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SEED DORMANCY AND GERMINATION OF TWO CULTIVATED SPECIES OF PASSIFLORACEAE*

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Abstract

Colombia is the country with the highest number of species of *Passiflora* in the world and most species have edible fruits and are cultivated. This investigation aims to better understand the range of seed dormancy exhibited in the fleshy fruits of Passifloraceae species, and to determine how to break this dormancy efficiently and reliably. Thus, in this investigation seed germination of *Passiflora edulis* and *P. maliformis* were studied. Seeds were extracted, conditioned, dried and scarified. Several chemical pre-treatments were applied (*e.g.* GA_3 500, 1000, 2000 ppm, KNO_3 1%, $KNO_3$1.5% - KH_2PO_4 1.5%). Illuminated germinators were used with constant temperature (*i.e.* 25, 30 ºC) and alternate temperature (*i.e.* 15/25, 15/30, 19/33, 20/30, 20/35 ºC). Germination was recorded as radicle protrusion. Removing the seed aril with water allowed a clean germination test in both species. For *P. edulis* the greatest germination was obtained at 20/30 ºC, while the poorest germination was found at constant temperature. Manually-scarified seeds provided the most rapid germination in all temperatures. For *P. maliformis* the highest germination was obtained for non-manually scarified seeds treated with boiling water and tested at 20/35 ºC. Pre-treatment with GA_3 or KNO_3 did not promote germination in both species. It is concluded that seeds of *P. edulis* and *P. maliformis* have physical dormancy. Scarifying seeds of *Passiflora* spp. enabled the water potential of the embryo to increase and for seeds to germinate. Meanwhile, the chemical pre-treatmeant of seeds showed no additional benefit. Thus, physiological dormancy does not occur, and physical barriers are the only factor preventing germination of viable seeds of the two *Passiflora* species studied.

Key words: *Passiflora edulis, Passiflora maliformis*, physical dormancy, scarification, maracuyá, gulupa, cholupa, passion fruit.

LATENCIA Y GERMINACIÓN DE SEMILLAS DE DOS ESPECIES CULTIVADAS DE PASSIFLORACEAE

Resumen

Colombia es el país con el mayor número de especies de *Passiflora* en el mundo y la mayoría de especies tiene frutos comestibles y son cultivadas. Esta investigación tuvo el objetivo de entender mejor el rango de latencia que exhiben las semillas de los frutos jugosos de las especies de Passifloraceae, y determinar cómo romper esta latencia eficientemente. Así, se investigó

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la germinación de semillas de *Passiflora edulis* y *P. maliformis.* Las semillas se extrajeron, acondicionaron, secaron y escarificaron. Se utilizaron pre-tratamientos químicos (*e.g.* GA_3 500, 1000, 2000 ppm, KNO_3 1%, KNO_3 1,5% - KH_2PO_4 1,5%). Se utilizaron germinadoras iluminadas con temperaturas constantes (*i.e.* 25, 30 ºC) y temperaturas alternadas (*i.e.* 15/25, 15/30, 19/33, 20/30, 20/35 ºC). El criterio de germinación fue salida de la radícula. Descartar el arilo de las semillas con agua permitió tener pruebas de germinación limpias en ambas especies. Para *P. edulis* la mayor germinación se obtuvo a 20/30 ºC, y la peor a temperaturas constantes. Semillas manualmente escarificadas tuvieron la germinación más rápida en todas las temperaturas. Para *P. maliformis* la mayor germinación se obtuvo para semillas no escarificadas manualmente tratadas con agua hirviendo e incubadas a 20/35ºC. Los pretratamientos con GA_3 o KNO_3 no promovieron la germinación en ambas especies. Se concluye que las semillas de *P. edulis* y *P. maliformis* tienen latencia física. La escarificación de semillas permitió el aumento del potencial de agua del embrión y la germinación, mientras que los pre-tratatamientos químicos no mostraron beneficios adicionales. Así, no ocurre latencia fisiológica y las barreras físicas son el único factor que previene la germinación de semillas viables de las dos especies estudiadas de *Passiflora*.

Key words: *Passiflora edulis, Passiflora maliformis*, latencia física, escarificación, maracuyá, gulupa, cholupa.

INTRODUCTION

The family Passifloraceae is native to the tropics and subtropics and comprises 20 genera and about 600 species. The genus *Passiflora* is widely distributed, with 400-500 species originating from America (HEYWOOD, 1998). With its high diversity in habitats and environments (ALBERT, 1991), Colombia is home to 226 species (BERNAL *et al.*, 2015), which makes it the country with the highest number of species of *Passiflora* in the world. Between 50 and 60 species of *Passiflora* have edible fruits and are cultivated, *e.g. P. quadrangularis* ("giant granadilla or badea"), *P. edulis* ("passion fruit, maracuyá or gulupa"), *P. maliformis* ("stone granadilla or cholupa"). In addition, about twenty species of *Passiflora* are cultivated as ornamentals for their attractive flowers (HEYWOOD, 1998).

Passiflora edulis is native to Brazil and was distributed throughout the tropics and subtropics during the 19th century (PATIÑO, 2002). Two forms are recognized: 1) *P. edulis f. edulis* with purple fruit of 4-5 cm in diameter and pulp with a sweeter flavour. It grows at an altitude of about 2,000 m. 2) *P. edulis f. flavicarpa* with yellow fruit of 5-6 cm in diameter, and a pulp that is more acid and better suited in lowlands (PURSEGLOVE, 1968). Commercial plantations of *P. edulis* grow plants from seeds sown in nursery beds. The seedlings of about 30 cm tall are transplanted after 3-4 months. Fruits are produced between 6 and 9 months after planting and full production is reached after 18 months (PURSEGLOVE, 1968). Colombia is the second seller of Passion fruit to the European Union after South Africa (BARNIER, 2010).

Further to this, *P. maliformis* has been recognized as one of the most promising species for cultivation in the North of South America (ALBERT, 1991) as it has historically been used by indigenous people in the lowlands of Colombia and Ecuador (PATIÑO, 2002).

Several species of *Passiflora* have reports of seed dormancy. Dormancy is a property of the viable seeds that blocks the capacity to germinate (BASKIN & BASKIN, 2014). Several classes of dormancy have been recognized, physical dormancy where the seed coat is water-impermeable and prevents germination, morphological dormancy where the seed has embryo underdeveloped or undifferentiated not ready to germinate, and physiological dormancy where the seed is water-permeable and have physiological (low or deep) inhibiting mechanisms that prevents germination (NIKOLAEVA, 1969; BASKIN & BASKIN, 2014). Dormancy is a result of an adaptative response to the environment in plants of many climatic regions (FINCH-SAVAGE & LEUBNER-METZGER, 2006). Recently, molecular studies have found that physiological dormancy is an active state, regulated by several genes (*e.g.* DOG1, delay of germination 1 in Arabidopsis; NCED6 and CYP707A2 which regulate ABA synthesis and catabolism; GA3ox1 and GA2ox2 wich regulate GA synthesis and catabolism), (FINCH-SAVAGE & FOOTITT, 2017).

Germination studies in *Passiflora* have concentrated on breaking the physical constraint of the seed coat seeds to germinate. Physical treatments have been tested, such us fracture of testa which improves germination in several *Passiflora* species (MORLEY-BUNKER, 1980). For *P. alata*, elimination of the aril produces higher and faster germination (ROSSETTO *et al.*, 2000). In *P. caerulea* seeds that have been cut exhibit higher germination than intact seeds (SEVERIN *et al.*, 2003). Chemical treatments and their combination with physical treatments have been successful to break dormancy in Passiflora species. For instance, PEREIRA & DIAS (2000) found better germination for *P. edulis* seeds with a combination of HCl abrasion with lime; MABUNDZA *et al.* (2010) achieved high germination in *P. edulis* seeds with chemical scarification (*i.e.* H_2SO_4); and PASSOS *et al.* (2004) found most promotion of germination in *P. nitida* for scarified seeds treated with gibberellic acid 1,000 ppm. In *P. alata* it was found that imbibition in GA_3 (500 ppm) produced the highest germination, but the imbibition duration (5-25 hours) had no effect on germination percentage (FERREIRA & MORO, 2001). However, other treatments such as NaOH and H_2SO_4 treatments were unsuccessful for the germination of seeds of *P. nitida* (MELO & VIEIRA, 2000).

In this paper, then, I report the results of my investigation that aims to better understand the range of seed dormancy exhibited in the fleshy fruits of Passifloraceae species (*i.e. Passiflora edulis, P. maliformis*), and to determine how to break this dormancy efficiently and reliably. Thus, the hypothesis I test is: the germination of seeds of fleshy fruits of *Passiflora edulis* and *P. maliformis* can be improved by seed cleaning, conditioning, alternating temperatures and/or chemical pre-treatments.

MATERIALS AND METHODS

Seed sources, extraction and conditioning

Mature fruits of the crop species *Passiflora edulis* were purchased from local markets in Santiago de Cali, Colombia, while fruits of the *Passiflora maliformis* were purchased from small farmers in the departments of Antioquia and Huila, Colombia. The sources and traits of these fruits are provided in Table 1.

Table 1. Details of fruit source of *Passiflora* species and subsequent seed storage period, temperature and moisture content (n.a.: seeds not available for seed moisture content test).

Species	Seed lot	Geographic origin of cultivation or purchase	Altitude (m)	Duration (months) and temperature of storage before investigation	Seed moisture content (%)
Passiflora edulis f. *flavicarpa* O. Deg.	---	Commercial market Cali, Colombia	---	0	8.0
Passiflora maliformis L.	A	Grown in Rionegro, Antioquia, Colombia	1,500	12 (20 ºC)	n.a.
Passiflora maliformis L.	B	Grown in Gigante, Huila, Colombia	1,000	7 (20 ºC)	4.4
Passiflora maliformis L.	C	Grown in Huila, Colombia	1,000	7 (20 ºC)	n.a.

All fruits were cut in half and seeds extracted with a spoon, on the day following purchase. Including their fleshy covering (*i.e.* aril), the seeds were deposited in a container with running tap water. The seeds were stirred manually for 30 minutes to dislodge the fleshy coverings. Thereafter, the seeds were placed in a sieve (*i.e.* aperture 2 mm), and squeezed gently by hand to release the fleshy cover. Running tap water was applied at least three times to rinse off the fleshy coverings, until the seeds were flesh free. All floating seeds were discarded, as it was assumed they were empty. Once completely free of all fleshy covering, the seeds were transferred to a flat tray in layers of one or two seeds deep.

The seeds were left at laboratory conditions overnight (24 hours, 20-25 ºC, 70% r.h.), to enable preliminary drying to occur. Subsequently, seeds were transferred to a desiccator over silica gel to reduce moisture content to 4.4 – 8 %, as it is recommended to reduce seed moisture content before dormancy-breaking treatments to stimulate germination of seeds (BEWLEY & DOWNIE, 1996; HONG & ELLIS, 1990).

The moisture content test of most seed lots was determined before the germination tests, according to seed availability (Table 1). The high-constant-temperature-oven method was applied, namely one hour at 130ºC (ISTA, 2016). Two samples of 1-2 g each of entire seeds were dried in Pyrex Petri dishes (85 x 15 mm) in an oven (Thermocenter) and weighed in an analytical balance (Metler Toledo) to the nearest 0.01 mg before and after drying. The moisture content was calculated as percentage wet basis (ISTA, 2016).

Germination tests

Each germination test consisted of four replicates of 50 seeds (*P. edulis*) or 25 (*P. maliformis*). The germination tests were carried out on rolled paper towels. The paper was soaked in deionised water and squeezed to discard excess water. Four papers (Kimberly-Clark Co., code 6803) were used for each replicate and four replicates were placed in a loosely-folded plastic bag. No fungicides were added to the substrate to avoid extra chemical factors affecting germination but, as these two species were very susceptible to fungal attack, sodium hypochlorite (1%) was pre-applied for 5 minutes only.

Scarification was needed because in preliminary tests non-scarified seeds did not germinate: a piece of testa from the opposite side to the radicle was cut off at the widest part of the seed, with a scalpel. Another pre-treatment was to soak non-scarified seeds in water just off the boil (96ºC) for 24 hours (as the water cooled). Some *Passiflora* species are self-incompatible and produce empty seeds or incompletely-developed seeds (GILMARTIN, 1958; SNOW, 1982). Thus, at the end of each test, the non-germinated seeds were each cut to confirm the presence or otherwise of embryo. Where empty embryos were detected, germination was recalculated as a percentage of the full seeds tested.

Illuminated constant and alternating temperature incubators were used for the germination tests, the latter with 16/8 hours thermoperiod, for constant temperature (*i.e.* 25, 30ºC) and alternate temperature (*i.e.* 15/25, 15/30, 19/33, 20/30, 20/35 ºC). Light was provided for 16 hours per day for both constant and alternating temperatures. In the latter case, the lighting period was synchronous with the longer thermoperiod. In each case, the first temperature listed for each alternating-temperature regime is that provided for 16 h of each daily cycle; *e.g.* 15/25 indicates 15ºC applied for 16 h d^{-1} and 25 ºC applied for 8 h d^{-1}.

Germination was recorded as radicle protrusion (≥ 2 mm length). The test duration was 28 days for *P. edulis* and 35 days for *P. maliformis*. The germination tests were checked periodically to record the progress of germination during tests. The checking frequency was once a week. The rate of germination was calculated by the formula (1)

$$R = \sum \frac{n}{(d * n)} \quad (1)$$

where n is the number of seeds germinated on day d
and, d is the number of days from the beginning of the germination test.

Chemical pre-treatments

Several chemical pre-treatments were applied for 24 hours at 20ºC before beginning germination tests; 30-60 ml of solution was used to soak 100-200 seeds. The controls and treatments used were: 1) Dry seeds, *i.e.* no pre-treatment; 2) Deionised water, as pre-applied control; 3) Gibberellic acid (GA_3): 500, 1000 and 2000 ppm; 4) Potassium nitrate (KNO_3), 1%; 5) Potassium nitrate (KNO_3), 1.5% + Potassium phosphate (KH_2PO_4), 1.5%.

Statistical analysis

In order to have normality of variances for analysis of variance, germination (percentage) was transformed to angles using the arcsine function by the formula (2):

$$Angles\ (Radians) = Arc\ sin\ \sqrt{\frac{\%\ ger\ min\ ation}{100}} \quad (2)$$

Angles were transformed from radians to degrees. The analysis of variance (ANOVA) was used to compare the variation among factors. The SAS software was used for these analyses (SAS, 1999). The factorial experiments differed slightly among species depending upon the numbers of seed lots, temperature regimes, and pre-treatments (Table 2). The level of α to determine significance in the F test was 0.05. When no interaction between factors was found, the a posteriori Tukey test was applied.

Table 2. Factorial design for the Passifloraceae species and factors tested for breaking seed dormancy.

Species	Factorial	Number of seed lots	Number of temperature regimes	Number of treatments
P. edulis	7x6	1	7	6
P. maliformis (lot A)	5x5	1	5	5
P. maliformis (lots B, C)	2x6x5	2	6	5

RESULTS

Passiflora edulis f. flavicarpa

Both factors, temperature and pretreatment, and their interaction, affected germination ($p<0.05$). The poorest germination was found at constant temperature (25, 30 ºC), while the greatest germination was at 20/30 ºC (Figure 1). Germination was also the most consistent among pre-treatments in this temperature regime.

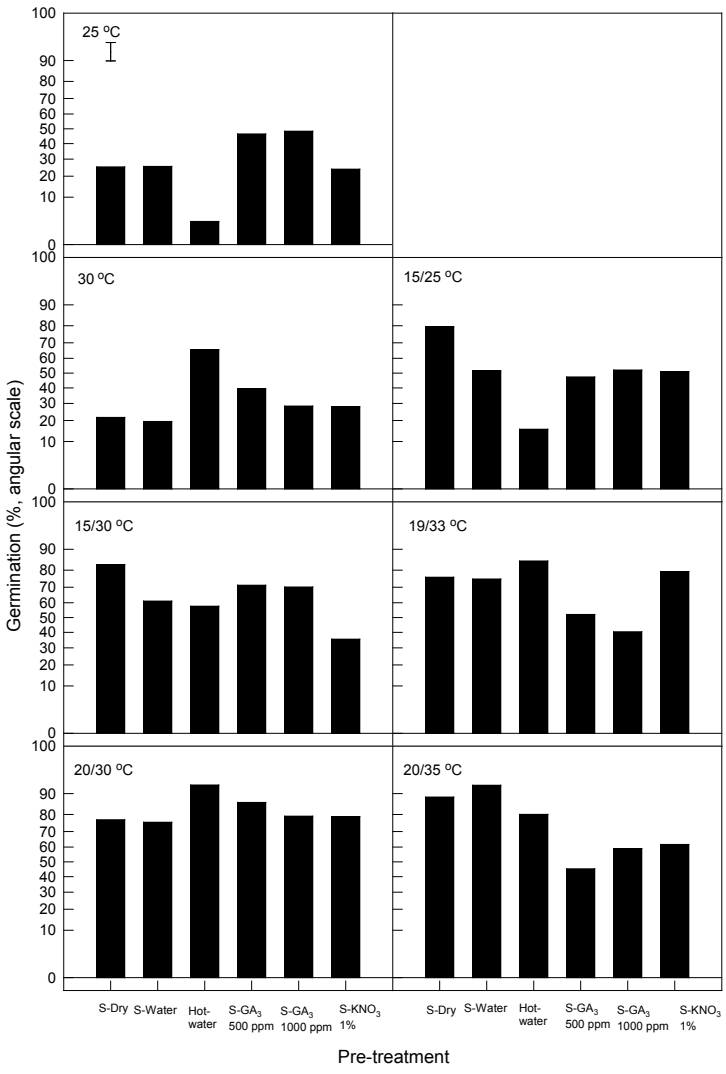

Figure 1. Effect of constant or alternating temperature and several pre-treatments on the germination of *Passiflora edulis* (S=scarified manually). The bar at the top left of the first is the standard error of difference of means for comparison of any pair of treatment combinations (126 df).

The rate of germination was affected by both factors and their interaction ($p<0.05$). Manually-scarified seeds provided the most rapid germination in all temperature regimes (Figure 2). Temperatures of 30, 19/33 and 20/30 ºC provided the most rapid germination.

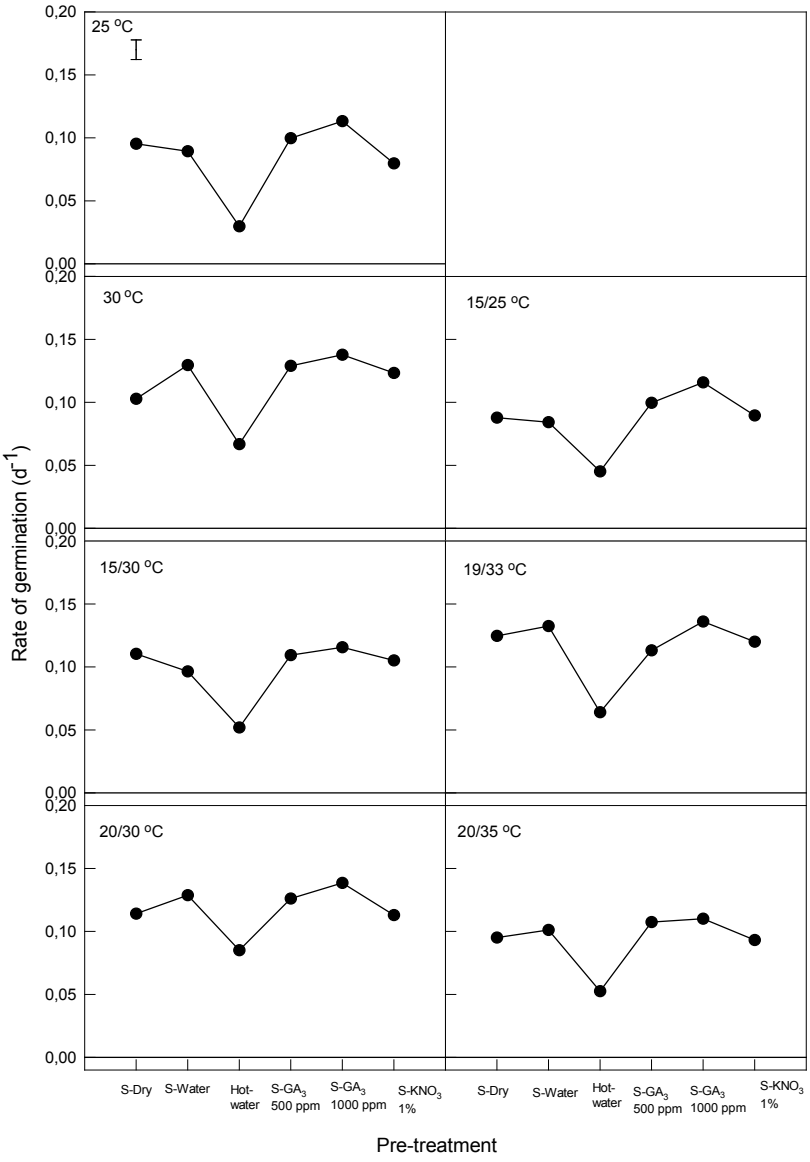

Figure 2. Effect of constant or alternating temperature and several pre-treatments on the rate of germination of *Passiflora edulis* (S=scarified manually). The bar at the top left of the first box is the standard error of difference of means for comparison of any pair of treatment combinations (126 df).

Passiflora maliformis

Only three treatment combinations provided more than 50% germination (Figure 3). Temperature regime, pre-treatment and their interaction have shown to affect germination ($p<0.05$). The highest germination of lot A was provided by non-manually scarified seeds pre-soaked in boiling water and then tested at 15/30ºC, and this was only 41%.

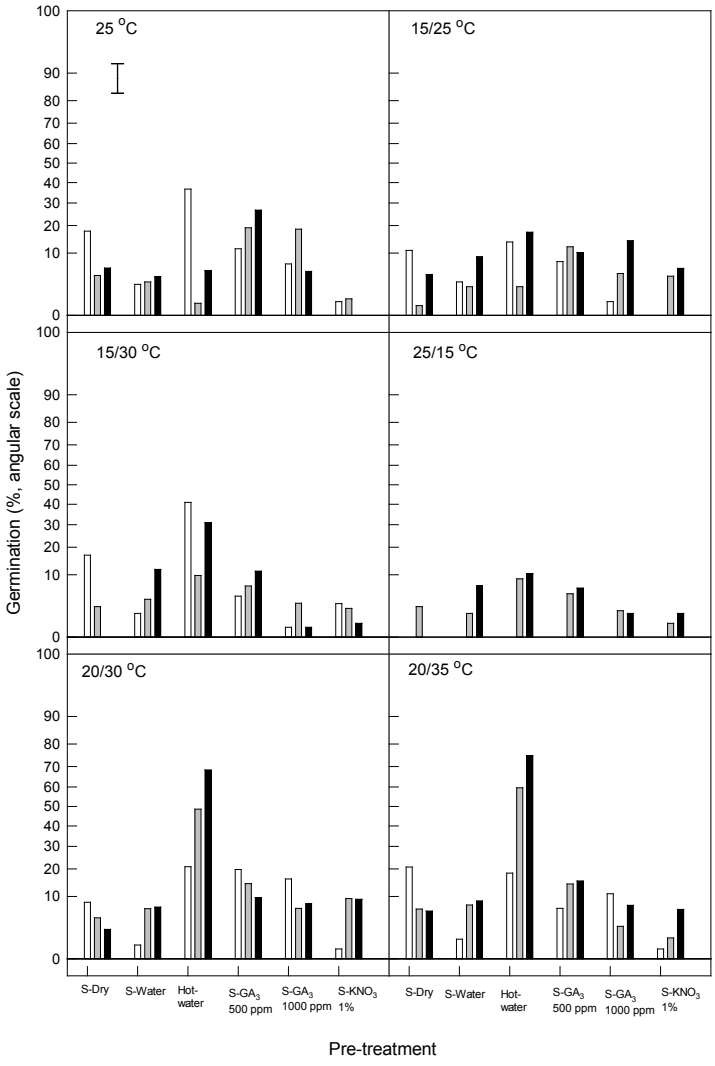

Figure 3. Effect of constant or alternating temperature and several pre-treatments on the germination of three seed lots of *Passiflora maliformis* (35 d duration, S=scarified manually), lot A: white, lot B: gray, lot C: black. Lot A was not tested at 25/15 ºC see text. The bar at the top left of the first box is the standard error of difference of means for comparison of any pair of treatment combinations (303 df).

Given the poor germination of lot A, another study was carried out with two further lots (B and C) and one additional alternating temperature (25/15 ºC) included. This resulted in significant differences among treatments ($p<0.05$).

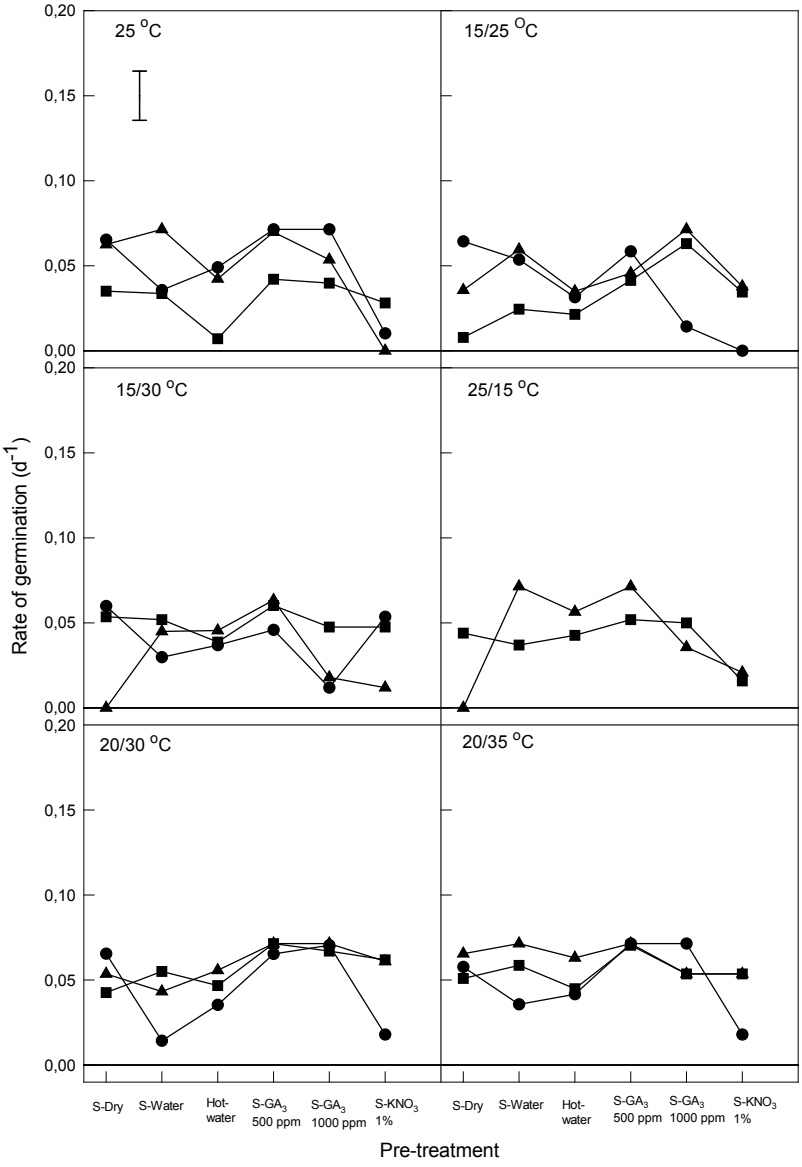

Figure 4. Effect of constant or alternating temperature and several pre-treatments on the rate of germination of *Passiflora maliformis* (S=scarified manually), Lot A (□), Lot B (¢), Lot C (p). Lot A was not tested at 25/15ºC see text. The bar at the top left of the first box is the standard error of difference of means for comparison of any pair of treatment combinations (303 df).

The highest germination was obtained for non-manuallyscarified seeds treated with boiling water and then tested at 20/35 (60 and 74%), followed by 20/30 ºC (49 and 68%) for seed lots B and C respectively (Figure 3). Pre-treatment with GA_3 or KNO_3 did not promote germination.

Although rate of germination was significantly affected by most of the factors and their interactions, germination of *P. maliformis* was slow compared to *P. edulis*. The low overall germination (Figure 3) and slow germination (Figure 4) confirms the difficulty in promoting germination in *P. maliformis*.

DISCUSSION

The conditioning of *Passiflora* seeds to remove the seed fleshy covering (*i.e.* aril) with running tap water was a good practice to allow for a clean germination test. This practice has been successfully used in *P. edulis*, however mediated by enzymes to digest the aril (POSADA *et al.*, 2014). Germination varied considerably among seed lots, the different temperature regimes, and the different pre-treatments employed. The seed dormancy of *Passiflora* species is due to seed hardness or physical dormancy as defined by NIKOLAEVA (1969), BEWLEY & BLACK (1985) and BASKIN & BASKING (2014). In these species, the hard testa provides a barrier to water uptake, a basic requirement for germination. This was confirmed in the preliminary test of germination for non-scarified seeds of *P. edulis* at 25ºC where non-germination at all was obtained (data not shown).

Among my scarified pre-treatments, seeds of *Passiflora edulis* were found to be better promoted to germinate by alternating temperature of 20/35ºC for manually scarified seeds soaked in cold water, and 20/30ºC for not scarified seeds soaked in boiling water. However, the latter pre-treatment is not recommended due to the slow rate of germination. When a regime of alternating temperature is applied, the most important feature to obtain high germination in *P. edulis* is to open the seed slightly, manually or by the action of the temperature of the boiling water. This result corresponds with the higher germination of cut seeds obtained in *P. caerulea* (SEVERIN *et al.*, 2003), seed apical point removed in *P. tricuspis* and *P. molissima* (DELANOY *et al.*, 2006), and *P. edulis* (GUTIÉRREZ *et al.*, 2011), and seeds scarified by using sulfuric acid in *P. edulis* (MABUNDZA *et al.*, 2010). This study, then, shows that the release of seed hardness is a way to obtain higher germination, because scarified dry or water-soaked seeds germinated better than the scarified seeds pre-treated with chemicals at alternating temperatures. This also confirms that the duration of soaking does not affect seed germination in *P. edulis* (ALEXANDRE *et al.*, 2004).

For *P. maliformis*, the boiling water pre-treatment for entire seeds and an alternating temperature of 20/35 or 20/30 ºC was the best way to promote seed germination and had no detrimental effect on the rate of germination in contrast to the slow rate of

germination in *P. edulis*. As found in *P. edulis*, the hard testa acts as a physical barrier to water uptake in the seeds of *P. maliformis*.

Thus, the barrier to germination in *Passiflora* species is the hard testa and as soon as the water potential of the embryo structures can increase by physically conditioning the seeds, they can germinate with higher results than seeds that have only been pre-treated chemically. This confirms the better germination found in *P. edulis* when abrasion or friction of seeds was applied (PEREIRA & DIAS, 2000), and in other *Passiflora* species when testa were cut or fractured (MORLEY-BUNKER, 1980; SEVERIN *et al.*, 2003). However, this result contradicts the data of PASSOS et al. (2004) for in vitro germination of *P. nitida*. In their study germination was increased by application of GA_3 (1,000 mg L^{-1}). The absence of any benefit of the chemical treatments for seed germination of *P. edulis* and *P. maliformis* confirm that these species do not have physiological dormancy.

On the other hand, the results of the present study with higher germination at alternated temperatures for both *Passiflora* species are contradicting to a study of *P. incarnata* which found optimal seed germination at a constant temperature (35ºC) in darkness (BIENVENUTI *et al.*, 2001).

The relatively low germination obtained for *P. maliformis* in this study (*i.e.* maximum 74%) indicates the difficulty to promote germination in this species. In accordance to this result, GUTIÉRREZ *et al.* (2011) did not found promotion of germination with any treatment in *P. maliformis* seeds. It is a limitation to handling seeds of this species and further investigation is required.

CONCLUSIONS

Seeds of Passifloraceae have physical dormancy or hardseededness. No scarification produced nil germination in *P. edulis* at constant temperature, whereas scarification with a scalpel or by immersion in boiled water increased germination in *P. edulis* and *P. maliformis* when alternating temperature was applied (*e.g.* 20/35 ºC, 16/8 h).

Scarifying seeds of *Passiflora* spp. enabled the water potential of the embryo structures to increase sufficiently for seeds to germinate, while seeds additionally pre-treated with potassium nitrate or gibberellic acid showed no additional benefit. That is, physiological dormancy does not occur, and physical barriers are the only factor preventing germination of viable seeds in *Passiflora* species.

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