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Evaluation of different methods to overcome *in vitro* seed dormancy from yellow passion fruit

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Seeds from yellow passion fruit (*Passiflora edulis* Sims) present dormancy imposed by the seed-coat. The present study aimed to evaluate some methods to overcome dormancy of seeds from *P. edulis* grown under *in vitro* conditions. The experimental design was completely randomized in factorial scheme (15 scarification methods × 2 substrates: PlantMax® and MS medium), constituting 30 treatments with 10 replicates. The following treatments were established: Control (untreated seeds); seeds soaked in water at room temperature for 12, 24 and 48 h; seeds soaked in hot water at 80°C for 1, 2.5 and 5 min; chemical scarification with sulphuric acid (98%) for 1, 5, and 10 min; immersion of the seeds in absolute ethanol for 5, 10 and 25 min; physical scarification with wood sandpaper (no. 125); and total seed coat removal with a Gavin® mini-vise. After each treatment, the seeds were inoculated *in vitro* in two sterile substrates (MS medium or PlantMax®). The results show that the best treatment to overcome dormancy of *P. edulis* seeds was total removal of seed coat with Garvin® mini-vise. This treatment yielded 100% seed germination under *in vitro* conditions either on PlantMax® or MS medium. Approximately 150 seeds per hour could be decoated according to the operator's skills. However, in some cases, there was physical damage to the zygotic embryo. Seed treated with sulphuric acid for 1 min also showed promising results (average 0.107). Seeds germinated within seven days after being treated with sulphuric acid and inoculated *in vitro* in PlantMax® sterile substrate. Moreover, poor germination rates were achieved when seeds were treated with sulphuric acid, followed by inoculation *in vitro* onto MS medium (0.0711). All seeds germinated on *in vitro* PlantMax® sterile substrate and presented an epinastic phenotype, possibly due the ethylene biosynthesis *in vitro*.

Key words: *Passiflora*, dormancy, seed germination.

INTRODUCTION

The yellow passion fruit (*Passiflora edulis* Sims.) is the most important and widely cultivated species of *Passiflora* genus, producing fruits for both fresh market and juice industry. Brazil is one of the major worldwide producers of yellow passion fruit, with a cultivated area of approximately 50,795 ha devoted to this crop (IBGE, 2009). This species can be propagated quite readily by seeds, cuttings and grafting onto seedling rootstocks (Alexandre et al., 2009a, b). Plants of the yellow form are almost exclusively seed-propagated, and seeds germinate within approximately 2 to 3 weeks (Vieira and Carneiro, 2005), although present dormancy imposed by

the seed-coat when cultivated *in vitro*.

Dormancy is referred to as the physiological state in which viable seeds fail to germinate when provided with water and adequate environmental conditions favorable for germination. Dormancy is considered as a strategy to avoid germination under certain conditions where seedling survival is likely to be low (Ellis et al., 1985a; Schmidt, 2000). Seed dormancy has been reported for different *Passiflora* species, example, *P. edulis* (Morley-Bunker, 1980; Hall et al., 2000; Alexandre et al., 2004), *P. incarnata* L. (Wehtje et al., 1985), *P. mollissima* (La Rosa, 1984), and *P. nitida* Kunth (Passos et al., 2004). According to Ellis et al. (1985b), *Passiflora* species possess non-endospermic seeds, meaning seeds with only residual or no endosperm, but with mature embryos. Their seeds have a hard coat with a semi-permeable inner layer. Despite ready uptake of water by the seeds during the imbibition, they might contain strong inhibitors which do not allow the embryo growth. Some of these inhibitors are located in the thin residual endosperm layer surrounding the embryo or within the embryo cotyledons. The inhibitors are blocked from leaching by the semi-permeable membranous testa incorporated in the coverings, and once excised, embryos germinate promptly. In summary, *Passiflora* species have exogenous dormancy (Morley-Bunker, 1980), which is probably due to the combination of mechanical and chemical dormancy.

Several studies have demonstrated that *in vitro* regeneration in yellow passion fruit has been achieved from pre-existing meristems located in shoot apices or nodal segments (Drew, 1991; Faria and Segura, 1997; Reis et al., 2003), from adventitious buds developed from leaf discs (Dornelas and Vieira, 1994; Appezzato-da-Glória et al., 1999), from the hypocotyl (Reis et al., 2003; Rêgo et al., 2011) and from internodal segments (Biasi et al., 2000), all derived from seedlings germinated *in vitro*. However, a basic problem related to *in vitro* growth of Passifloraceae species is the seed germination, which still presents technical difficulties. In fact, the great difficulty found for the *in vitro* culture establishment of yellow passion fruit is related to unsuccessful germination of the seeds, even when it was applied any of the already published protocols (Kantharajah and Dodds, 1990). According to Morley-Bunker (1980), seeds from yellow passion fruit present dormancy imposed by seed-coat tegument. Hall et al. (2000), suggested that only after the total removal of the seed coat the dormancy is overcame; scarification of the seeds may work, but it is technically difficult to execute, since the mature passion fruit seeds are small and hard.

Various methodologies used to overcome seed dormancy were published for different species: chemical scarification with sulphuric acid in *Cotinus coggygria* Scop (Olmez et al., 2009), *Tamarindus indica* (Muhammad and Amusa, 2003), and *Parkia biglobosa* (Aliero, 2004); and chemical scarification using ethylic alcohol in *Euphorbia heterophylla* L. (Kern et al., 2009). In addition, for the seeds of *Passiflora* species, the induction of germination has been done by using gibberellic acid (Morley-Bunker, 1980; Passos et al., 2004), hot water (Oliveira et al., 2010), water at room temperature (Pinto et al., 2010), scarification with wood sandpaper (Oliveira et al., 2010; Lombardi et al., 2007), and seed coat fracture or fermenting seed with cytase (Morley-Bunker, 1980). The objective of this study was to develop an efficient, simple, rapid, and reliable method to overcome the lack *in vitro* germination of passion fruit seeds.

MATERIALS AND METHODS

The experiment was conducted in Tissue Culture Laboratory at the Federal University of Roraima, Roraima Brazilian State. Three hundred seeds from the same fruit of yellow passion fruit cultivar, Yellow Giant, were collected from a commercial plantation in county of Boa Vista, Roraima, Brazil. Seeds were surface-sterilized and then submitted to chemical and physical scarification methods, before placing them for *in vitro* germination.

Scarification methods to overcome *in vitro* seed dormancy

The treatments applied to overcome *in vitro* seed dormancy as shown in Table 1 include: Control (non treated seed); hot water (80°C for 1, 2.5 and 5 min); absolute ethanol (for 5, 10 and 15 min); sulphuric acid 98% (for 1, 5 and 10 min); scarification with wood sandpaper (no. 125); and total seed coat removal utilizing a Garvin mini-vise® (jaw width = 1 inch) (Figure 1). The scarification was made before the seeds' surface sterilization, as recommended by Kantharajah and Dodds (1990) and d'Utra Vaz et al. (1993). After the total removal of the seed coat, seeds were submitted for the surface sterilization process.

Germination media and growth conditions

Seeds were germinated *in vitro* in a sterile commercial pot mix (Plantmax® (Eucatex, SP, Brazil) and MS (Murashige and Skoog, 1962) medium. For surface sterilization, the seeds were soaked into a dilute commercial bleach solution (0.7% hypochlorite) for 20 min, followed by three rinses with sterile distilled water. For *in vitro* germination, the seeds were transferred to test tubes (150 × 15 mm) containing either 10 ml MS medium, supplemented with 3%

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Abbreviations: **MS**, Murashige and Skoog (1962); **B5**, Gamborg medium (1968); **BA**, 6-benzyladenine; Kin, kinetin; **IAA**, indole-3-acetic acid.

Table 1. Relation of the different scarification methods for overcoming *in vitro* seed dormancy from yellow passion fruit (*Passiflora edulis* Sims.)

Treatment	Methods for break seed dormancy
T1	Seed with intact coat (control)
T2	Soaking in water at room temperature for 12 h
T3	Soaking in water at room temperature for 24 h
T4	Soaking in water at room temperature for 48 h
T5	Soaking in hot water at 80°C for 1 min
T6	Soaking in hot water at 80°C for 2.5 min
T7	Soaking in hot water at 80°C for 5 min
T8	Soaking in sulphuric acid for 1 min
T9	Soaking in sulphuric acid for 5 min
T10	Soaking in sulphuric acid for 10min
T11	Soaking in ethilic alcohol for 5 min
T12	Soaking in ethilic alcohol for 10 min
T13	Soaking in ethilic alcohol for 25 min
T14	Seed scarifications with wood sandpaper (no. 125)
T15	Total removal of seed coat with mini-vise

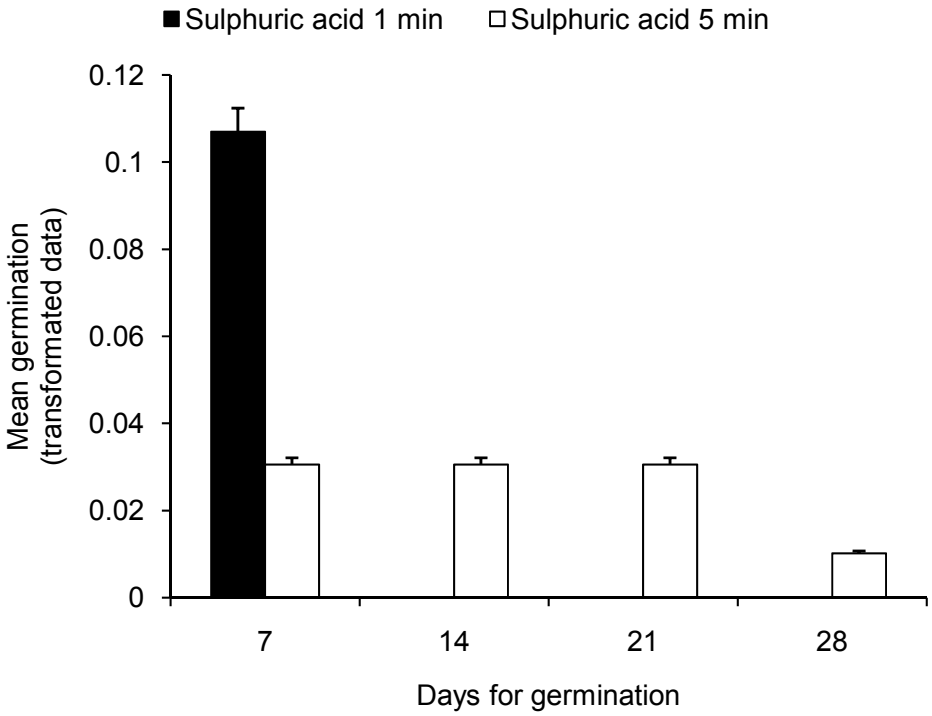

Figure 1. *In vitro* germination dynamics of *Passiflora edulis* Sims., as affected by different time of chemical scarification using sulphuric acid at 98% (for 1 and 5 min).

sucrose, 0.8% agar (Sigma-Aldrich, St. Louis, MO) and 0.01% inositol or to a dish containing 2.65 cm^3 Plantmax® substrate. The pH of the medium was adjusted to 5.8 before been autoclaved (121°C, 1.1 atm, 20 min). The tubes were sealed with one layer of PVC film (Goodyear, Brazil). The cultures were incubated at 27 °C, in the dark to favor hypocotyl etiolation. After 20 days of incubation, the tubes containing germinated seeds (hypocotyl approximately 12 cm high) were transferred to a growth chamber with a day and night cycle of 16 and 8 h for 20 days, respectively. Eight to ten explants were collected from each *in vitro* grown plantlet.

Statistical analysis

The experiment was arranged in entirely randomized design in factorial scheme (15 × 2). Fifteen treatments were combined with

Table 2. Variance analysis of the eight treatments to overcome *in vitro* seed germination and following seed inoculation in two substrates: PlantMax® and Murashige and Skoog medium (1962).

Source of Variation	df	Square mean	F		
Scarification (Scar)	7	0.00487	18.76937**	-	-
Substrates (Sub)	1	0.00658	25.32513**	-	-
Scar x Sub	7	0.00152	5.83453**	-	-
Scar /Sub	14	0.0032	12.30195	P = 0.0	-
Scar /PlantMax®	1	0.02045	0.00292	11.24919	P = 0.0
Scar /MS medium	2	0.02424	0.00346	13.33187	P = 0.0
Sub/ Scar	8	0.00215	8.27086	P= 0.00002	-
Sub/ SA. 1 min	1	0.00645	0.00645	24.8528	P = 0.0
Sub/ SA. 5 min	2	0.00645	0.00645	24.8528	P = 0.0
Sub/ SA. 10 min	3	0.00118	0.00118	4.55972	P = 0.03442
Sub/ EA. 5 min	4	0.00013	0.00013	0.51656	P = 100.0
Sub/ EA. 10 min	5	0.00053	0.00053	2.03445	P = 0.15593
Sub/ EA. 25 min	6	0.00118	0.00118	4.55972	P = 0.03442
Sub/ SP	7	0.0012	0.0012	4.6311	P = 0.03306
Sub/ Mini-vise	8	0	0	0	P = 100.0
Error	144	0.00026			
Total	159				
Average	0.088				
CV(%)	18.66				

SA, Sulphuric acid; EA, ethilic alcohol; SP, sandpaper.

Table 3. Comparison of means between different treatments to overcome *in vitro* seed germination from yellow passion fruit, inoculated in PlantMax® substrate and Murashige and Skoog (1962) medium, respectively.

Treatment	Mean and percentage (%) of seed germination in two substrates	
	PlantMax®	MS medium
Soaking in sulphuric acid for 1 min	$0.107(70)^{Aab}$	$0.0711(0)^{Bb}$
Soaking in sulphuric acid for 5 min	$0.107(70)^{Aab}$	$0.0711(0)^{Bb}$
Soaking in sulphuric acid for 10min	$0.086(30)^{Abc}$	$0.0711(0)^{Bb}$
Soaking in ethylic alcohol for 5 min	$0.076\ (10)^{Ac}$	$0.0711(0)^{Ab}$
Soaking in ethylic alcohol for 10 min	$0.081\ (20)^{Ac}$	$0.0711(0)^{Ab}$
Soaking in ethylic alcohol for 25min	$0.086(30)^{Abc}$	$0.0711(0)^{Bb}$
Manual seed scarification with wood sandpaper	$0.0762(40)^{Bc}$	$0.0918(10)^{Ab}$
Total removal of seed coat with a mini-vise	$0.1225(100)^{Aab}$	$0.1225(100)^{Aa}$

two types of substrates for *in vitro* seed germination. Each treatment was composed by 10 replicates (one seed per tube). The data were transformed by arcsin $[(x + 0.5)/n]^{1/2}$ before variance analysis and compared by F-test at significance level of $P \leq 0.05$. The averages were compared by Tukey's test at the same significance level. The analyses were made using the software Genes (Cruz, 2006).

RESULTS

The experimental results revealed that soaking *P. edulis* seeds in water at room temperature, or hot water at 80°C for different periods of time prevented germination, similar to results observed for the control treatment (untreated seed) (Figure 3c). From the 15 scarification methods utilized in this study (Table 1) attempting to overcome seed dormancy, only ten were able to induce seed germination, followed by inoculation in both sterile PlantMax® substrate and MS medium without the presence of any plant growth regulator (Tables 2 and 3). The raw data from the 10 treatments were transformed by arcsin and then submitted to variance analysis (Table 2). There were significant differences among the tested scarification methods, substrates, and by the interaction between the scarification methods × substrates. When we unfolded the scarification methods into the substrates, significant differences were also observed, indicating different responses among scarification methods used in

Figure 2. Physical scarification of the seed from yellow passion fruit (*P. edulis* Sims.) (a). Garvin® Mini-vise table with integrated clamp (jaw width = 1 inch). (b) and (c). Detail of intact and decoated seeds using mini-vise.

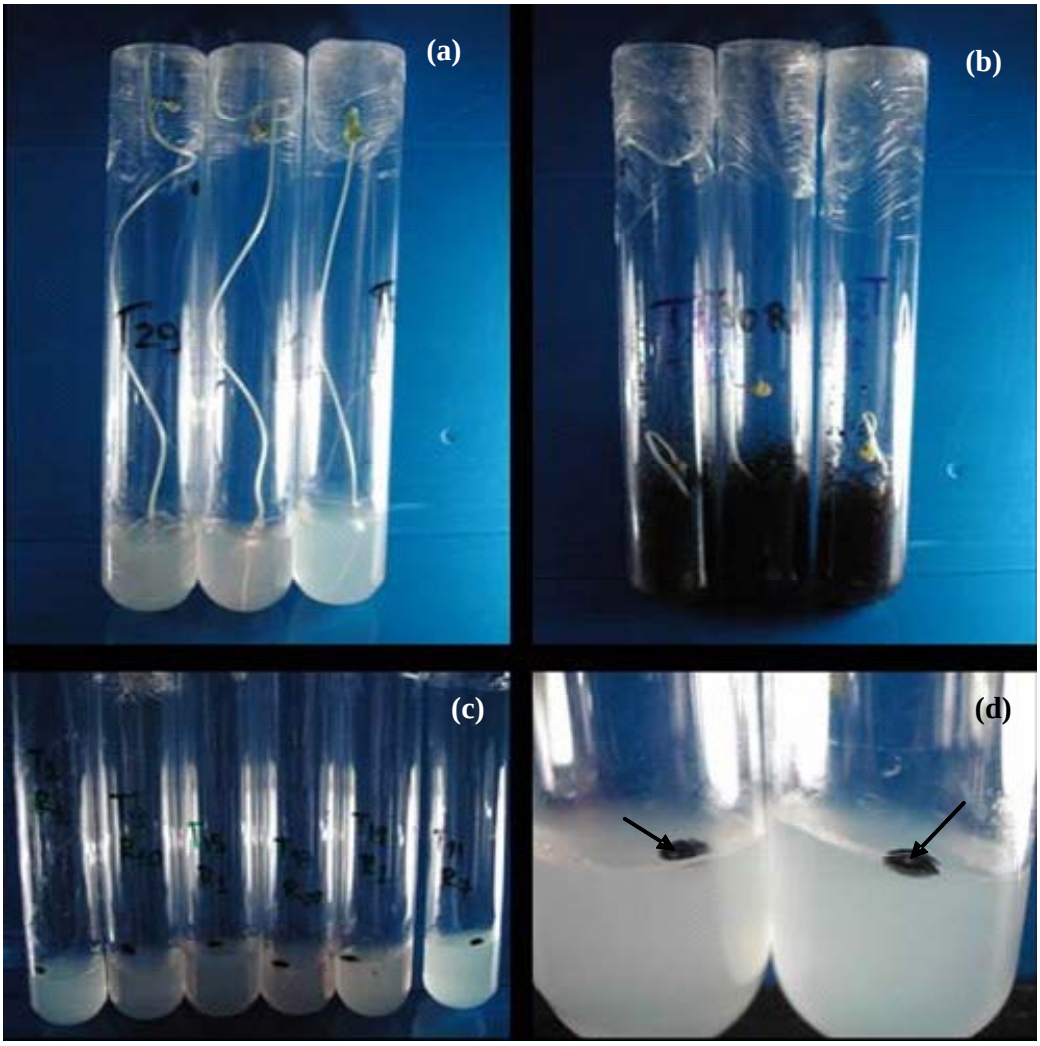

Figure 3. Overcoming *in vitro* seed dormancy of yellow passion fruit (*P. edulis* Sims.). (a) Etiolated plantlets derived from seeds germinated in MS medium 21 days after total removal of the seed coat. (b) Plantlets derived from seeds germinated in PlantMax® substrate 21 days, displaying epinastic phenotype. (c) Non-germinated seeds; from left to right: Control (untreated seeds), soaked in water at room temperature (for 12, 24 and 48 h), and in hot water at 80°C (for 1, 2.5 and 5 min). (d) Detail of non-germinated seeds after scarification with wood sandpaper (number 125).

each substrate (Table 2). However, when unfolding substrates into scarification methods, there were significant differences only for seeds that were chemically scarified with sulphuric acid 98% (1, 5 and 10 min), absolute ethanol (25 min) or scarified with wood sandpaper (Figure 3d). On the other hand, there were no significant differences if the seeds were scarified by soaking in ethanol (for 5 and 10 min) or by total removal of the seed coat.

The comparisons among the treatments (Table 3) show that there were significant differences between scarification method for each substrate (PlantMax® and MS medium), and the best result to overcoming the *in vitro* seed dormancy was the total removal of the seed coat with mini-vise, resulting in 100% germination under sterile conditions (Figure 3a and b). The second better treatment was chemical scarification with sulphuric acid 98% (for 1 or 5 min). Seeds soaked in sulphuric acid (98%) for 1 min followed by the transfer to PlantMax sterile substrate induced faster germination compared to 5 min treatment. After seven days after seed inoculation, the seeds treated with sulphuric acid for 1 min reached 70% germination, whereas in the seeds soaked in sulphuric acid for 5 min had germination rate ranging from 7 to 28 days after inoculation (Figure 1). The lowest germination rates were achieved when seeds were immersed into sulphuric acid 98% for 10 min (Table 3). Prolonged submersion in sulphuric acid was rather harmful for the germination and further emergence; hence this procedure should be avoided in seeds of *P. edulis*.

Low germination rates were also observed when chemical scarification (sulphuric acid or absolute ethanol) was followed by inoculation in MS medium. The chemical scarification produced better results when scarified seeds were inoculated into PlantMax® substrate. All seeds chemically or physically scarified developed a pronounced hook located just below the shoot apex when inoculated on *in vitro* PlantMax® substrate (Figure 4e to g). This response resembled to an epinastic phenotype as observed in “triple response to ethylene”.

DISCUSSION

Under *in vitro* growing conditions, seeds of yellow passion fruit presented a high degree of dormancy as found in the control treatment which did not germinate in both PlantMax® substrate and MS medium. Similar results were described by Morley-Bunker (1980) working with *Passiflora* species (but mainly *P. edulis*) from South Africa, Ghana, Hawaii, Malawi, and Kenya. The author verified that seeds germinated better when mechanically fractured and kept under alternated temperature regimes of 12 /12 h at 20/30°C, rather than constant at 30°C. Scarifying the seed coats with sandpaper or by fermenting seeds with cytase for 24 h did not promote germination. Likewise, the incorporation of gibberellic acid to the germination medium with agar also did not improve germination. Germination of fractured seeds sown in sand bed was even slower than that in agar. Previous works dealing with the difficulties of *in vitro* seed germination of yellow passion fruit were reported elsewhere by Manders et al. (1994) and Hall et al. (2000).

Furthermore, lack of germination was also observed when seeds were soaked into water at room temperature for 12, 24 or 48 h, followed by inoculation in both PlantMax® and MS-based medium. Morley-Bunker (1980) reported that when passion fruit seeds were soaked in water at room temperature for 24, 48, and 72 h, no germination was recorded. Likewise, no improvement on germination occurred when seeds of *Passiflora mollissima* (Kunth) L.H. Bailey, *Passiflora tricuspis* Mast. and *Passiflora nov* sp. was soaked for 24 h in water at room temperature (Delanoy et al., 2006). Conversely, Ellis et al. (1985b) claimed that the treatment for 48 h improved the germination percentage only for *P. mollissima*, when compared to the control.

The use of hot water (80°C) for different times (1, 2.5 and 5.0 min) did not stimulate seed germination. Conflicting results were reported by Oliveira et al. (2010) working with seed dormancy in *P. cincinnata* Mast. The best results to overcome seed dormancy were achieved by drying seeds under shade conditions, followed by treatment with hot water at 50°C or alternatively, seeds should be dried under shade followed by scarification. Soaking the seeds in hot water may lead to the loosening of the seed coat wall, allowing water to permeate into the tissues, leading to physiological changes promoting embryo growth (Agboola and Adedire, 1998; Sabongari, 2001; Aliero, 2004). Treatment with hot water was also efficient to reduce seed dormancy in some species of the genus *Cassia* (Skerman, 1977; Rodrigues et al., 1990). Moreover, when the seeds were treated with sulphuric acid (98%) followed by inoculation of the seeds in MS medium, a poor germination rate took place. However, when the seeds were scarified and transferred to PlantMax® substrate, variable levels of germination occurred (Table 3). Excellent results using sulfuric acid were also obtained for some Leguminosae species (Olmez et al., 2009, Muhammad and Amusa, 2003, Aliero, 2004), but no previous records were found for the genus *Passiflora*.

Chemical scarification by soaking in absolute ethanol for different times (5, 10 and 25 min) also led to variable levels of seed germination (Table 3). No significant differences were observed between the exposure for 5 or 10 min in absolute ethanol, followed by the inoculation in both MS medium or PlantMax® (Table 2 and 3), being the lowest germination percentages observed among the treatments. By increasing the exposure time in alcohol to 25 min remarkably improved germination rate was obtained after transferring to PlantMax® substrate sterile, but still a poor germination response when seeds were

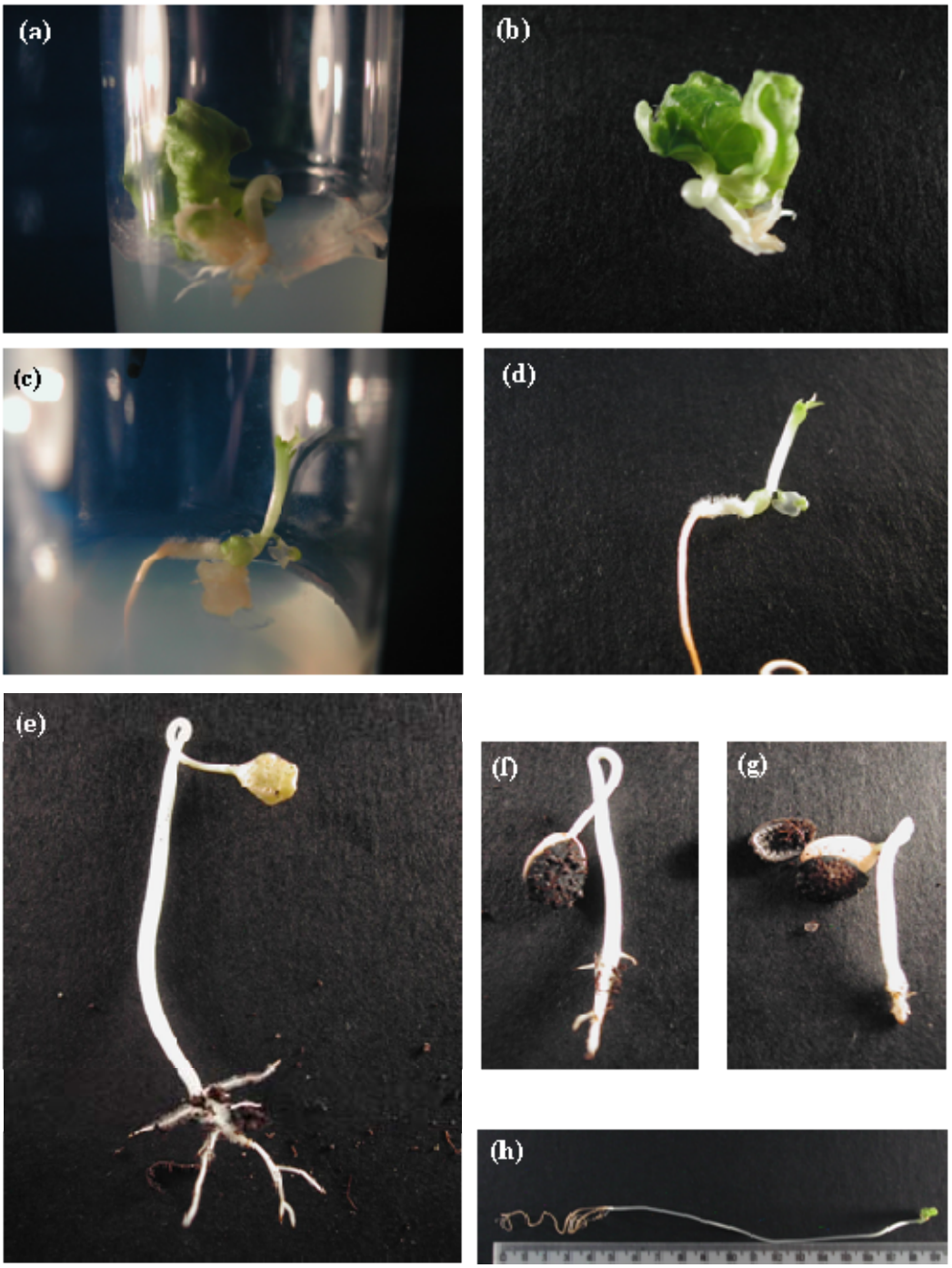

Figure 4. Different physical damages in seedlings from yellow passion fruit (*P. edulis* Sims.) caused by mini-vise. (a) to (c) Detail of triple response (epinastic phenotype) after total removal seed coat with mini-vise. (f) and (g), Seedlings germinated after chemical scarification with sulphuric acid (98%). Note that seed coat yet is present. (h) Seedlings germinated in MS medium, after total removal seed coat, measuring approximately 19 cm 21 days old.

transferred onto MS medium (Table 3). Kern et al. (2010) compared the effects of methanol, ethanol, propanol and acetaldehyde on germination and growth of *E. heterophylla*. Ethanol at concentrations ranging from 0.25 to 1.5% showed a dose-dependent inhibition of germination and growth of *E. heterophylla*. Measurements of respiration and alcohol dehydrogenase activities during seed water uptake and initial seedling growth revealed that ethanol induced an extended effect under hypoxic conditions in the growing tissues. These effects were

caused by alcohol dehydrogenase activity, which is required for the conversion of ethanol to acetaldehyde, though plays a role in the ethanol-induced injuries.

Scarified seeds from yellow passion fruit of both chemical and physical methods, followed by transfer to PlantMax® displayed epinastic phenotype (Figure 3e to g), possibly due to *in vitro* ethylene biosynthesis. Similar results were described for the first time by Stewart and Freebairn (1969), linking this phenotype to ethylene biosynthesis during germination of lettuce and tomato seeds. According to Guzman and Ecker (1990), ethylene concentrations above 0.1 $\mu L.L^{-1}$ induce changes in the growth pattern of seedlings by reducing the rate of elongation and increasing lateral expansion, which leads to swelling of the region below the hook. These effects of ethylene are common shoot culture of several dicots species, also the case of passion fruit, whose climacteric rise of respiration would be expressed at vegetative tissue level as already reported (Reis et al., 2003). Yellow passion fruit is indeed very sensitive to ethylene, which affects the shoot apex development, rhizogenesis, axillary shoot development from nodal segments, and adventitious shoot regeneration from hypocotyl explants (Faria and Segura, 1997; Naik and Chand, 2003; Reis et al., 2003; Trevisan and Mendes, 2005; Mensuali-Sodi et al., 2007).

Etiolated dicot seedlings are usually characterized by a pronounced hook located just behind the shoot apex. This shape facilitates the penetration of the seedlings through the soil, protecting the tender apical meristem (Taiz and Zeiger, 2010). Like epinasty, hook formation and maintenance result from ethylene-induced asymmetric growth. The closed shape of the hook is consequence of the faster elongation of the outer side of the stem compared with inner side. However, when the hook is exposed to light, it opens because the elongation rate of the inner side increases, equalizing the growth rates on both sides (Guzman and Ecker, 1990). Possibly the pronounced hook present in yellow passion fruit *in vitro* cultivated is associated with the fact that passion fruit produces ethylene at high rates (Ludford, 1995), and the possible accumulation of ethylene within tissue culture flasks need to be taken into account. Perhaps the use of ventilated culture vessels is an alternative to modify vessel atmosphere and improve gas exchange, improving the seed germination *in vitro* (Trevisan and Mendes, 2005). In this sense, further is needed to evaluate the utilization of the use of vented lids for seed germination of passion fruit, which can be applied to enhance elimi-nation of ethylene in PlantMax® substrate during seed germination.

The physical scarification methods, like wood sandpaper and total removal seed coat with mini-vise showed different results. The former produced poor germination of the seeds from yellow passion fruit, in both MS medium and PlantMax® substrate. Morley-Bunker (1980) also stated that this method is not efficient to overcome seed dormancy imposed by seed coat in Passifloraceae. Contrarily, Kuhne (1968), Ellis et al. (1985b), and Morton (1987) working with *P. edulis* Sims., reported that scarification with sandpaper improved germination. It is important to observe that seed germination was carried out under *ex vitro* conditions. On the other hand, the total removal seed coat with a mini-vise (Tables 2 and 3) was the best scarification method to overcome seed dormancy in both substrates (PlantMax® and MS medium), where 100% of the seed germinated (Figure 2a and b). This indicates that seed dormancy in yellow passion fruit is imposed by seed coat as described by Morley-Bunker (1980) and Hall et al. (2000). This scarification method (mini-vise) is easy to execute, cheap and also is not dangerous to the handler. Furthermore, it is possible to produce seedlings measuring approximately 18 cm length, at 30 days after inoculation *in vitro* MS medium, producing around at 15 explants (1 cm length) (Figure 3a and h).

Whole decoated seeds germinated, including those that had damaged embryos (Figure 3a to d). The seed coat from yellow passion fruit is very hard and possibly the main consequence is the interference with water uptake, mechanical restraint to radicle protrusion, interference with gas exchange and supply of inhibitors to the embryo (Morley-Bunker, 1980). In conclusion, the results confirm that *P. edulis* Sims seeds showed physical dormancy, which can be easily overcome by total removal of the seed coat with a mini-vise.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Agboola DA, Adedire MO (1998). Response of treated dormant seeds of three species to germination promoters. Nig. J. Bot. 11:103-109.

Alexandre RS, Júnior AW, Negreiros JRS, Parizzotto A, Bruckner CH (2004). Germinacão de sementes de genótipos de maracujazeiro. Pesquisa Agropecuária Brasileira, 39: 1239-1245.

Alexandre RS, Lopes JC, Bruckner CH, Dias DCFS (2009a). Propagation of passion fruit by seeds. In: Alexandre RS, Bruckner CH, Lopes JC (eds.) In: Alexandre RS, Bruckner CH, Lopes JC (eds). Propagation of Passionfruit: Morphological, Physiological and Genetic Aspects. EDUFES, (In Portuguese). pp. 25-72

Alexandre RS, Otoni WC, Dias JMM, Bruckner CH, Lopes JC (2009b). Vegetative propagation of passionfruit: rooting cutting and grafting. In: Alexandre RS, Bruckner CH, Lopes JC (eds.) In: Alexandre RS, Bruckner CH, Lopes JC (eds). Propagation of Passionfruit: Morphological, Physiological and Genetic Aspects. EDUFES, (In Portuguese). pp. 73-116.

Aliero BL (2004). Effects of sulphuric acid, scarification and wet heat treatments on germination of seeds of African locust bean tree, *Parkia biglobosa.* Afr. J. Biotechnol. 3: 179-181.

Appezzato-da-Gloria B, Vieira MLC, Dornelas MC (1999). Anatomical studies of *in vitro* organogenesis induced in leaf-derived explants of passionfruit. Pesquisa Agropecuária Brasileira, 34: 2007-2013.

Biasi LA, Falco MC, Rodriguez APM, Mendes BMJ (2000). Organogenesis from internodal segments of yellow passion fruit. Scientia Agricola 57: 661-665.

Cruz CD (2006). Programa Genes. Aplicativo Computacional em Genética e Estatística, 1st edn. Editora UFV, ViçosaDelanoy M, Van Damme P, Scheldeman X, Beltran J (2006). Germination of *Passiflora mollissima* (Kunth) L.H.Bailey, *Passiflora tricuspis* Mast. and *Passiflora nov* sp. seeds. Scientia Horticulturae 110:198-203.

Dornelas MC, Vieira MLC (1994). Tissue culture studies on species of *Passiflora*. Plant Cell, Tiss. Organ Cult. 36:211-217.

Drew RA (1991). *In vitro* culture of adult and juvenile bud explants of *Passiflora* species. Plant Cell, Tiss. Organ Cult. 26: 23-27.

Ellis RH, Hong TD, Robrechts EH (1985a). Handbook for seed technology for Genebanks, Principles and Methodology. IBPGR, Rome, Italy. Vol. 1.

Ellis RH, Hong TD, Robrechts EH (1985b). Handbook for Seed Technology for Genebanks 2: Compendium of Specific Germination. Information and Test Recommendations. IBPGR, Rome, Italy.

Faria JLC, Segura J (1997). *In vitro* control of adventitious bud differentiation by inorganic medium components and silver thiosulfate in explants of *Passiflora edulis* f. *flavicarpa. In Vitro* Cell. Dev. Biol. Plant, 33: 212-219

Guzman P, Ecker JR (1990). Exploting the triple response of *Arabidopsis* to identify ethylene-related mutants. Plant Cell 2:513-523.

Hall RM, Drew RA, Higgins CM, Dietzgen RG (2000). Efficient organogenesis of an Australian passion fruit hybrid (*Passiflora edulis* x *Passiflora edulis* var. *flavicarpa*) suitable for gene delivery. Aust. J. Bot. 48:673-680.

IBGE (2009). Levantamento sistemático da produção agrícola. Rio de Janeiro.

Kantharajah AS, Dodds WA (1990). *In vitro* micropropagation of *Passiflora edulis* (purple passionfruit). Ann. Bot. 65:337-339.

Kern KA, Pergo EM, Kagami FL, Arraes LS, Sert MA, Ishii-Iwamoto EL (2009). The phytotoxic effect of exogenous ethanol on *Euphorbia heterophylla* L. Plant Physiol. Biochem. 47:1095-1101.

Kuhne FA (1968). Cultivation of granadillas. Farming South Africa, 43:29-32.

LaRosa AM (1984). The biology and ecology of *Passiflora mollissima* in Hawaii. Cooperative National Park Resources Studies Unit. Technical Report 50.

Lombardi SP, Passos IRS, Nogueira MCS, Appezzato-da-Glória B (2007). *In vitro* shoot regeneration from roots and leaf discs of *Passiflora cincinnata* Mast. Brazilian Arch. Biol. Technol. 50:239-247.

Ludford PM (1995). Postharvest hormone changes in vegetables and fruit. In: Davies PJ ed. Plant hormones, physiology, biochemistry and molecular biology. Dordrecht, Netherlands: Kluwer Academic Publishers, pp. 725-750.

Manders G, Otoni WC, d'Ultra Vaz E B, Blackhall NW, Power JB, Davey MR (1994). Transformation of passionfruit (*Passiflora edulis* f. *flavicarpa* Degener.) using *Agrobacterium tumefacines*. Plant Cell Rep. 13:697-702.

Mensuali-Sodi A, Lucchesini M, Maltinti S, Serra G, Tognoni F (2007). Leaf senescence in tissue culture of *Passiflora incarnata* L.: the role of ethylene. In: Ramina A, Chang C, Giovannoni J (eds.). Advances in Plant Ethylene Research. Proceedings of the 7th International on the Plant Ethylene Hormone, pp.151-152.

Morley-Bunker MJS (1980). Seed coat dormancy in *Passiflora* species. Annu. J. 8:72-84.

Morton J (1987). Fruits of Warm Climates. Creative Resource Systems. Winterville, Miami, USA.

Muhammad S, Amusa NA (2003). Effects of sulphuric acid and hot water treatments on seed germination of tamarind (*Tamarindus indica* L). Afr. J. Biotechnol. 2:276-279.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15:473-497.

Oliveira Jr MX, São Jose AR, Rebouças TNH, Morais OM, Dourado FWN (2010). Overcoming seeds dormancy of passion fruit (*Passiflora cincinnata* Mast.). Revista Brasileira de Fruticultura, 32:584-590.

Olmez Z, Gokturk A, Karasah B, Yilmaz H (2009). Effects of cold stratification and sulphuric acid pretreatments on germination of three provenances of smoke-tree (*Cotinus coggygria* Scop.) seeds in greenhouse and laboratory conditions. Afr. J. Biotechnol. 8:4964-4968.

Passos IRS, Matos GVC, Meletti LMM, Scott MDS, Bernacci LC, Vieira MAR (2004). Gibberelic acid utilization for dormancy break in *Passiflora nitida* Kunth seeds *in vitro* germinated. Revista Brasileira de Fruticultura 26:380-381.

Reis LB, Paiva Neto VB, Picoli EAT, Costa MGC, Rego MM, Carvalho CR, Finger FL, Otoni WC (2003). Axillary bud development of passionfruit as affected by ethylene precursor and inhibitors. *In Vitro* Cell. Dev. Biol. Plant 39:618-622.

Rêgo MM, Rêgo ER, Bruckner CH, Finger FL, Otoni WC (2011). *In vitro* induction of autotetraploids from diploid yellow passion fruit mediated by colchicine and oryzalin. Plant Cell Tissue Organ Cult. DOI 10.1007/s11240-011-9995-6

Rodrigues EH, Aguiar IB, Sader R (1990). Quebra de dormência de sementes de três espécies do gênero *Cassia*. Revista Brasileira de Sementes, 12:17-27.

Sabongari S (2001). Effect of Soaking duration on germination and seedling establishment of selected varieties of Tomato (*Lycopersicum esculentum* Mill). M.Sc. Thesis, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. Research17: 122-127.

Skerman PJ (1977). Tropical forage legumes. 2 edth FAO. Plant Production and Protection. Rome.

Stewart ER, Freebairn HT (1969). Ethylene, seed germination, and epinasty. Plant Physiol. 44:955-958.

Taiz L, Zeiger E (2010). Plant Physiology. 5th ed. Sinauer Associates, Inc., Sunderland, MA, p. 690.

Trevisan F, Mendes BMJ (2005). Optimization of *in vitro* organogenesis in passion fruit (*Passiflora edulis* f. *flavicarpa*). Scientia Agricola 62:346-350.

Vieira MLC, Carneiro MS (2005). *Passiflora* spp. Passionfruit. In: Biotechnology of Fruit and Nut Crops Edit by Richard E. Litz Cromwell Press,, London, pp. 436-453.

Wehtje G, Reed RB, Dute RR (1985). Reproductive biology and herbicidal sensitivity of maypop passionflower (*Passiflora incarnata*). Weed Sci. 33: 484-490.