EFFECTS OF PRE-GERMINATION TREATMENTS, DESICCATION AND STORAGE TEMPERATURE ON GERMINATION OF *CARISSA EDULIS*, *VANGUERIA MADAGASCARIENSIS* AND *XIMENIA AMERICANA* SEEDS

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MAARA, N. T., KARACHI, M. & AHENDA, J. O. 2006. **Effects of pre-germination treatments, desiccation and storage temperature on germination of *Carissa edulis*, *Vangueria madagascariensis* and *Ximenia americana* seeds.** Effects of seed pre-germination treatments, desiccation, storage temperature and duration of storage on germination of freshly harvested *Carissa edulis*, *Vangueria madagascariensis* and *Ximenia americana* seeds were investigated in three experiments in germination cabinets set at 20 to 25 °C. In experiment 1, nine seed pre-germination treatments were applied. Germination tests were done immediately after the pre-treatments and after three months of storage at room temperature. In experiment 2, seeds were dried to target seed moisture contents (TSMC) and then germinated. In experiment 3, seeds at TSMC were stored at 25 to -20 °C for one, two and three months prior to germination. The pre-treatments did not provide significant differences in germination. Good germination (> 70%) was obtained from all treatments that did not involve injury to the seed. Nipping reduced germination whereas boiling water was lethal. Ageing resulted in the reduction of germination of all seeds. Drying *C. edulis* seeds resulted in a marked decline in seed germination but only marginal reduction in *X. americana* and no effects on *V. madagascariensis*. All control seeds maintained > 70% viability after three months of storage. Low seed moisture content and temperature reduced viability of *C. edulis* and *X. americana* seeds but had no effects on *V. madagascariensis* seeds.

Keywords: Seed moisture content, viability, longevity, wild fruits

MAARA, N. T., KARACHI, M. & AHENDA, J. O. 2006. **Kesan rawatan pracambah, pengeringan dan suhu storan terhadap percambahan biji benih *Carissa edulis*, *Vangueria madagascariensis* dan *Ximenia americana*.** Kesan rawatan pracambah biji benih, pengeringan, suhu storan dan tempoh penyimpanan terhadap percambahan biji benih *Carissa edulis, Vangueria madagascariensis* dan *Ximenia americana* yang baru dikutip dikaji. Tiga uji kaji dijalankan di dalam kabinet percambahan yang ditetapkan pada suhu antara 20 °C hingga 25 °C. Dalam uji kaji 1, sembilan rawatan pracambah biji benih diasaskan. Ujian percambahan dijalankan sebaik sahaja rawatan pracambah dan selepas tiga bulan biji benih disimpan pada suhu bilik. Dalam uji kaji 2, biji benih dikeringkan kepada kandungan lembapan biji benih sasar (TSMC) dan kemudiannya dicambah. Dalam uji kaji 3, biji benih pada TSMC disimpan pada suhu antara 25 °C hingga -20 °C selama satu, dua dan tiga bulan sebelum dicambah. Rawatan pracambah tidak mempengaruhi percambahan dengan ketaranya. Percambahan yang baik (> 70%) diperhatikan dalam semua rawatan yang tidak merosakkan biji benih. Gentasan mengurangkan percambahan manakala air didih membunuh biji benih. Tempoh penyimpanan yang lama mengurangkan percambahan biji benih. Pengeringan menyebabkan pengurangan ketara dalam percambahan biji benih *C. edulis* tetapi pengurangan sedikit dalam *X. americana* dan tiada kesan dalam *V. madagascariensis*. Biji benih kawalan mempunyai percambahan > 70% selepas disimpan selama tiga bulan. Kandungan lembapan biji benih dan suhu yang rendah mengurangkan percambahan biji benih *C. edulis* dan *X. americana* tetapi tidak mempengaruhi percambahan biji benih *V. madagascariensis*.

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INTRODUCTION

Indigenous wild fruits in Kenya provide an important supplement to diets of local people (Muok *et al.* 2001). Some fruits are rich in sugars, essential vitamins and proteins (Maundu *et al.* 1998). In southern Africa, the sale of *Zizyphus mauritana* fruits is beginning to contribute to commercial trade (Kwesiga *et al.* 1996), whereas fruits of *Vitellaria paradoxa* and *Parkia bigloea* are harvested and marketed (Cooper *et al.* 1996).

The expansion of agriculture into habitats previously occupied by undomesticated species and over-exploitation of these species are threatening this genetic resource with extinction as trees can no longer recover through natural regeneration (Cunningham & Mberikum 1993). In addition, wild fruit trees have received little attention in research and development compared with exotic varieties because they are perceived as slow growing, producing fruits after several years and are irregular in fruiting.

There is a need to gather, cultivate, select and improve these wild fruits in order to exploit their potential and conserve a valuable genetic pool. This requires understanding of the propagation requirements and seed behaviour under storage. The three main categories of seed storage behaviour based on physiological storage potential are orthodox, recalcitrant and intermediate seeds (Hong & Ellis 1996, Schmidt 2000). Orthodox seeds can be stored at low seed moisture content and low temperatures (Dulloo *et al.* 2000) and longevity of seeds increases with decrease in seed storage moisture content (Roberts 1973). Recalcitrant seeds are shed when seed moisture is high and usually start germinating soon after shedding and often before shedding (Rajeswari & Kaveriappa 2000). These seeds are sensitive to desiccation and lose viability on drying (Berjak & Pammer 1995). Chilly temperatures also damage seeds of tropical species. Intermediate seeds exhibit some degree of desiccation tolerance and freezing sensitivity. This category of seeds is damaged after desiccation to seed moisture content of 7–12%. Seeds of tropical species die more rapidly when temperatures are below 0 °C and in some cases temperatures below 10 °C kill seeds (Hong *et al.* 1998).

The objective of this study was to determine the germination requirements and seed longevity under varied temperature and moisture regimes for *Carissa edulis*, *Vangueria madagascariensis* and *Ximenia americana* seeds. These species are among the important indigenous dryland fruit species that require domestication. They are threatened with over-exploitation and in addition there is widespread destruction of their natural habitats for human settlement (Kigomo 2001).

MATERIALS AND METHODS

Seeds were collected from Kisanana division of Koibatek district (0° 25' S, 0° 25' N; 35° 30 W', 35° 15' E; 880–1000 m asl) in the Rift Valley, Kenya. Rainfall ranges from 300–750 mm per annum and is erratic and unreliable. Ripe fruits were harvested by hand. A total of 5 kg of *C. edulis*, 10 kg of *V. madagascariensis* and 20 kg of *X. americana* fruits were harvested from at least 25 trees maintaining an isolation distance of 100 m between trees. The fruits were transported in perforated polythene bags to the Kenya Forestry Research Institute Seed Centre where seeds were extracted and cleaned. Fruits were de-pulped by hand to minimize mechanical damage. *Carissa edulis* seeds were squeezed through a 2 mm sieve leaving the pulp; *V. madagascariensis* fruits were soaked in cold water for two days and the nut cracked using a knife to release the seed; while *X. americana* fruits were rubbed against a wire under continuous running water until all the pulp was removed. Seed quality was assessed visually whereby damaged, infested or deformed seeds were discarded.

Experiment 1: Germination capacity after 0 and 3 months of storage

Two hundred seeds each of *C. edulis* and *V. madagascariensis* and 80 of *X. americana* were subjected to the following treatments: T1—control; T2—washed in tap water; T3—nipped, i.e. seeds were nipped at the distal end taking care that no injury occurred to the embryo; T4—soaked in tap water for 24 hours at room temperature; T5—soaked in tap water for 24 hours and then washed in running tap water; T6—nipped then soaked in tap water for 24 hours; T7—soaked in hot water for 1 hour, i.e. seeds were soaked in water previously heated to boiling point; T8—soaked in hot water for 1 hour and then washed in running tap water and T9—nipped then soaked in hot water for 1 hour. Seeds from each treatment were sub-divided into two lots.

Seedlot A was used to test germination after applying the pre-germination treatments, while seedlot B was used to test germination after three months of storage at room temperatures (20–25 °C). Seeds were soaked in 1% sodium hypochloride solution for 10 min to sterilize the surface then dried using blotter sheet prior to germination test. Seeds were germinated on 1% agar solution in a germination cabinet set at 20–25 °C. The design was a complete randomized design with four replicates. Ten seeds were used per replicate for *X. americana* due to lack of seeds and 25 seeds for the other species (ISTA 1993a, b). The numbers of germinated seeds were removed after counting and observations terminated when no further germination occurred.

Experiment 2: Effects of desiccation on seed germination

The initial and target seed moisture contents (TSMC) were determined according to Hong and Ellis (1996). Anhydrous silica gel at the ratio of 1 seed: 2 silica gel by weight was used to desiccate the seeds: *C. edulis*—29.7, 25.3, 20.1, 14.7, 9.7 and 5.1%; *V. madagascariensis*—17.7, 11.5, 9.9, 5.8 and 3.1% and *X. americana*—22.5, 12.5, 9.5, 5.4 and 3.8%. Germination test was conducted on 25 seeds per species replicated five times and the procedures were as in experiment 1.

Experiment 3: Effects of storage temperature on seed longevity

Seeds at TSMC were stored in airtight plastic containers for one, two and three months at either 25, 10, -3, or -20 °C. Each treatment consisted of 25 seeds replicated four times. Seed germination was tested after each duration of storage as in experiment 1.

Statistical analysis

The cumulative number of seeds that had germinated was expressed as a percentage of the total number of seeds germinated in each treatment and subjected to ANOVA. Least significant difference was used to separate significant means.

RESULTS AND DISCUSSION

Germination after pre-treatments and after three months of storage

The pre-treatments did not improve overall germination (Table 1). Simple treatments such as the control (TI) and soaking in tap water (T4) resulted in good germination (> 80%), which are in agreement with the findings of Prins and Maghembe (1994). Germination started on day 3 and was completed by day 8 in control treatments of *C. edulis* and *V. madagascariensis*. The satisfactory (> 90%) and quick germination indicates lack of seed dormancy. However, germination in *X. americana* started on day 16 and continued till day 38, which suggests the existence of some form of physiological dormancy. Rapid germination may be an adaptation to produce seedlings to escape the adverse effects of unfavourable moisture conditions found in the area.

In nature, seeds ripen towards the beginning of the rainy season, facilitating germination soon after fruits have fallen from the tree. Washing *C. edulis* seeds (T2 and T5) resulted in reduction in germination by 21–26%. Seeds of this species are small with a fairly exposed embryo. The embryo may have been damaged during the pre-treatments, resulting in reduced germination. This observation is consistent with that of Msanga (1995).

Nipping (T3) seeds also resulted in reduced germination for all species. It appears that either the treatment caused embryo injury or that the seed's internal structures might have been damaged by unimpeded swelling (Willan 1985).

Exposure to boiling water was lethal to all seeds (T7, T8 and T9). This is in agreement with other observations on tropical seeds (Prins & Maghembe 1994, Schmidt 2000).

Similarly, seed ageing resulted in reduction of germination of all seeds but with greater reduction on the germination of nipped *C. edulis* and *X. americana* seeds. Seeds of these species should be sown fresh at ripening.

Effects of drying on seed germination

Drying seeds did not improve germination. The pattern of germination on *C. edulis* seeds was typical of that exhibited by intermediate seeds (Hong & Ellis 1996). The sensitivity to desiccation of these seeds suggests that the critical lower moisture threshold was about 20% below which germination was reduced to less than 50% (Table 2). However, the initial SMC was lower than that suggested for this category of seeds (Hong & Ellis 1998). The relationship between SMC and viability of *X. americana* indicates that the seeds exhibit intermediate storage behaviour which concurs with the observation by Fletcher and Pritchard (2000) but contradicts that of Msanga (1995) in that *X. americana* seeds tolerate desiccation. Germination of *V. madagascariensis* was not affected by drying indicating that they are orthodox seeds.

Effects of seed moisture content and storage temperature on seed longevity

There were variable survival patterns under different seed storage conditions. Germination of *C. edulis* seeds with 20.1–29.7% SMC stored at 25 °C exceeded 80% during the three-month storage period (Table 3). However, a sharp reduction in germination was observed for seeds with < 20.1% SMC irrespective of storage temperature and was lowest in seeds stored at -20 °C. This behaviour confirms the intermediate seed storage characteristics of *C. edulis*.

The best consistent germination (≥70%) for

Table 1 Effects of treatments and storage on germination (%) of seeds

Treatment	*C. edulis*		*V. madagascariensis*		*X. americana*	
	Initial	After 3 months	Initial	After 3 months	Initial	After 3 months
T1	96	88	90	80	90	79
T2	70	37	86	72	93	80
T3	52	34	61	52	83	10
T4	91	86	82	78	91	75
T5	75	74	89	76	94	75
T6	34	17	66	58	72	3
T7	0	0	0	0	0	0
T8	0	0	0	0	0	0
T9	0	0	0	0	0	0
LSD ($p < 0.05$) within treatment	5.6	5.3	5.6	4.5	4.5	20.4
LSD ($p < 0.05$) between treatments	5.3		4.9		14.4	

T1—control; T2—washed in tap water; T3—nipped; T4—soaked in tap water for 24 hours at room temperature; T5—soaked in tap water for 24 hours and then washed in running tap water; T6—nipped then soaked in tap water for 24 hours; T7—soaked in hot water for 1 hour, i.e. seeds were soaked in water previously heated to boiling point; T8—soaked in hot water for 1 hour and then washed in running tap water; T9—nipped then soaked in hot water for 1 hour.

Table 2 Effects of desiccation on germination of seeds

C. edulis		*V. madagascariensis*		*X. americana*	
Moisture content (%)	Germination (%)	Moisture content (%)	Germination (%)	Moisture content (%)	Germination (%)
29.7	97	17.7	74[a]	22.5	90
25.3	89	11.5	77[a]	12.5	86
20.1	86	9.9	87	9.5	85
14.7	47	5.8	95	5.4	79
9.7	41	3.1	93	3.8	79
5.1	30				
LSD ($p < 0.05$)	6.1		6.6		6.8

Moisture is initial moisture content.
[a]Fungus infection observed

X. americana seeds was recorded at 25 and 10 °C across all SMC after one month of storage (Table 4). The combination of low temperature and low SMC resulted in rapid ageing and poor germination of seeds. This response is similar to those reported by Jayanthi *et al.* (1998), Schmidt (2000) and Dudley *et al.* (2001) on seeds of some tropical tree species. The results indicate that seeds of *C. edulis* and *X. americana* could be sown fresh at ripening or stored without complicated pre-treatments for three months at ambient temperature without major loss in viability.

Germination of *V. madagascariensis* was not affected by storage conditions (Table 5). These seeds can therefore be stored at the range of experimental treatments of this study for three months without loss of viability. However, there is a need to determine whether or not viability can be maintained for a longer period.

Table 3 Effects of moisture content and storage temperature on germination of *Carissa edulis* seeds over a 3-month period

Seed moisture content (%)	Storage temperature (°C)	Germination (%) Duration (months)		
		1	2	3
29.7	25	92	89	90
	10	84	83	72
	-3	67	66	61
	-20	38	32	30
25.3	25	86	87	83
	10	83	80	74
	-3	65	47	40
	-20	32	14	9
20.1	25	85	83	80
	10	77	71	67
	-3	62	52	29
	-20	37	13	4
14.7	25	48	44	38
	10	52	42	38
	-3	44	32	26
	-20	32	27	23
9.7	25	37	38	28
	10	38	36	31
	-3	42	26	22
	-20	29	22	19
5.1	25	25	25	20
	10	29	31	34
	-3	30	31	26
	-20	23	21	18

Table 4 Effects of seed moisture content and storage temperature on germination of *Ximenia americana* seeds over a 3-month period

Seed moisture content (%)	Storage temperature (°C)	Germination (%) Duration (months)		
		1	2	3
22.5	25	87.5	78.8	73.8
	10	82.5	75.0	60.0
	-3	43.8	10.0	0.0
	-20	25.0	0.0	0.0
12.5	25	82.5	55.0	47.5
	10	81.3	47.5	28.8
	-3	28.8	8.8	0.0
	-20	21.3	0.0	0.0
9.5	25	86.3	48.8	48.8
	10	83.8	47.5	37.5
	-3	27.5	5.0	0.0
	-20	17.5	0.0	0.0
5.4	25	70.0	66.3	50.0
	10	80.0	38.8	35.0
	-3	20.0	5.0	0.0
	-20	18.8	1.3	0.0
3.8	25	77.5	38.8	27.5
	10	73.8	37.5	31.3
	-3	20.0	7.5	0.0
	-20	22.5	1.3	0.0

Table 5 Effects of seed moisture content and storage temperature on germination of *Vangueria madagascariensis* seeds over a 3-month period

Seed moisture content (%)	Storage temperature (°C)	Germination (%) Duration (months)		
		1	2	3
17.7	25	69	65	62
	10	71	74	70
	-3	72	76	59
	-20	79	60	67
11.5	25	67	74	61
	10	64	75	61
	-3	69	72	64
	-20	67	74	64
9.9	25	86	76	73
	10	83	88	78
	-3	90	73	80
	-20	80	76	77
5.8	25	90	78	78
	10	89	85	83
	-3	88	82	70
	-20	90	85	75
3.1	25	88	90	79
	10	89	76	71
	-3	92	86	87
	-20	89	88	90

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