CHAPTER 6

PRE-TREATMENT METHODS FOR IMPROVING JACKET PLUM (*PAPPEA CAPENSIS*) SEED GERMINATION

6.1 Abstract

A trial was carried out with the objective of determining effective methods to improve *Pappea capensis* seed germination and culture asepsis. Seed germination tests were carried out using different growth chambers with 12 °C and 24 hours of darkness (D), 17 °C and 24 hours of light (L), 20 °C and D, 25 °C and D, 25 °C and L, or 32 °C and L. Seed germination was assessed using water floatation (sinking or floating seeds). They were primarily scarified in 98% H_2SO_4 and seed decontamination was assessed using 70% ethanol, 0.1% mercuric chloride ($HgCl_2$) or 1.75% NaOCl. The results indicated high seed germination (80%) with 25 °C and D followed by 20 °C and D (60%) treatments, but 12 °C and D, 17 °C and L, 25 °C and L, or 32 °C and L treatments inhibited seed germination (0%). Dark conditions with temperature ranges of 20 ^{o}C to 25 ^{o}C stimulated seed germination, but low temperatures (<20 ^{o}C) adversely affected seed germination. High seed germination (76%) was observed from submerged seeds. NaOCl decontamination method improved *in vitro* seed germination to 76%. $HgCl_2$ improved asepsis (89%), but with many abnormal seedlings.

6.2 Introduction

Jacket plum (*Pappea capensis* L.) tree species produce seeds which are rich in edible oil (74%) and this seed oil is used for making soap (Palmer & Pitman, 1972; Swart, 1991). They are alternative sources of bio-diesel fuel, and hence scientific research trials have been initiated at the University of Pretoria. *P. capensis* tree species are mainly propagated by seeds, but seedling growth is extremely slow (Anonymous, 1997; Venter & Venter, 1996; Van Wyk & Gericke, 2000). Seed morphology shows a relatively tough seed coat (testa) which is hard to crack or break by hand. According to Venter & Venter (1996), *P. capensis* seeds do not require any pre-treatment when sown soon after collection from trees. However, there have been no scientific reports on the percentage germination of fresh or stored seeds. Moreover, there have been no reports on identification of any seed treatment that would lead to an improvement in seedling growth.

P. capensis trees are widely distributed in the tropical ecological areas (Venter & Venter, 1996). According to Venter & Venter (1996), tree fruiting occurs from December to July period, while Fivaz & Robbertse (1993) reported April - May as a fruit development period. Differences in fruiting time or duration can be attributed to the prevailing environmental conditions within a particular ecological area. *P. capensis* tree species can be either deciduous or evergreen depending upon the prevailing environmental conditions (Palmer & Pitman, 1972; Venter & Venter, 1996). Furthermore, it is believed that late rains promote *P. capensis* seed germination. However, we observed no seed germination or seedling growth under the trees or in proximity during the dry season (August – October).

Many planting materials derived from *in vitro* culture of tree species have exhibited rapid growth (George, 1993), but *in vitro* propagation for mass multiplication of planting materials requires aseptic explants. Therefore, there is a need to select a pre-treatment that effectively decontaminates the seeds before germination. Moreover, the seed coats might harbour *in vitro* contaminants.

Many environmental factors such as temperature, light and water have a major influence on seed germination (Abdul-Baki & Anderson, 1972). According to Naidu, Rajendrudu & Swamy (1999), temperature has a strong influence on breaking seed dormancy in some plant species. For example, incubating *Prosopis juliflora* seeds at 60 ^{o}C for 12 – 24 hours improved seed germination and 30 - 100 ^{o}C temperature regimes improved *Sapindus trifoliatus* (soapnut) seed germination (Naidu *et al*., 1999). *S. trifoliatus* plants belong to the family Sapindaceae. Furthermore, it is known that high temperature treatments improve water imbibition and gaseous exchange rates. Generally, manipulation of such factors might provide an optimal condition for seed germination. However, a propagation method to enhance rapid *P. capensis* seedling growth has not been established. Therefore, the objective of the study was to determine effective pre-treatment methods that improve *P. capensis* seed germination and culture asepsis.

6.3 Materials and methods

6.3.1 Site description

P. capensis seeds were collected from Pretoria National Botanical Gardens in August 2005. This site lies at an altitude 1360 m above sea level, latitude 25^{o} 44'S, longitude 28^{o} 16'E

and an annual rainfall of 750 mm (Botha, Willis & Winter, 2000). Matured seeds were collected from the ground under the trees, but these seeds could be lying on the ground for more than a month after dropping from *P. capensis* trees. They were kept in paper bags and taken to the laboratory for different seed germination experiments.

6.3.2 Effects of temperature and light on seed germination

P. capensis seeds were stored at room temperature in paper bags for four weeks. They were soaked in a warm-bath (28 °C for 12 h) in order to weaken the seed coat, and hence improving water permeability. The seeds were then disinfected in 3.5% sodium hypochlorite (NaOCl, 8 min) and rinsed three consecutive times in sterile water. Ten seeds were placed on moist two layer-filter papers (Whatman No. 1) in each Petri dish (9-cm diameter), sealed and placed in transparent plastic bags. They were then exposed to different temperature and light regimes (treatments) in growth chambers maintained at (i) 12 °C with 24 hours of darkness (D), (ii) 17 °C with 24 hours of light (L), (iii) 20 °C with D, (iv) 25 °C with D, (v) 25 °C with L or (vi) 32 °C with L. There were six Petri dishes per treatment and the experimental design was a complete randomized block with six treatments and three replicates.

6.3.3 Germination of floating and submerged seeds

The reason for this experiment was to determine methods to separate viable from non-viable seeds. It was suspected that some seeds could have lost their viability due to a long storage period and perhaps the condition of storage. *P. capensis* seeds were stored in paper bags at room temperature for 12 or 16 weeks after collection. They were then placed in a

basin of water and floating seeds were separated from submerged seeds. Treatments were (i) sank seeds (ii) floating seeds or (iii) a mixture of sank and floating seeds. The seeds were sown in seedling trays filled with a mixture of fine sand and coconut bark growing medium and placed on a mist bed. Water (mists) was supplied for eight seconds every four minutes. The conditions in the mist propagation chamber were maintained at 70- 90% relative humidity and 23 ^{o}C to 26 ^{o}C temperature. There were 200 seeds per treatment and the experiment was laid out in a complete randomised block design with four replicates.

6.3.4 Seed decontamination

The reason for carrying out this experiment was due to high *in vitro* contamination (>50%) observed when seeds were only decontaminated in 98% H_2SO_4 (3 min). This experiment was laid out in a completely randomised design with three replicates. Seed coats were easily removed when the seeds were scarified in 98% H_2SO_4. Seed decontamination treatments were (i) 70% ethanol (40 sec), (ii) 0.1% w/v $HgCl_2$ (8 min) or (iii) 1.75% NaOCl (10 min). These seeds were rinsed in sterile water for four consecutive times and germinated on 10 ml aliquot of hormone-free half strength MS media (see section 6.3.4).

6.3.5 Incubation condition

Refer to section 2.3.8 for incubation conditions.

6.3.7 Statistical analysis

Data on seed germination and culture asepsis were expressed in percentages and then transformed where necessary (Steel & Torrie, 1980). Mean germination time (MGT) was calculated using the formula: MGT = $\sum(\eta \times d)/N$, where η is the number of seeds germinated

each day, d is the number of days from the beginning of the test and N is the total number of seeds germinated at the end of the experiment. The data were subjected to analysis of variance (ANOVA) using GenStat 4.24DE (Rothamsted Experimental Station) and mean separation was done using LSD test.

6.4. Results and discussion

6.4.1 Effects of temperature and light on seed germination

Significant differences ($P \leq 0.05$) in percentage germination of *P. capensis* seeds were obtained (Figure 6.1). An effective seed pre-treatment was 25 °C with dark (D) (80%) followed by 20 °C with D (60%). Seed germination was inhibited (0%) at 12 °C with D, 17 °C with L, 25 °C with L and 32 °C with L. *P. capensis* seeds that failed to germinate were still intact (there were no indications of rotten or damaged seeds) (Figure 6.2A). These results show that low temperatures (≤17 ^{o}C) and light inhibited seed germination. Observations showed that 25 ºC with D treatment resulted in more vigorous seedling growth than 20 ºC with D treatment (Figure 6.2B-C).

No significant differences were obtained in root and shoot lengths between 20 ^{o}C with D and 25 ^{o}C with D treatments. Roots were longer (9.4 cm) than shoots (2.6 cm). 20 ^{o}C with D treatment resulted in seedlings with thin roots and shoots (Figure 6.2). These results demonstrate that 25 ºC with D was an optimal condition for *P. capensis* seed germination.

The calculated mean germination time (MGT) shows that early germination (16.3 days) was at 20 ºC with D treatment and late seed germination was at 25 ºC with D (19.3 days) treatment. Apart from this rapid seed germination obtained at 20 ºC with D treatment, the final seed germination at 25 ºC with D treatment was high (80%).

Generally, seed germination responses to temperature and light vary with species. Pandey & Palni (2005) reported that dark conditions inhibited *Parthenium hysterophorus* seed germination (8%) while 12 h of light and dark conditions improved germination (56%). In this trial, dark conditions promoted *P. capensis* seed germination. This could be genetically controlled or an ecological adaptation to avoid exposure to dehydration above ground which may limit natural multiplication of *P. capensis* trees.

6.4.2 Germination of floating and submerged seeds

Significant differences ($P \leq 0.05$) in percentage seed germination were obtained amongst floatation treatments (Figure 6.3). Submerged seeds sown 12 weeks (November) after collection resulted in 65% germination. Light or floating seeds resulted in 9% germination while the mixture resulted in 37% germination. There was 20% germination for submerged seeds sown 16 weeks (December) after collection, but none of the floating seeds germinated (Figure 6.3). Low germination (3%) was obtained from a mixture of floating and submerged seeds. The findings from 16 weeks stored seeds could be attributed to deteriorating embryos due to storage or that stored seeds entered some form of dormancy. Seed germination occurred once this speculated dormancy was broken, but this needs further research to confirm the speculated embryo deterioration or dormancy phenomenon.

Piña-Rodrigues & Figliolia (2005) reported 69% germination for *Virola surinamensis* seeds that sank and 85% germination for those that floated. This was attributed to ecological adaptation in that these trees were found in flood prone areas. Therefore, light-weight seeds enabled these species to survive the floods while heavy seeds were buried deep and hence failed to emerge. Consequently, this reduced germination chances of *V. surinamensis* seeds. In the case of *P. capensis*, the opposite is true due to the fact that these tree species are found on a high ground without floods.

Limitation in soil moisture as the dry season progresses does not seem to support *P. capensis* seedling growth. Many seeds falling onto the ground might be dehydrated and embryos of such seeds might be deteriorated if collected late in the dry season. Therefore, water floatation method is important to separate deteriorated *P. capensis* seeds (floating) from viable seeds (submerged). This method is cheap, easy and can be used to improve germination of stored seeds and can save labour and ensure seed germination uniformity.

6.4.3 Seed decontamination

H_2SO_4 (98%) was not effective as a primary seed decontaminant. Its use is still justified because it was easy to remove the seed coats. Figure 6.4 shows effects of ethanol, $HgCl_2$ and NaOCl on *P. capensis* percentage seed decontamination and germination. There were significant differences ($P \leq 0.05$) between treatments with respect to asepsis and germination. Seed germination was significantly higher for NaOCl (76%) than either the $HgCl_2$ (23%) or ethanol (60%) treatment. Clear MS media were observed for seeds decontaminated either in NaOCl or ethanol and seedlings were normal (Figure 6.5A). In contrast, $HgCl_2$ was effective in seed decontamination (87%), but resulted in low

percentage seed germination and stunted seedlings. MS medium turned brown indicating presence of $HgCl_2$ residues which were detrimental to seed germination and growth. There was no clear distinction between roots (radicle) and shoots (plumule) (Figure 6.5B).

$HgCl_2$ exposure time and concentration can be optimised, but $HgCl_2$ is toxic to aquatic life, especially fish (Crompton, 1997). Its use is not justified if other less toxic sterilants can decontaminate explants. Efficacy of a sterilant depends on contact with the contaminated area, size and choice of explants (Hammerschlag, 1986). A sterilant can be ineffective if it does not reach the contaminated area. In this trial, seed testa removal facilitated good contact between the seed and sterilant. Consequently, this improved culture asepsis, but loss of a single cotyledon (seed leaf) delayed or caused seed germination failure as shown with $HgCl_2$. Mild stirring of seeds was important to avoid breaking the union of the two cotyledons. Generally, *P. capensis* seed germination started six days after culturing on the MS media for all the treatments except where $HgCl_2$ was used as a sterilant.

6.5 Conclusion

Improved *P. capensis* seed germination was achieved at 25 ºC with 24 hours of darkness. Germination was high when seeds were sown soon after collection (fresh seeds). 1.75% NaOCl improved seed asepsis while 0.1% $HgCl_2$ was detrimental to seed germination. Submerged seeds exhibited high germination rate and seedling growth uniformity. Based on these results, *P. capensis* seeds should be scarified with 98% H_2SO_4 and sterilised with 1.75% NaOCl and incubated at 25 ^{o}C with 24 hours darkness in order to obtain high germination rates.

Table

Table 6.1 Effect of temperature (°C) and darkness (D) on mean root length (cm) and shoot length (cm) of jacket plum (*Pappea capensis*) seedlings

Treatments	seed length	seed width	root length	shoot length
20 °C and D	0.45	0.40	9.08	2.52
25 °C and D	0.40	0.40	9.72	2.70
Mean	0.43	0.40	9.40	2.61
Probability	ns	ns	ns	ns

ns = not significantly different at P≤0.05

Figures

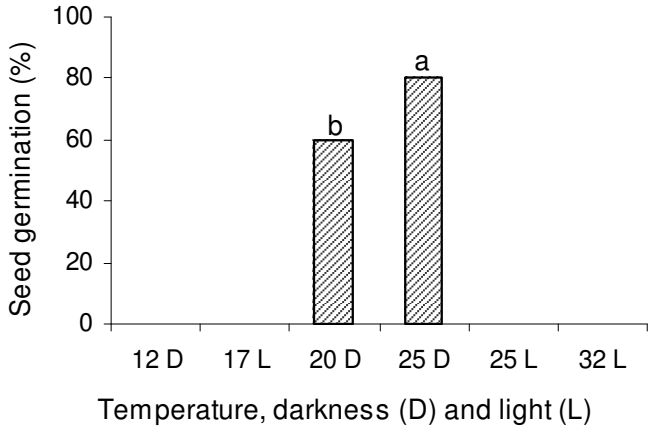

Figure 6.1 Percentage germination of jacket plum (*Pappea capensis*) seeds exposed to different temperatures (^{o}C), darkness (D) and light (L) four weeks after sowing. (Bars with different letters are significantly different at P≤0.05)

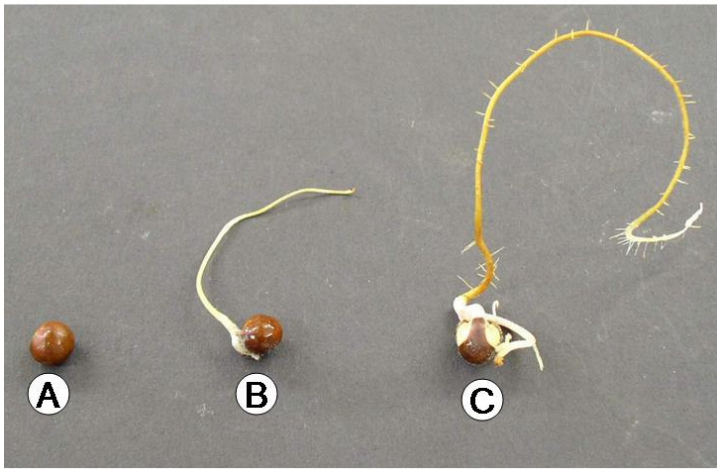

Figure 6.2 Jacket plum (*Pappea capensis*) seed/seedlings exposed to 24 hours of darkness and at (A) 12 ºC (B) 20 ºC and (C) 25 ºC temperatures (note that vigorous seedling growth was obtained at 25º C)

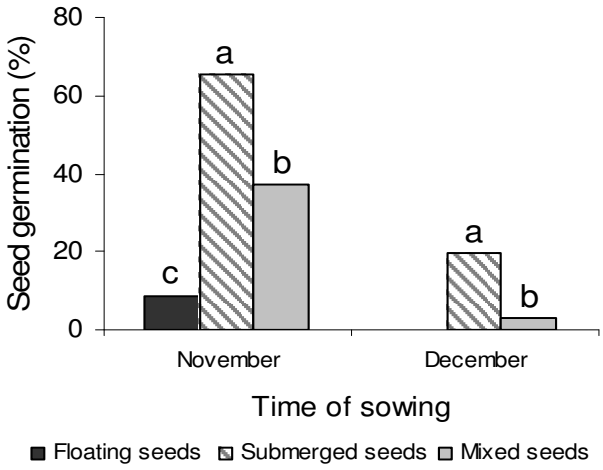

Figure 6.3 Jacket plum (*Pappea capensis*) seed germination (%) separated by water floatation method into floating, submerged and a mixture of submerged and floating seeds. Different letters within the same column indicate significant differences (P ≤0.05)

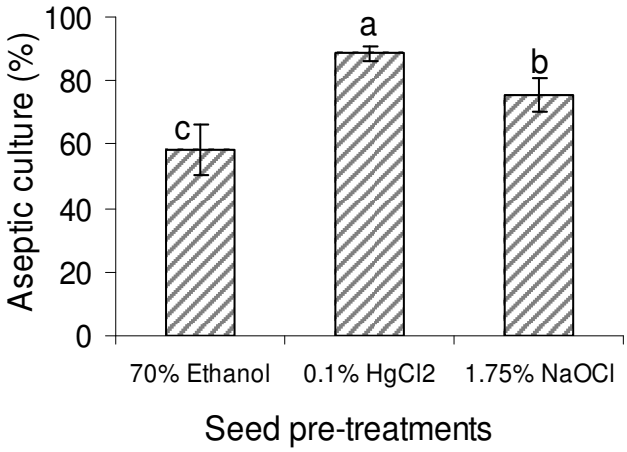

Figure 6.4 Percentage asepsis of jacket plum (*Pappea capensis*) seeds to 70% ethanol, 0.1% $HgCl_2$ or 1.75% NaOCl pre-treatments. Bars with the different letters are significantly different at P≤0.05

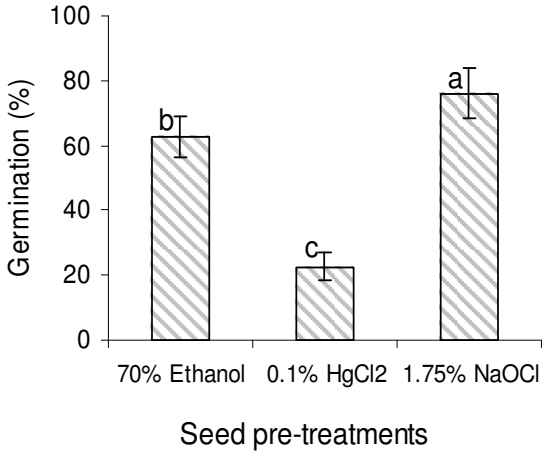

Figure 6.5 Percentage germination of jacket plum (*Pappea capensis*) seeds to 70% ethanol, 0.1% $HgCl_2$ or 1.75% NaOCl pre-treatments. Bars with the different letters are significantly different at P≤0.05

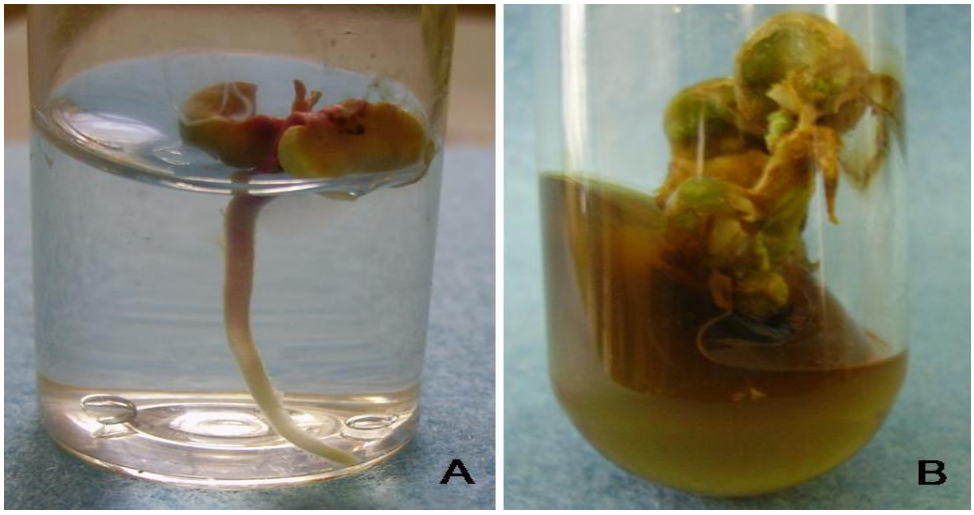

Figure 6.6 Jacket plum (*Pappea capensis*) seedlings in test tubes (A) normal seedling six days old; (B) abnormal seedling four weeks old (note brown culture medium in test tube B)