contributed to better water absorption in T3 and T4 than in T1, but did not stimulate the germination performance of the seeds. The highest germination percentages and germination speed indexes were obtained from T2 seeds (92% and 8, 4595), T5 (96% and 11.7438) and T6 (94% and 11.6728) as well as the lowest mean germination times T2 (11.73 days), T5 (9.49 days) and T6 (8 .81 days), which did not differ statistically from each other (α 0.05). However, the germination process took less time for T2 seeds, 21 days. The

combination of aryl removal and phytohormone solution in a bain-marie at 45 °C/20min. with or without osmopriming with KNO_3 stimulated the germination process of *P. setacea* seeds.

Keywords: physical dormancy, physiological dormancy, phytohormones, osmotic agent.

RESUMO

Sementes de *P. setacea*, após remoção do arilo, foram submetidas aos tratamentos pré-germinativos: T1(controle); T2 (pré-embebição em solução de Promalin® [giberelina (GA_{4+7}) + N-(fenilmetil)- 1H -purina 6- amina (6-benziladenina) ambos na concentração de 18,8 $g.L^{-1}$] em banho-Maria, 45 °C/20 min.; T3 (osmocondicionamento com KNO_3 à concentração de 2,6$g.L^{-1}$ H_2O, 25 °C/1 dia, com aeração); T4 (osmocondicionamento com KNO_3 à concentração de 2,6$g.L^{-1}$ H_2O, 25 °C/2 dias, com aeração); T5 (T3 seguido de T2) e T6 (T4 seguido de T2). Os teores de umidade iniciais, variando de 6,7% a 8% não diferiram entre si (α 0,05). O maior incremento de massa fresca às 168 h e o maior teor de umidade ao fim do teste de germinação foram atingidos por T2, 40,9% e 44,3%, respectivamente. O menor incremento de massa fresca às 168 h foi de T1 (13,1%). O osmocondicionamento com KNO_3 e aeração contribuiu para melhor absorção de água de T3 e T4 que de T1, mas não estimulou o desempenho germinativo das sementes. Os maiores percentuais germinativos e índices de velocidade de germinação foram obtidos pelas sementes de T2 (92% e 8,4595), T5 (96% e 11,7438) e T6 (94% e 11, 6728) bem como, os menores tempos médios de germinação T2 (11,73 dias), T5 (9,49 dias) e T6 (8,81 dias), os quais não diferiram estatisticamente entre si (α 0,05). Entretanto, o processo germinativo foi em menor tempo para as sementes de T2, 21 dias. A combinação entre remoção de arilo e solução de fitormonios em banho-Maria a 45 °C/20min. com ou sem osmocondicionamento com KNO_3 estimulou o processo germinativo de sementes de *P. setacea*.

Palavras-chave: dormência física, dormência fisiológica, fitormonios, agente osmótico.

1 INTRODUCTION

One of the most important evolutionary adaptations that differentiate plant species is the occurrence of seed dormancy, a trait that allow plants to adapt and survive in adverse environmental conditions. Physiological, biochemical, morphoanatomical, genetic, molecular and other mechanisms are involved in the induction of different types of dormancy (Klupczyńska & Pawlowski, 2021; Willis et al., 2014). The seed coat of several Neotropical species of the genus *Passiflora* retains primitive or non-primitive morphological traits, such as the presence of bitegument, aril, semipermeability and integumentary concavities that can contribute to the occurrence of dormancy in these species (Pérez-Cortéz et al., 2002; 2009; Cárdena-Hernández et al., 2011; Posada et al., 2014).

The intensity of the processes that restrict germination in seeds of *Passiflora* spp., domesticated or wild, is variable and species-specific. Freshly harvested seeds of *P. alata*, *P.*

cincinnata, *P. gibertii*, *P. morifolia*, *P. mucronata* and *P. tenuifila* show pronounced dormancy, those of *P. suberosa* exhibit moderate dormancy and by contrast those of *P. edulis* Sims show no signs of dormancy (Ferreira, 2020). Depending on the species, seeds could have physical, physiological or physico-physiological dormancy (Cadorin et al., 2017; Gutiérrez et al., 2011). For instance, there is physical dormancy in seeds of *P. laurifolia* (Rezazadeh et al., 2018) and *P. maliformis* (Torres-G, 2018), physiological dormancy in seeds of *P. elegans* (Silva et al., 2019) and *P. suberosa* (Oliveira et al., 2020) and physicophysiological dormancy in seeds of *P. actinia* (Grzybowski et al., 2019) and *P. mollissima* (Delanoy et al., 2006).

Previous research has demonstrated that *P. setacea* DC. seeds have physical and physiological dormancy (Santos et al., 2016). Germination of these seeds, with numerical expressiveness and significant levels of uniformity, could be obtained only after pre-germination treatments with gibberellins or KNO_3 (Pádua et al., 2011), followed or not by physical scarification (Santos, 2015), gibberellins in combination with cytokinins (José et al., 2019), and removal of the aril that covers the seeds (Kohl & Duarte, 2019) were carried out.

The germination-promoting action exerted by phytohormones and osmotic agents will be different depending on many factors, like the type of dormancy, the seed morphology and even the genotype of the species, as well as the dosage and time of exposure to these products (Duermeyer et al., 2018; Santos et al., 2008). The most commonly used phytohormones to promote germination are gibberellins, auxins, cytokinins and ethylene; salts, sugars, polyethylene glycol and glycerol are the osmotic agents most frequently used to obtain the same effect (Ferreira, 2022; Santos et al., 2008). The combination of phytohormones and osmotic agents can be more effective, as it increases the regulation of complex metabolic pathways and physiological events that will enhance seed germination and uniformity.

The objective of this work was to evaluate the effect of pre-germination treatments (phytohormones associated or not with an osmotic agent) on the germination process of seeds of *P. setacea* cultivar BRS Pérola do Cerrado.

2 MATERIAL AND METHODS

P. setacea fruits, were obtained from a grower in Brasília, Federal District, Brazil. In laboratory, seeds were sanitized according to José et al., 2019 and the pulp was removed from the fruits. To remove the aril attached to the seeds the pulp was rubbed against the most abrasive

side of a dishwashing sponge and subsequently against the nylon mesh of a kitchen sieve, under running water. Seeds were washed with a neutral detergent solution, at a concentration of 2% (v/v), under running water, followed by successive rinses. Aril free, thoroughly rinsed seeds were spread over germination paper for drying at room temperature (25 ± 2 ºC) for 24h.

The seeds were subdivided into six samples of 200 seeds and each sample was submitted to the following pre-germination treatments: T1: untreated seeds (control); T2: pre-soaking in Promalin® [gibberellin [GA_{4+7}) + N-(phenylmethyl)-1H-purine 6-amine (6-benzyladenine)] solution, both at a concentration of 18.8 $g.L^{-1}$, in bain-marie, at 45 °C/20min; T3: seeds immersed in a potassium nitrate solution (KNO_3), concentration of 2.6$g.L^{-1}$, and kept in a germinator at 25 °C/1 day, with constant aeration; T4: seeds immersed in a KNO_3 solution, concentration of 2.6$g.L^{-1}$ and kept in a germinator at 25 °C/2 days, with constant aeration; T5: seeds submitted to T3 and then to T2; T6: seeds submitted to T4 and then to T2.

After each of the treatments described above, the seeds were washed with water and dried at room temperature, 25 ± 2 °C/24h, in order to adjust their moisture content to similar values. The moisture content of the seeds before (Mci) and at the end (Mcf) of the germination tests were determined by the oven method at 105±3 °C/24 h (Brasil, 2009) and the results were expressed as percentages, in a fresh weight basis.

Each sample of treated, dried seeds were divided into four subsamples of 50 seeds, and the germination tests were conducted with four repetitions of 50 seeds. Seeds were planted on paper substrate, moistened with water in the volume of three times the dry mass of the substrate, placed inside "gerbox" type acrylic germination boxes. Seeds were kept in a germinator under alternating temperatures (20 - 30 ºC), 8h light/16h dark photoperiod. Germination assessment was done by daily counts for up to 35 days. The germination criterion adopted in this study was protrusion of the root, longer than 1 cm. The increment of fresh weight (IFW) of the seeds was evaluated by weighing them during the first five hours of imbibition and the first, second, sixth and seventh days after sowing (DAS). For this, the seeds of each repetition/treatment were removed from the boxes, moisture from the seed surface was blotted with filter paper, then seeds were transferred into a sanitized container and weighed. After weighing the seeds were placed again in the germination boxes and returned to the germinator. The IFW evaluation period for *P. setacea* seeds, seven days or 168 h, was determined previously by establishing seed imbibition curves (Salomão et al., 2023).

Calculation of the percentage of increment of fresh weight (IFW) [Nascimento et al., 2022]:

$$IFW = (Wf-Wi)/Wf \times 100$$

Where:

IFW = increment of fresh weight (%); Wi = seeds initial weight at time 0 h; Wf = seed final weight at final time.

Calculation of the times for the beginning (Ti) and for the end (Tf) of germination:

Where:

Ti = first day of root protrusion > 1cm since the beginning of the test; Tf = last day of root protrusion > 1cm in test duration period

Calculation of the average germination time (Tm) (Labouriau, 1983):

$$Tm = \sum_{i=1}^{k} niti / \sum_{i=1}^{k} ni$$

Where:

Tm = mean germination time (days); ti = time between the beginning of the test and the i-th observation; ni = number of seeds germinated in time ti; k = last germination time.

Calculation of emergence speed index (ESI) (Cetnarski Filho & Carvalho, 2009):

$$ESI = \sum (ni/ti)$$

Where:

ESI = emergence speed index; ni = number of seeds germinated in the time between the beginning of the test and the i-th observation; ti = time elapsed between the beginning of the test and the i-th observation

The values of initial and final moisture content (Mci and Mcf), IFW, Ti, Tf, Tm, ESI and the final percentage of germination were submitted to analysis of variance (ANOVA), followed by Tukey's multiple comparison test (RM one-way ANOVA, α 0.05). The program used for statistical analysis was GraphPad Prism (@2017 Graph Pad Software Inc.

3 RESULTS

The initial moisture contents of the seeds before they were exposed to the six treatments did not differ significantly from each other (α 0.05), and neither had they a direct influence on seeds IFW or on seeds final moisture contents. Seeds exposed to T1, T4, T5 and T6 showed similar moisture content values, ranging between 6.7% and 7% (Table 1). Seeds treated with T2 and T3 started the germination process with the highest moisture contents, 7.6% and 8%, respectively (Table 1). At the end of the germination tests, the lowest value of moisture content was verified for the seeds submitted to T3 (20.3%) and the highest value was for the seeds of T2 (44.3%). The variation between the final moisture contents (Table 1) was statistically significant (α 0.05).

The lowest IFW values, in all weighing periods, were verified for T1 seeds, which differed (α 0.05) from the values of the other treatments (Figure 1). The IFW of these seeds was 15.9% at 144h, decreasing to 13.1% at 168h.

Table 1. Initial moisture contents (Mci) and final moisture contents (Mcf) of *Passiflora setacea* DC. seeds submitted to different pre-germination treatments.

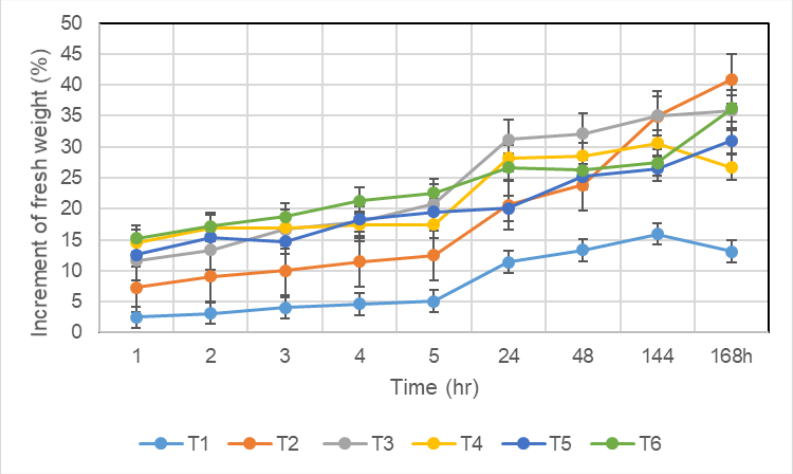
Treatments	Mci (%)	Mcf(%)
T1	6,9 ab	23,1bcd
T2	7,6 a	44,3 a
T3	8,0 a	20,3 cde
T4	6,7 ab	39,6 ab
T5	7,1 ab	37,5 abc
T6	7,0 ab	30,1 abcd

In each column, means followed by the same letters do not differ significantly by Tukey's multiple comparison test (α 0,05).

The trend towards a decrease in IFW from 144h to 168h was also observed in seeds treated with T4. The IFW of these seeds at 144h was 30.6% and 26.7% at 168h. However, these seeds behaved like those treated with treatments T3, T5 and T6, which absorbed a greater amount of water in the first five hours of imbibition (Figure 1). In contrast, the IFW in the first five hours of imbibition of seeds exposed to T1 and T2 were 5.1% and 12.5%, respectively (Figure 1). These values differed significantly (α 0.05) from each other and from the other IFW values. The highest final IFW was obtained by seeds treated with T2 (40.9%), which was significantly different from the other final IFW values (Figure 1). The IFW of seeds treated with T5 and T6 at the end of the evaluation time (168h) were 31% and 36.2%, respectively (Figure 1).

Seeds exposed to T1 started to germinate (2% germinated seeds), according to the adopted criterion of root protrusion greater than 1 cm, at 18 DAS. At 35 DAS, these seeds reached a final germination percentage of 22%, with a Tm of 23.7 days (Figure 2 and Table 2). T2 seeds showed a peak of root protrusion greater than 1 cm at 7 DAS with 21% of germination. The Tm of these seeds was 11.7 days and the germination process ended at 21 DAS (Figure 2 and Table 2).

Figure 1. Increment of fresh weight (IFW %) during 168 h of imbibition of *Passiflora setacea* DC seeds submitted to different pre-germination treatments.



Seeds treated with T3 started germinating at 15 DAS with 2% root protrusion, Tm of 26.5 days and final germination occurred at 35 DAS, with 12% germination (Figure 2 and Table 2). Following treatment with T4 seeds germinated only at 35 DAS, with a total germination percentage of 20%, with a Tm of 35 days (Figure 2 and Table 2). Seeds exposed to T5 and T6 started to germinate at 6 DAS, with 20% and 18% of germination, respectively. For these seeds Tm were 9.5 days (T5) and 8.8 days (T6), with final germination percentages at 30 DAS of 96% (T5) and 94% (T6) (Figure 2; Table 2). The times for the beginning of germination and the Tm of seeds treated with T2, T5 and T6 did not differ statistically from each other (α 0.05). Ti and Tm of seeds exposed to T1 and T3, did not differ from each other. However, Ti and Tm values of seeds treated with T4 differed from the other treatments (Figure 3). The time required to complete the germination process after treatment with T2 differed significantly from the other treatments (Figure 2), whereas there was no significant difference between the Tf of seeds treated with T1, T3, T4, T5 and T6 (Figure 2).

The lowest final germination percentages were obtained for seeds treated with T1 (22%), T3 (12%) and T4 (20%) [Table 2]. These percentages did not differ significantly (α 0.05) from

the germination percentages of seeds exposed to T2, T5 and T6. ESI values were 0.8193 (T1); 0.4856 (T3) and 0.5714 (T4), with no significant difference between them (Table 2). Final germination percentages and ESI were 92% and 8.4595 (T2); 96% and 11.7438 (T5) and 94% and 11.6728 (T6) [Table 2]. There was no significant difference between the final germination percentages and the ESI for seeds treated with these three treatments (Table 2).

Figure 2. Initial time (Ti), mean time (Tm) and final time of root protrusion (Tf) in *Passiflora setacea* DC. seeds submitted to different pre-germination treatments.

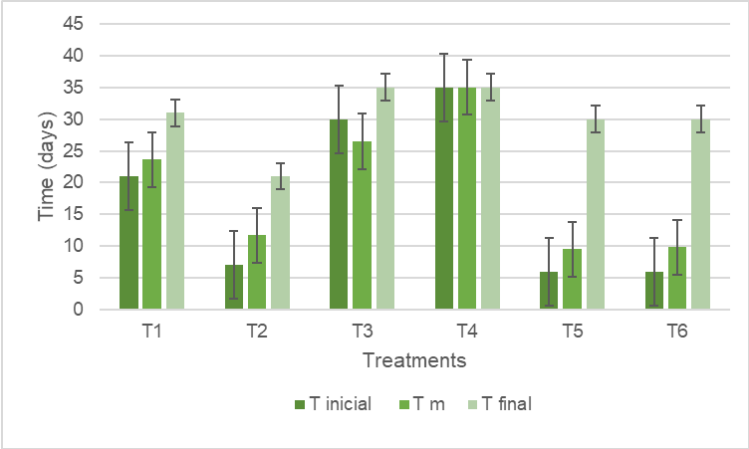


Table 2. Final germination percentages (G%) and emergence speed index (ESI) of *Passiflora setacea* DC. seeds submitted to different pre-germination treatments.

Treatments	G (%)	ESI
T1	22 d	0,8193 c
T2	92 abc	8,4595 ab
T3	12 def	0,4856 cd
T4	20 de	0,5714 cd
T5	96 a	11,7438 a
T6	94 b	11,6728 a

In each column, means followed by the same letters do not differ significantly by Tukey's multiple comparison test (α 0,005).

4 DISCUSSION

Dormancy in seeds of species of the genus *Passiflora* occurs at different intensities and generally results from factors such as tegumentary semipermeability, presence of shallow concavities in the tegument, presence of aril and dependence on germination promoting

substances (Martins et al., 2010; Pérez- Cortez et al., 2002). The results obtained in the present work bring evidence that tegumentary semipermeability, presence of aril and hormonal dependence have a negative effect on the regulation of *P. setacea* seed germination.

In *Passiflora* spp. seeds, tegumentary semipermeability compromises both the volume and the speed of water absorption during imbibition (Ferreira et al., 2007). The values of IFW and seed moisture contents at the end of the germination tests, carried out with *P. setacea* seeds after exposure to the six pre-germination treatments, indicate restriction to water absorption during imbibition, even in untreated seeds (T1). Control seeds (T1) reached the lowest final IFW value, 13.1% and final moisture content, 23.1% (Table 1, Figure 1). T3 and T4 seeds showed IFW higher than T1 seeds (control), in the first five hours and at the end of imbibition, 35.9% (T3) and 26.7% (T4), and final moisture content of 20.3 % (T3) and 39.6% (T4). However, these higher IFW values did not correspond to an increase in germination of seeds treated with T3 and T4.

The gelatinous aril present in the testa of the seeds can disrupt germination through two different mechanisms. On the one hand, hydrophobic molecules present in the aryl, such as unsaturated triglycerides, can act as a barrier or create very negative water potential conditions, preventing seed imbibition; on the other hand, steroids present in the aril, such as brassins, can function as hormones, competing for the active binding sites or even inhibiting the action of germination promoting hormones present in the seed (Freitas et al., 2016; Martins et al., 2010; Silva et al., 2015). As found in seeds of other species of the genus *Passiflora*, both tegumentary semipermeability and the presence of aril residues had a negative effect on germination (Rodríguez et al., 2020). Aryl residues were observed in seeds of *P. setacea* exposed to treatments T1, T3 and T4. Possibly, aryl residues and tegumentary semipermeability interfered with the germination process of seeds treated with T1. In the case of seeds treated with T3 and T4, conditioning with KNO_3 and aeration did not favor germination per se (Figure 1; Table 2), these results suggest the need for specific phytoregulators to trigger the germination process of *P. setacea* seeds.

Comparing the amount of water absorbed by seeds after treatment with T2, T5 and T6 in the first five hours of imbibition, there was a higher IFW in seeds exposed to T5 and T6. Constant aeration during osmopriming with KNO_3 promoted greater oxygen diffusion, contributing to better water absorption for seeds exposed to T3, T4, T5 and T6, without, however, stimulating

the germination performance of T3 and T4 seeds. However, after 168h of evaluation, T2 seeds reached an IFW of 40.9% and the final IFW of T5 and T6 seeds were 32% and 36.2% (Figure 1). At the end of the germination tests, the moisture contents of these seeds were 44.3% (T2), 37.5% (T5) and 31% (T6) [Table 1].

P. setacea seed germination was stimulated by the combination of pre-soaking in Promalin® solution and water bath at 45 °C/20 min. (T2). This temperature probably contributed to change the profile of secondary metabolites of the aril (Diniz et al., 2007) and the structure of the seed coat, softening or cracking it due to thermal shock; as a result, the integuments were no longer a barrier to water absorption, which can be inferred by the higher final value of IFW (40.9%) after this treatment [Figure 1]. These changes probably facilitated the absorption of Promalin®, resulting in a more effective action of the phytohormones (gibberellins and cytokinins) present in the solution, greater stimulation of the metabolic machinery, and 92% of final germination, in less time, 21 DAS (Figure 2; Table 2). A favorable effect of temperature on the germination performance of *Passiflora* seeds was also reported for *P. cincinnata* seeds kept in a water bath at 50 °C/5min. (Oliveira Júnior et al., 2010), *P. edulis f. flavicarpa* immersed in water at 40°C/15min. (Welter et al., 2011) and *P. actinia* by immersion in water at 40°C or 50°C for 5min. or 10 min. (Grzybowski et al., 2019). The interaction between osmopriming treatments with KNO_3 and pre-soaking in Promalin® solution in bain-marie at 45 °C/20 min. resulted in higher germination percentages 96% (T5) and 94% (T6) of *P. setacea* seeds, but at 30 DAS (Figure 2, Table 2).

The time required for seed germination, adopting root protrusion as a germination criterion, is variable among *Passiflora* species and often will depend on the type of pre-germination or germination treatment to which the seeds are submitted. In seeds of *P. edulis f. flavicarpa*, *P. edulis*, *P. alata*, *P. alata* spp. and *P. mucrunata* that received an incision on the seed coat as a treatment to break dormancy, root protrusion occurred only at 7.5 DAS (Nascimento et al., 2022). In the case of *P. alata* seeds, it was reported that root protrusion started at 8.33 days for seeds that were submersed in water, but only 5 days when the seeds were sowed on moist paper (Ferrari et al, 2007). In the wild species *P. morifolia*, *P. suberosa littoris* and *P. palmari* var. *sublanceolata* the beginning of seed germination occurred at 8 DAS (Pires et al, 2012). In *P. setacea* seeds, the time required for the beginning of germination, that is, root protrusion greater than 1 cm, was variable among seeds exposed to the six different treatments

(Figure 2). In seeds treated with treatment T1, the onset of root protrusion was at 18 DAS, with 2% of germinated seeds. In seeds exposed to T3, Ti occurred at 15 DAS, with 2% of root protrusion and seeds treated with T4 germinated only at 35 DAS, with Ti, Tm and Tf equal to 35 days, with a total germination percentage of 20%. In contrast, for seeds exposed to treatments T2, T5 and T6, Ti values were smaller and with higher percentages of protruded radicles. Seeds exposed to T2 started germination at 7 DAS with 21% of germination, and in seeds treated with treatments T5 and T6 the beginning of germination was at 6 DAS, with 20% and 18%, respectively, of protruded radicles (Figure 2).

According to the species of the genus *Passiflora*, there may be a better germinative response to specific treatments. Conforming to Rezazadeh et al. (2018), the best germination performances were obtained with the combination of the following treatments: scarification and fermentation for *P. laurifolia*; scarification and use of GA_3 for *P. maliformis* seeds and immersion in water or scarification and use of GA_3 for *P. tripartita* var. *mollissima*. In seeds of *P. ligularis*, only scarification, or immersion in GA_3 for 15min. were favorable to germination performance (Rezazadeh et al., 2018; Cadorin et al., 2017). Seeds of *P. edulis f. flavicarpa* pre-soaked with GA_3 showed an increase in both final germination and germination speed (Zanini et al., 2016). The best method for overcoming dormancy in *P. elegans* seeds was the removal of the seed coat, followed by immersion in a GA_3 solution. (Silva et al., 2019).

Phytohormones act antagonistically or in synergism both in regulating germination and in overcoming dormancy (Shu et al., 2016). The complex metabolic and physiological mechanisms that stimulate germination are stimulated by cytokinins that complement the action of gibberellins (Ferreira, 2022). The treatment with phytohormones activates development and stimulates the germination process in seeds of some species of the genus *Passiflora* (Cárdenas-Hernández et al., 2013). The final germination of the control treatment (T1) was 22% at 35 DAS with a Tm of 23.7 days and ESI 0.8193. In the present work overcoming dormancy in *P. setacea* seeds was achieved by combining gibberellins and cytokinins (Promalin®) in a bain-marie at 45 ºC/20min (T2), with a final germination percentage of 92% at 21 DAS, with a Tm of 11.7 days ESI 8.4595 (Table 2). These results corroborate those obtained by José et al. (2019) in which the use of Promalin® stimulated the best germination performance in tests of vigor and germination of *P. setacea* seeds. The beneficial effect of Promalin® in inducing germination was also reported for seeds of other *Passiflora* species such as cultivars BRS RC 024/15 and BRS RC 028/14 of *P.*

edulis (Luz et al., 2021), *P. cincinnata* (Moura et al., 2018), *P. alata* (Ferrari et al., 2008), *P. caerula*, *P. hatsbachii*, *P. sidifolia*, *P. suberosa* (Oliveira et al., 2020) and *P. tenuifila* (Junghans et al., 2020).

Osmopriming in seeds can favor germination in numerical terms and promote repair and metabolic activation during imbibition (Santos et al., 2008). There are reports that KNO_3 can stimulate germination under stress conditions and favor the metabolic routes that improve the germination process, as observed in *P. eichleriana* and *P. setacea* seeds (Marostega et al., 2017; Pádua et al., 2011). It should be noted, however, that the role of KNO_3 in overcoming dormancy is controversial. The use of KNO_3 as an osmotic agent for 1 or 2 days (T3 and T4) in *P. setacea* seeds was not favorable to germination performance (Table 2). Seeds treated with KNO_3 showed the lowest final germination percentages and ESI. Seeds treated with T3 reached 12% of final germination with ESI of 0.4846 and seeds exposed to T4 had 20% of final germination with ESI of 0.5714. This negative effect of osmopriming on *P. setacea* seeds can be attributed to a combination of factors, such as the concentration of the KNO_3 solution ($2.6 g.L^{-1}$), the exposure periods tested (1 and 2 days) or the intrinsic properties of this osmotic agent. Due to the low molecular weight of this salt, it is easier for it to pass through the seminiferous envelopes, modulating both the effect of ABA and gibberellins; however, its accumulation causes phytotoxicity, physiological drought and delay or inhibition of the germination process (dos Santos et al., 2022; Hernández et al., 2021).

Depending on the characteristics of the plant species, the combination of phytoregulators with the osmotic agent KNO_3 can have a positive or negative effect on the germination process (Çavuşoğlu et al., 2017). The results obtained with *P. setacea* seeds when treated with phytoregulators present in the Promalin® solution (T2) at 45 ºC/20 min. and the combination of this treatment with the osmotic agent KNO_3 (T5 and T6) were expressed by the values of the germinative attributes Ti, Tm, ESI and G%, validating the favorable effect of the interaction between these treatments. Seeds primed with KNO_3 for 1 day and immersed in Promalin® (T5) started germination at 6 DAS and reached the final percentage of germination of 96% at 30 DAS, with a Tm of 9.5 days and ESI 11.7438. Seeds primed with KNO_3 for 2 days and immersed in Promalin® (T6) starting the germination process at 6 DAS, reached a final germination percentage of 94% at 30 DAS, with a Tm of 8.8 days and ESI 11.6728. These results can be attributed to the reversal of the negative effects of KNO_3 on *P. setacea* seeds by treatment with

Promalin® at a temperature of 45 ºC/20 min. Such results suggest that seeds of *P. setacea*, when submitted to appropriate treatments to overcome dormancy, such as T2, T5 and T6, had their germination performance stimulated and expressed their real vigor, but does not standardize the germination process.

5 CONCLUSIONS

Dormancy in *P. setacea* seeds results from a combination of intrinsic and extrinsic factors, which characterizes dormancy as physical-physiological. The regulation of germination in these seeds was obtained by removing the aril in association with the supply of phytohormones (gibberellins and cytokinins) at a temperature of 45 ºC/20min., with or without the osmotic agent (KNO_3). It should be noted that in terms of economics and to shorten the time necessary to obtain seedlings, it is recommended to remove the aril associated with treatment of seeds with Promalin® phytohormones (gibberellins and cytokinins) in a water bath at 45 ºC/20min.

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