JOURNAL OF AGRICULTURE & SOCIAL SCIENCES
ISSN Print: 1813–2235; ISSN Online: 1814–960X
09–036/MFA/2010/6–3–57–60
http://www.fspublishers.org

Full Length Article

Effects of Different Pre-germination Treatment Methods on the Germination of Passion (*Passiflora edulis*) Seeds

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ABSTRACT

Freshly extracted passion fruit seed exhibit slow and low germination percentage. Pre-germination treatments may enhance and improve percent germination in passion fruit seeds. This experiment was conducted to determine the appropriate pre-germination treatment in improving the germination potential of passion fruit seeds. The pre-germination treatments included chemical scarification (with 98% H_2SO_4), fermentation (in 10% sucrose solution) and soaking seeds in water for 7 or 14 days. Chemical scarification of passion fruit seeds for 5 min significantly significantly ($P<0.05$) germination percentage (94%), seedling height (3.0 cm) and number of leaves (4.3) compared with un-treated seeds, which had lower germination percentage (20%), seedling height (1.6 cm) and number of leaves (3.0). Fermentation significantly ($P < 0.05$) increased number of roots per-seedling (11.0), length of roots (2.7 cm), fresh mass (2.4 g) and dry mass (0.73 g) of seedlings six weeks after germination. Un-treated seeds had lower number of roots per-seedling (5.8), length of roots (1.1 cm), fresh mass (1.1 g) and dry mass (0.3 g) of seedlings six weeks after germination. Soaking seeds in water for 7 days gave a higher germination percentage (71%) than soaking seeds for 14 days (40%). Results obtained in this experiment indicate that the pre-germination treatment of passion fruit seeds by using (H_2SO_4) enhanced germination of the seeds by breaking dormancy. Thus for the production of passion fruit, sulfuric acid can be used to promote germination of passion seeds.

Key Words: Dormancy; Germination; Passion fruit seeds; Pre-germination treatment

INTRODUCTION

The purple passion fruit (*Passiflora edulis* var. edulis) thrives in sub-tropical and tropical highlands (Samson, 1986). The granadilla or purple passion fruit has potential importance as a source of vitamin A in copious amounts and fair amounts of B_1 and B_2 in the human diet and as a possible export crop in the form of juice and syrup for flavouring (Rice *et al*., 1987). The purple passion fruit is preferred for fresh consumption, juice processing and the making of preserves (Anonymous, 2006). The seed contains about 12% protein and 50 to 55% fibre, but however it has been found un-suitable for cattle feed (Anonymous, 2006). There is currently a revival of interest in the pharmaceutical industry, especially in Europe, in the use of the glycoside, *passiflorine*, especially from *P. incarnata* L., as a sedative or tranquilizer. Italian chemists have extracted *passiflorine* from the air-dried leaves of *P. edulis* (Anonymous, 2006).

Passion fruit vines are usually grown from seeds. It is assumed that fruits should be stored for a week or two to allow them to shrivel and become perfectly ripe before seeds are extracted. If planted soon after removal from the fruit, seeds will germinate in 4 to 5 weeks. Cleaned and stored seeds have a lower and slower rate of germination. Thus there is need to sow seeds as soon as possible after harvesting (Thomson, 1979). Passion fruit seeds have a fleshy aril and may show considerable dormancy and treatments to the seed covering structures have been reported to promote germination (Obroucheva, 1999). Seed scarification involves breaking, scratching or softening the seed coat so that water can enter and germination can begin (Hartmann *et al*., 2002).

In many plant species, seed development and germination are separated by a period of low metabolic activity referred to as dormancy or quiescence. Seed dormancy can be defined as the failure of seeds to germinate owing to factors associated with their seed coat or embryo. It is due to the seed coat being impermeable to water or oxygen or both, hard seed coat, immature embryo, embryos that require an 'after-ripening period` or as a result of endogenous chemical germination inhibitors (Wareing & Phillips, 1981).

Several studies have been carried out on pre-germination treatments of seeds (Thakur & Sharma, 2005; Farooq *et al*., 2005; Basra *et al*., 2007). Pre-sowing salicylicate treatments improved the germination rate and uniformity and early seedling growth in both cucumber and melon (Basra *et al*., 2007). However, use of commercial fertilizers as osmotica for rice priming resulted in lowering seed germination and seedling growth (Farooq *et al*., 2005).

Naturally, dormancy is broken by changes in the environmental conditions around the seed. These changes

To cite this paper: Mabundza, R.M., P.K. Wahome and M.T. Masarirambi, 2010. Effects of different pre-germination treatment methods on the germination of passion (*Passiflora edulis*) seeds. *J. Agric. Soc. Sci.*, 6: 57–60

are normally indicative of a change in the growing season. The changes include increased temperatures, changes in the wetting and drying cycles of the soil, which is normally associated with the beginning of the summer or rainy season. However, there are also artificial ways of breaking seed dormancy. Hot water is used for small to medium-sized seeds or large quantities of seeds. This treatment requires dipping seeds into four to five times their volume of hot water (77 to 100°C). Mechanical scarification is a technique for overcoming the effect of an impermeable seed coat. Mechanical scarification can be done by rubbing seeds between two pieces of sandpaper or using a file, a pin, or a knife to rupture the seed coat, cracking with hammer or a vice (Hartmann *et al*., 2002). Seed may be mixed with coarse sand and shaken vigorously in a jar. Care must be taken not to injure the embryo. Acid treatments are often used to break down especially thick impermeable seed coats. In this treatment, dry seeds are placed in containers and covered with concentrated H_2SO_4 in a ratio of about one part seed to two parts acid (Heydecker, 1973; Hartmann *et al*., 2002).

MATERIALS AND METHODS

Experimental site: The experiment was conducted in Greenhouse of Crop Production Department, the University of Swaziland, Luyengo. The experimental site is located at 26°34’S, 31°12’E co-ordinates and is 750 m sl, has a mean annual rainfall of 800 mm and mean temperature of 18°C.

Pre-germination treatments: Passion fruit seeds were extracted from fruits and subjected to (H_2SO_4) treatment. The seeds were placed in 500 mL glass beaker and then 200 mL of the acid was poured onto the seeds. After 5 min, the sulfuric acid, was poured off and the seeds were rinsed with tap water. Other passion fruit seeds were subjected to soaking in tap water for 7 or 14 days in order to promote germination. The two sets of seeds were soaked in 400 mL water in 500 mL-beakers. The seeds that floated were discarded. The water was changed after every two days to prevent potential microbial growth. The feasibility of enhancing germination by fermentation of seeds in sucrose solution was investigated. Passion fruit seeds were extracted and placed in 10% sucrose solution and kept for 14 days. Floating seeds were discarded after 48 h. For the control, **s**eeds were extracted and sown immediately without any pre-germination treatment.

After the pre-germination treatments, seeds were sown in seed trays (200 seed holes). The pre-germination treatments were scheduled to be completed at the same time to allow for the seeds to be sowed at the same time. Fifty seed holes represented a plot. For each treatment, fifty seeds were sown. There were five treatments, which were randomly allocated. Compost was used as the germination medium. The experiments were carried out between November 2006 and February 2007.

Experimental design: The experiment was laid out in randomized complete block design (RCBD) with four replications. Data collected included the number of days to germination, number of germinated seeds, height of seedlings, number of leaves, number of roots per seedling, length of root systems, dry and fresh mass of roots. Five plants were randomly selected per treatment and used for data collection. Data were collected three weeks after sowing, three weeks after germination and six weeks after germination. The data were subjected to the Analysis of Variance (ANOVA) using MSTAT C statistical programme. Where significant differences were detected the means were seperated using Duncan’s New Multiple Range Test (DNMRT) at $P < 0.05$.

RESULTS

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Effect of pre-germination treatments: Pre-germination treatments of passion fruit seeds by chemical scarification gave the highest germination percentage of 94%, followed by fermentation of seeds in 10% sucrose solution with 86%, while the minimum germination (20%) observed from un-treated seeds (Table I).

Soaking passion fruit seeds for 7 days in tap water had significantly ($P < 0.05$) higher germination percentage of 77% compared to soaking in tap water for 14 days and control, which gave germination percentage of 41% and 20%, respectively (Table I).

Growth characteristics: Fermenting seeds in 10% sucrose solution gave the highest height (3.4 cm) of passion fruit seedlings three weeks after germination, followed by sulfuric acid pre-germination treatment, which gave seedling height of 3.0 cm, while the control gave the lowest seedling height of 1.6 cm (Table I).

There were no siginificant differences between sulfuric acid pre-germination treatment and fermentation in 10% sucrose solution treatments in respect to leaf numbers of passion fruit seedlings (Table I). Similarly, 7 days soaking in water, 14 days soaking in water and control treatments exhibited no significant difference between themselves in terms of number of leaves of the seedlings (Table I). The highest number of leaves per-seedling was observed in both seeds treated with sulfuric acid (4.3) and fermentation in 10% sucrose solution (4.0).

Sulfuric acid gave the highest number of roots per seedling (11) followed by sucrose (10%) pre-germination treatment (Table II). The lowest number of roots per seedling was observed in seeds soaked in water for 14 days and control, both of which had six roots per-seedling.

Fermentation of seeds in 10% sucrose solution gave the highest length of roots (2.7 cm) of passion fruit seedlings measured six weeks after germination followed by sulfuric acid scarification, which gave the length of roots of 2.1 cm, while the control gave the lowest length of roots of 1.1 cm (Table II).

Fermentation of passion fruit seeds in 10% sucrose gave the highest fresh mass of roots of 2.4 g, measured six weeks after germination, followed by sulfuric acid

Table I: Effects of pre-germination treatments of passion fruit seeds on germination percentage (%), seedling height and number of leaves, three weeks after sowing

Pre-germination seed treatment	Germination percentage (%)	Seedling height (cm)	Number of leaves per seedling
Sulfuric acid	94.0a	3.0b	4.3a
10% sucrose	86.5b	3.4a	4.0a
7 days soaking in water	71.5c	2.1d	3.3b
14 days soaking in water	41.5d	2.5c	3.0b
Control	20.0e	1.6e	3.0b

Means with the same letter along the same column are not significantly different from each other at $P < 0.05$. Mean separation by DNMRT

Table II: Effects of pre-germination treatments of passion fruit seeds on number of roots, length of root system, root fresh mass and root dry mass, six weeks after sowing

Pre-germination seed treatment	Number of roots per seedling	Length of root system (cm)	Root fresh mass (g)	Root dry mass (g)
Sulfuric acid (H_2SO_4)	11.0a	2.1b	2.2a	0.6b
10% sucrose	8.5b	2.7a	2.4a	0.7a
7 days soaking in water	7.0c	1.7d	1.6b	0.3d
14 days soaking in water	5.5d	1.9c	1.8b	0.4c
Control	5.8d	1.1e	1.1c	0.3d

Means with the same letter along the same column are not significantly different from each other at $P < 0.05$. Mean separation by DNMRT

scarification, which gave fresh mass of roots of 2.2 g, while the control gave the lowest fresh mass of roots of 1.1 g (Table II).

Fermentation in sucrose (10%) gave the highest dry mass of roots of 0.7 g recorded six weeks after germination followed by H_2SO_4 scarification, which gave dry mass of roots of 0.6 g (Table II). The 7 days water soaking and control, although not significantly ($P > 0.05$) different from each other gave the lowest dry mass of roots of 0.3 g.

Fermenting passion fruit seeds in 10% sucrose for 14 days significantly ($P < 0.05$) increased length of dry and fresh mass of roots six weeks after germination (Table II). Soaking seeds in tap water for 14 days gave a significantly ($P < 0.05$) higher plant height of seedlings three weeks after planting as compared to soaking seeds for 7 days and control (Table I). However, there was no significant ($P < 0.05$) difference in the number of leaves of seedlings three weeks after planting, when seeds were soaked for 7 days, 14 days and control (Table I). Soaking seeds for 14 days, significantly ($P < 0.05$) increased the length of roots, fresh and dry mass of roots compared to soaking of seeds for 7 days and control (Table II).

DISCUSSION

This study has shown that chemical scarification of passion fruit seeds increased germination percentage. Previous studies have shown that chemical seed treatment improved seed germination and growth under various conditions (Kozlowski, 1972; Anonymous, 2007a; Ehiagbanare & Onyibe, 2007; David & Midcap, 2007). Seed priming allows for some of the metabolic processes necessary for germination to occur without actual germination (Basra *et al*., 2007).

According to Ehiagbanare and Onyibe (2007) seed pre-treatments and pre-treatment duration affected commencement of germination in *Tetracarpidium conophorun.* The seeds were soaked in concentrated sulfuric acid for 5, 10, 15, 20 and 30 min and in concentrated nitric acid for 5, 10, 15, 20 and 30 min. Germination was first observed among the seeds treated with concentrated sulfuric acid for 10 min at 23 days after sowing (DAS), while those treated with the same acid for 20 min and 30 min both commenced germination at 30 DAS. Seeds treated for 5 min with concentrated sulfuric acid had the highest percentage germination of 66% followed by de-pulped, but un-treated seeds, which had 57% (Ehiagbanare & Onyibe, 2007). Kozlowski (1972) attributed early germination of seeds after scarification to cracks, which permit water and gases into the seed resulting in enzymatic hydrolysis and thus transforming the embryo into a seedling.

Scarification of seeds of *Tamarindus indica* L. with 95% H_2SO_4 for 5, 10 or 15 min, methanol, ethanol, iso-propanol or butanol for 10 or 20 min, or boiling water for 10, 15, 20 or 30 min, was used to determine the effect of the various treatments on the development and vigour of the resultant seedlings (Anonymous, 2007a). Seeds immersed in methanol, ethanol and sulfuric acid for 10 min produced seedlings with high vigour (Anonymous, 2007a). The optimum soaking period depends on the species, it is usually in the range 20-60 min, but soaking for 120 min gave very good results for charlock (*Sanapis arvensis*) seeds (David & Midcap, 2007). Scarification of seeds on the other hand for short periods of time (5-20 min) with concentrated sulfuric acid stimulated sub-sequent germination in *S. arvensis* seeds (David & Midcap, 2007).

In this study the use of sucrose solution as a priming agent resulted in increased number and length of seedling roots, fresh mass and dry mass. According to Bewley and Black (1983), germination and early growth requires a supply of readily available substrate (e.g. sucrose) other than that derived from hydrolysis of major stored reserves. Sucrose is utilised before radicle emergence prior to mobilization of the major carbohydrates stored reserves. Therefore, treating passion fruit seeds with sucrose probably provided a supply of readily available carbohydrates for respiration during germination and development of seedling's roots. Sucrose is a major form in which the products of carbohydrates catabolism are translocated into developing seedlings (Esau, 1977). Seed priming increases crop yield possibly by modulating enzymes of sucrose metabolism in chickpea (Anonymous, 2007a). The number of seeds and seed yield per plant were higher in chickpea crops raised from water and mannitol (4%) primed seeds in comparison with the control non-primed seeds. In primed plants, an enhanced acid invertase (sucrose enzyme) activity in the apical part of the main stem and the part immediately below it at 100 and 130 days after sowing might result in an increased availability of hexoses to these plant parts (Anonymous, 2007b).

The root length is an important parameter for studying the process of nutrient uptake by plant roots (Bohm, 1979). In addition, the use of root length as a parameter encourages the possibility that relationships will be found between root length and development stages of the plant. However, when considering the efficiency of a root system for plant growth, root distribution in the soil profile is the more important factor rather than total root mass (Bohm, 1979).

Soaking of seeds for 7 days had a higher germination percentage than soaking seeds for 14 days in this study. This is attributed probably due to harmful effect of complete submersion in water for extended periods. Kramer (1983) attributed the harmful effect of extended pre-soaking to restriction of oxygen supply during some critical metabolic stage of germination. Aeration of water during soaking may reduce harmful effects in cereals and bean seed.

CONCLUSION

Passion fruit seeds exhibit physical dormancy due to the hard seed coat, which affects the seed germination. Results obtained in this experiment indicate that the pre-germination treatment of passion fruit seeds by using H_2SO_4 enhanced germination of the seeds, height and number of leaves of seedlings. Fermentation of passion fruit seeds in sucrose solution also gave the next best results in breaking seed dormancy, promotion of growth and development of seedlings. Soaking seeds in tap water for 7 days or 14 days did not effectively improve germination, growth and development of seedlings against the control.

It is recommended that farmers, who are interested in production of passion fruit in Swaziland, should be advised to use H_2SO_4 acid for the scarification of seeds before sowing. Sulfuric acid was most effective in breaking dormancy in passion fruit seeds in this investigation. However, in cases whereby farmers have got limited resources (e.g., capital), they can ferment the passion fruit seeds in 10% sucrose solution, which gave the next best results.

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(Received 08 July 2009; Accepted 14 September 2009)