Effects Of Various Pre-Sowing Treatments On Germination Of Kei Apple *(Dovyalis Caffra)* Seeds Harvested At Different Maturity Stages.

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Abstract: The effect of pre-sowing treatments on the germinating Kei apple seeds harvested at different stages of maturity is being investigated. Germination values recorded were Germination Percent, (GP) Mean Germination Time (MGT), and Time to 50% Germination (T_{50}) compared between seed collected at varying maturity stages as; Green (GN), ripe (RP) and over ripe (OR). Pre-sowing treatments tested were soaking in cold water (CW), soaking in boiling water (HW), and treatment with Indole Acetic Acid (IBA), Abrasion against sand paper (AB), Sulphuric acid (SA) and the control (CNT). Seed maturity was significant ($P<0.0001$) with the greatest GP for the GN (88.14±0.54) and RP (88.25±0.54) stages. GP from the OR stage differed significantly ($P<0.0001$) compared to the other two maturity stages and it was associated with the GP (83.46±0.54). The effect of pre-sowing treatments was significant (ANOVA).The comparison between means showed significant differences in germination due to pre-sowing treatments. IBA, CW, HW, AB, gave the highest GP. MGT corresponding to these pre-sowing treatments ranged between 7.9 – 8.0 days. Intermediate GP of 77.01±0.77 was obtained in untreated seeds while the lowest GP which failed to reach 50% was obtained where Sulphuric acid was used. However, the corresponding MGT to sulphuric acid was the least of 4.3±0.04 days and that corresponding to the CNT was 11.0±0.04 days. Seed maturity x pre-sowing treatments interacted significantly to influence germination. It was concluded that since a reasonable GP was obtained from the control, dormancy is not responsible for hindrance of germination and that all pre-sowing treatment are effective in improving germination but SA is not suitable pre-sowing treatment.
[Hae. M and Funnah S.M. **Effects Of Various Pre-Sowing Treatments On Germination Of Kei Apple *(Dovyalis Caffra)* Seeds Harvested At Different Maturity Stages.** Life Science Journal. 2011;8(S2):100-105] (ISSN: 1097 – 8135).

Keywords: Kei apple; Germination; Pre-sowing treatments; Dormancy

1. Introduction

Dovyalis caffra is a recent crop, which is currently under intensive research. This is because its fruits have been identified as one of the indigenous forest products which have a potential to improve people's health (Swanepoel, 2003; Loots *et al.*, 2006). However, there are a number of challenges surrounding propagation of *D. caffra* trees through seeds. These problems include the fact that the seeds have hard and fibrous coats resulting in erratic germination, which may occur only after 18-20 days after planting (Joker, 2000; Bujulu & Mkenda , 2002). One of the factors that limit germination of seeds is dormancy. Seeds of *D. caffra* display a very confusing physiology hence it is difficult to apply methods that improve germination (Albrecht, 1993). Joker (2000) showed that the seeds possess a certain degree of dormancy. It is also known that dormancy does not exist in recalcitrant seeds of Kei apple since after harvest they proceed straight to germination (Hacker, 2003). It has been argued that where there is no seed dormancy in recalcitrant seeds, lack of germination may be hindered by hard seed coats. Whichever physiology is possessed by *D. caffra* seeds, there is a need to improve germination.

Seed germination is affected by other factors other than dormancy and hard-seed coats including maturity of fruits when harvested (Epkong, 2008; Samarah *et al.*, 2003). This is because fruit maturity stage, at which seeds are extracted, has a marked influence not only on the longevity but also on viability period of seeds. Hamilton & Midcap (1981) are of the view that there are no set rules in determining when seeds of a particular plant can be collected. However, physical appearance such as color can be used as a visual guide to judge the correct harvest time.

There are a number of ways used to improve germination of seeds (Hossain *et al.* 2005). Scarification by Sulphuric acid is one of the effective and extensively used ways of germination enhancement (Narbona *et al.*, 2006); Nadjafi *et al.*, 2006; Travlos *et al.*, 2007., Olvera-Carillo *et al.*, 2003). Hot water treatment has also proven effective in improving seed germination (Delanoy *et al.*, 2006). As a result it has been used by a number of workers in the field (Hossain *et al.*, 2005; Travlos *et al.*, 2007; Harris, 1996; Ren & Tao., 2004). The other way of improving seed germination is by abrasion against sandpaper until part of the seed coat has been removed and treatment with auxins (Chuanren *et al.*, 2004). *D. caffra* is a new crop which is under a lot of research on how to domesticate it. There is therefore lack of documented information on various ways of enhancing germination of its seeds

and the appropriate maturity stage at which seeds should be extracted. The aim of this study was to investigate the influence of pre-germination treatments and seed maturity stage on germination of *D. caffra* seeds.

2. Materials and Methods

Seed source and collection

Ripe Kei Apple fruits (with dark yellowish colour) were collected from a population of 20 female trees grown as a hedge at a location in Mafikeng (25º51S´ 25º38 E´ – 25.85, 25.633). The fruits were immediately put in brown paper bags and taken to the experimental laboratory of the Department of Crop Science of the Northwest University at Molelwane. Upon arrival, the fruits were depulped against a wire mesh and then washed clean using a stream of running tap water. Seeds were then immersed in 500 ml beaker filled with water. Some of the seeds which failed to sink were eliminated from the trial. After thorough cleaning the seeds were then soaked in 1% NaOCl solution for 10 minutes and dried with cloth to limit decay (Daws *et al.,* 2004; Gamènè *et al., 2004*).

Seed pre-sowing treatments

To facilitate germination, seed coats were either weakened or removed by subjecting them to various pre-sowing treatments. Mechanical scarification of seed coats was carried out by abrasion of thin layers of seeds lightly rubbed against two sheets of sandpaper (Brummer & Klos, 2000) until seed coats were removed using the hand palm. Acid scarification was undertaken by immersion of seeds in concentrated sulphuric acid and allowing it to swirl for 10 seconds in order to remove the seed coats. The seeds were then washed using a stream of running tap water until all the acid had been removed. Adhering acid was removed by washing seeds in a bath of sodium bicarbonate (Hartman & Kester, 1983) and then rinsing with distilled water. Hot water treatment was achieved by immersion of seeds in boiling water and then allowed to cool overnight, while cold water treatment was done by immersion of seeds in cold tap water and allowed to soak overnight. Hormone treatment was done by preparing Dynaroot solution (10g in 500ml of distilled water) and soaking seeds overnight. Untreated seeds were used as control.

Effect of seed maturity on germination of D. caffra seeds

To determine the influence of pulp condition on seed germination of *D. caffra*, the seeds were obtained from fruits at different stages of maturity (green, ripe, overripe). Five seed pre-treatments to enhance germination methods were used on each batch as follows: (1) mechanical scarification by abrasion of seeds against sandpaper; (2) immersing seeds in boiling distilled water and left to cool overnight; (3) dipping seeds in concentrated sulphuric acid for 10 seconds; (4) priming seeds with water; (5) soaking in Dynaroot solution and (6) untreated seeds.

Bioassays

Germination tests were conducted by placing 50 seeds in a petri dish (10 cm diameter) lined with No. 42 Whatman filter paper. The filter papers were moistened daily with distilled water throughout the experiment. The petri dishes were placed in different incubators set at 27^0C. Germination was recorded daily and seeds were considered to have germinated once the radicle had protruded 2mm in length (Gonzalez-Benito & Prez-Gorcia, 2006).

Data collection

Mean germination time (MGT) was calculated by using the formula of Van Staden and Street (2007) and Muhammed & Amusa (2003): $MGT = \sum (n \times d) / N$, where, n = number of seeds germinated on each day, d = number of days from the beginning of test, and N = total number of seeds germinated at the termination of the experiment. Percent germination (GP) was determined following the equation: GP = SG / TS x 100 where: GP = Percent Germination, SG = Seeds Germinated, TS = Total Seeds Planted (Yang *et al*., 1999). For each treatment, final germination percent was arcsine transformed before analysis (Mg'omba *et al*., 2007).

Experimental design

A factorial experiment of 3 x 5 combinations using three groups of seeds collected from fruits at different maturity stages and five seed pre-treatment methods was used. Each treatment was replicated seven times. The experiment was laid in a Randomized Complete Block Design. All data collected was subjected to Analysis of variance using Statistical Analysis System (SAS) program (SAS Institute, 1985). Mean separation was performed using Tukey's Test where significant differences existed.

3. Results

Results presented in this experiment were obtained after failure of seeds to germinate twice. The effects of seed maturity stage on germination of *D. caffra* seeds are presented in Table 1. Final GP was significantly ($P<0.0001$) different between seed maturity stages. The mean GP for seeds harvested GN and RP gave the highest GP values (which were not significantly ($P<0.0001$) different from one another but significantly different from (83.49±0.54) OR stage. Mean germination time (MGT) was significantly affected by seed maturity stage (Table 1). MGT varied between 6.0 and 7.9 days among seed maturity treatments. There were no significant differences between GN and RP stages. However, the two maturity treatments differed significantly compared to that at OR stage. The effect of seed maturity stage on days to

50% germination (T_{50}) is also presented in Table 1. The analysis of variance shows that seed maturity stage had an influence on T_{50}. The first seeds to reach 50 % germination were those from GN and OR and they were not statistically different from one another ($P<0.0001$). For GP and MGT, where GN and RP maturity stages were associated with the highest values, the T_{50} followed a different pattern. There was a decline in germination speed to 50% at RP stage as indicated by an increase in T_{50}.While T_{50} in GN (8.84±0.13) and OR (8.81±0.13) did not differ significantly ($P<0.0001$) from one another. The effect of pre-sowing treatments on germination of Kei-apple seeds is shown in Table 2. Seeds treated with IBA solution reached 50% germination (T_{50}) faster (8.5±0.18 days) than other pre-sowing treatments. The next treatment to reach T_{50} was AB. IBA was followed by HW (11.0±0.18 days) and soaking in cold water (11.3±0.18 days) which did not differ significantly ($P<0.0001$). The control took a much longer time to reach T_{50} (14.3 ±0.18 days) and scarification by sulphuric acid resulted in germination percentages which never reached 50%.

The analysis of variance (ANOVA) shows that pre-treatment methods had a significant influence ($P<0.0001$) on MGT (Table 3). When seeds were scarified with sulphuric acid (SA), germination occurred after only 4.3±0.04 days after incubation. Intermediate MGT was obtained due to AB (7.9±0.04), CW (8.0±0.04), HW (7.9±0.04) and IBA (8.0±0.04) which did not differ significantly ($P<0.0001$). All treatments were compared with the control which had the longest MGT (11.0±0.04). The interaction between seed maturity factors and pre-sowing treatments was highly significant ($P<0.0001$) according to ANOVA (Table 3). At all seed maturity stages, the highest GP was obtained where IBA treatments, soaking in cold water and hot water treatments were used (Fig 1). GP in all these treatments was almost constant across all seed maturity stages. Intermediate results were associated with control which performed better than sulphuric acid. Germination pattern observed in untreated seeds showed that seeds germinated better with an increased maturity stage. On the contrary, where sulphuric acid was used, germination was at its highest for seeds obtained from ripe fruits but declined with an increase in maturity (Fig 1).

Results recorded due to the interactive effect of seed maturity stage and pre-sowing treatments on days to 50% germination (T_{50}) show significant ($P<0.0001$) effects (Fig 3). The control gave the highest MGT which declined with increasing maturity stage. Seeds treated with IBA reached T_{50} faster than other treatment regardless of maturity stage of seeds. There were no significant differences ($P<0.0001$) between seed soaking in cold water, hot water treatment and abrasion with sand paper.

4. Discussion

This experiment was undertaken to investigate the effect of seed maturity and pre-sowing treatment on germination (GP, MGT and T_{50}) of *D. caffra* seeds. These parameters are important in decision making to produce large quantities of seedlings using seeds for commercial purposes. The hard seed coat is a possible barrier to successful germination of Kei apple seeds as seeds showed no possibility of dormancy. The ANOVA (Appendix 2) showed that seed maturity influenced germination of Kei apple seeds. Seeds harvested at GN and RP maturity stages gave high GP and germinated within a very short time. A significant difference was evident in seeds harvested at OR maturity stage. Germination speed to 50% did maintain the same tendency. Instead seeds harvested at GN and OR maturity stages performed well with no significant differences between them. These results indicate that when seeds are obtained for planting, it is very important that the quality of seeds cannot be judged by the color or condition of pulp. Based on the data obtained in this experiment, it was concluded that the most suitable stage for the collection of Kei apple seeds is at GN and RP for better GP and to experience rapid germination. However, reasonable GP can still be obtained at OR maturity stages.

Pre-sowing treatments had a significant effect on the enhancement of *D. caffra* seeds (Table 3). The greatest GP was obtained on seeds treated with CW, HW, AB and IBA. Intermediate GP was obtained from the control suggesting that *D. caffra* seeds do not possess dormancy. The results from the current study is not in agreement with the report by Albrecht, (1993) who indicated that *D. caffra* seeds possess dormancy. Even though pre-treatment with SA resulted in the lowest GP, which never reached 50%, this is where the fastest germination was experienced. It is therefore concluded that soaking in SA is not a suitable technique to enhance GP of *D. caffra* seeds. This could mean that the concentration used in the current study was high. Future research on the use of SA should use serial dilution to determine the right concentration.

Different pre-sowing treatments have been widely used before to enhance germination of seeds and variable results were reported by various workers. Travlos *et al*. (2007) whose objective was to break dormancy in hard coated seeds of *Tylosema esculentum* (Burch) also tried various pre-sowing methods. These included abrasion against sand paper, scarification with sulphuric acid and immersion in water. Sulphuric acid scarification resulted in higher GP while the other treatments gave intermediate germination values. On the contrary, in the current experiment, sulphuric acid

gave the lowest germination. These results, on the other hand agree with those of Narbona *et al*., (2006) who used sulphuric acid scarification to induce germination, and found out that it resulted in lower germination percentages compared to the control. Their results showed a high variability as in one of their experiments, mechanical scarification out-performed other pre-sowing treatments.

It must also be borne in mind that different seeds respond differently to different pre-sowing treatments and that improper seed pre-sowing treatment can lead to poor germination (Ren & Tao, 2004). Hence, different workers experience variable results with the same pre-sowing treatments depending on the species. Furthermore, sometimes pre-sowing treatment may not be necessary at all. For example, Kulkarmi *et al*., 2006 reported that scarification treatment used to enhance germination of *Eucomis autumnalis* subsp. *Autumnalis* seeds had no significant effect. From the results of the current study it can be concluded that even though SA is not suitable to enhance germination of *D. caffra* seeds, CW, HW, AB and IBA pre-sowing treatment can be safely used. What is fascinating about the results of this study is the fact that successful germination of seeds can be achieved through use of simple technology that farmers can afford and easily adopt. This is very important in the success of domestication of this species. The interaction between the main factors was significant (Table 3). IBA, CW and HW gave the highest GP regardless of seed maturity stage at which seeds were collected while the lowest germination was experienced with SA.

5. Conclusion

From these results, it can be concluded that not only seed maturity should be considered in successful germination of *D. caffra* seeds but even the type of pre-sowing used is important.

Acknowledgements

The authors are would like to extend their gratitude toProfessor S.M. Materechera for his input, Ms K. Mooketsi for her contribution in the preparation of the manuscript and to Dr. S.D. Mulugeta for statistical assistance.

Table 1. Germination of *D. caffra* seeds harvested at various maturity stages

Seed maturity stage	GP	MGT	T_{50}
Green (GN)	88.14±0.54[a]	7.97±0.03[a]	8.84±0.13[a]
Ripe (RP)	88.25±0.54 [a]	7.98±0.03[a]	9.830.13[b]
Over ripe (OR)	83.49±0.54[b]	6.0±0.03[b]	8.810.13[a]

Values are means ± SE. Values bearing the same letters are not significantly different according to Tukey's Test.GP= Germination percentage; MGT=Mean Germination time (days); T_{50} = Days to 50% Germination

Table 2. Effect of seed pre-sowing treatment on germination of *D. caffra* seeds

Pre-sowing treatment	GP	MGT	T_{50}
CNT	77.01±0.77[a]	11.0±0.04[a]	14.3±0.18[a]
AB	99.36±0.77[b]	7.9±0.04[b]	9.7±0.18[b]
CW	98.10±0.77[b]	8.0±0.04[b]	11.3±0.18[c]
HW	98.41±0.77[b]	7.9±0.04[b]	11.0±0.18[c]
SA	25.80±0.77[c]	4.3±0.04[c]	9.7±0.18[d]
IBA	100±0.77[b]	8.0±0.04[b]	8.57±0.18[d]

Values are Means ± SE of seven replicates of 50 seeds. Figures on the same column bearing the same letter are not significantly different according to Tukey's Test.CNT = control, AB = abrasion, CW = Cold water abrasion, HW = Hot water treatment, SA = Sulphuric acid, CW =cold water treatment.

Table 3. Analysis of variance for GP, MGT and T_{50} of *D. caffra* seeds harvested at different maturity and exposed to various pre-sowing methods.

Source of variation	d.f	GP	MGT	T_{50}
Block	6	8.60^{ns}	0.05^{ns}	1.27^{ns}
Seed Maturity (M)	2	309.85***	2.45***	14.31***
Pre-sowing treatment (P)	5	18656.46***	1.37***	501.59***
M x P	10	174.71***	1.48***	18.37***
Error	95			
Total	121			

ns= not significant
**** = highly significant

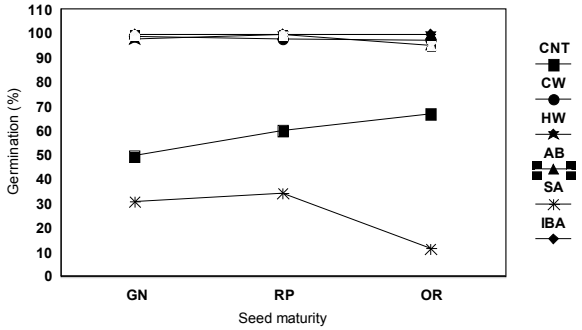

Fig. 1 Effect of various pre-sowing treatments x maturity on germination percent of *D. cafra* seeds. GN=Green pulp; CNT=Control; CW=Soaking in cold water; HW=Hot water treatment; SA= Sulphuric acid; AB=Abrasion with sand paper and IBA=Soaking in IBA. Bars represent statistical differences (means).

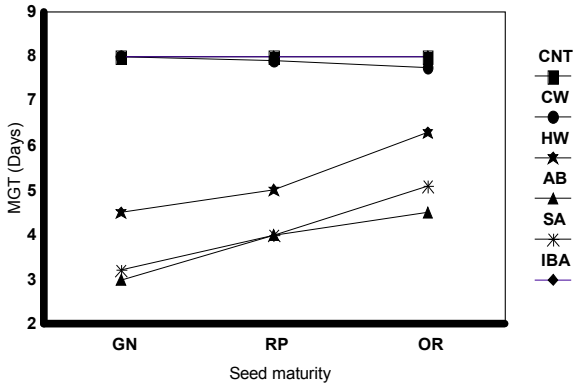

Fig. 2 Effect of various pre-treatment x Mean Germination time of *D. caffra* seeds. GN=Green pulp; CNT=Control; CW=Soaking in cold water; HW=Hot water treatment; SA= Sulphuric acid; AB=Abrasion with sand paper and IBA=Soaking in IBA. Bars represent statistical differences (means).

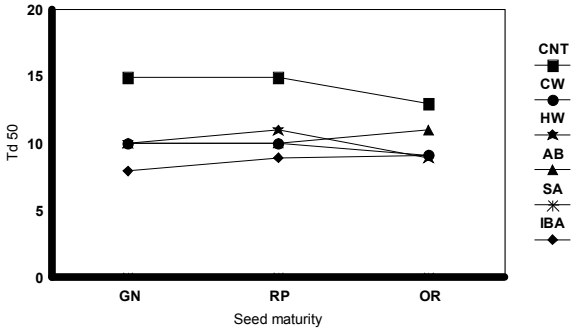

Fig. 3 Effect of various pre-sowing treatments x on T_{50} of *D. cafra* seeds. GN=Green pulp; CNT=Control; CW=Soaking in cold water; HW=Hot water treatment; SA= Sulphuric acid; AB=Abrasion with sand paper and IBA=Soaking in IBA. Bars represent statistical differences (means).

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5/28/2011