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The Effect of Hot Water, Sulphuric Acid, Nitric Acid, Gibberellic Acid and Ethephon on the Germination of Corchorus (*Corchorus tridens*) Seed

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Abstract: Laboratory trials were carried out to evaluate the effects of concentrated sulphuric acid (98%), concentrated nitric acid (65%), simmering hot water 98.5°C, gibberellic acid (GA_3) and ethephon on the germination capacity of one-and-two-year old *Corchorus tridens* seeds. The results showed that treating one-year-old *Corchorus tridens* seeds with concentrated sulphuric acid (98%) for 10, 20, and 30 min significantly broke the seed dormancy and promoted the germination of the seeds compared to control seeds (treated with distilled water), hot water treatment, nitric acid (65%) (seeds treated for 10, 20 and 30 min), gibberellic acid and ethephon. Treating Corchorus seeds with concentrated sulphuric acid for more than 10 min significantly decreased germination capacity. Concentrated nitric acid, gibberellic acid and ethephon had no effect on the germination capacity of corchorus seed. Simmering hot water also broke seed dormancy of *Corchorus tridens* seed but was not as effective as concentrated sulphuric acid. The results also showed that *Corchorus tridens* seed stored for two years lost their viability by up to 91.5%. It was concluded that *Corchurus tridens* seeds from Botswana have hard seed coat or impervious seed coat dormancy but not physiological dormancy as an adaptation to arid and desert conditions.

Key words: Seed dormancy, Corchorus, hot water, acid treatment, growth regulators

INTRODUCTION

Corchorus belongs to the family Tiliaceae and the genus *Corchorus* consists of some 50-60 species of which about 30 are found in Africa. *Corchorus* is mainly known for its fibre product jute and for its leaf vegetables[1]. Jute is mainly extracted from *C. olitorius* L. and *C. capsilaris* L., a species from India[2,3]. Several species of *Corchorus* are used as a vegetable of which *C .olitorius* is most frequently cultivated. *C. olitorius* called Jew's mallow or Jute mallow in English and corete potagete in French and locally called delele (in Botswana) is popular as a vegetable in both dry and semi- arid regions and in the humid areas of Africa. It is referred to as derere in Zimbabwe, called tege in Cameroon and otege in Uganda[4]. Some Nigerian names include ewedu in Yorubo, ahuhura in Igbo, whereas the Hausa people call it malafiya, somewhat similar to molukhia as used by Arabs in North-eastern Africa.

The most frequently cultivated species in Africa is *C. olitorius* and *C. capsilaris* in India[4]. In Sudan the crop is much appreciated in 'malachia' soup and considerable research attention has been given to this crop. In Egypt farmers occasionally grow the crop in greenhouses during the winter months to cater for its out-of-season demand. It is very old vegetable that was mentioned in early Greek literature. The cultivation of *C. olitorius*, *C. tridens* and *C. trilocularis* L. is similar but *C. trilocularis* is least frequently cultivated for its leaves[4].

In Botswana, the crop is collected from the wild or only cultivated to a limited extent and used as an indigenous vegetable, eaten as spinach leafy vegetable. All the Corchorus species collected in Botswana have a problem of seed dormancy thus limiting its cultivation. Seed dormancy constitutes a major difficulty in *Corchorus* cultivation[4]. To commercialise the production of jute mallow in Botswana, seed dormancy must be broken because this is the present method of cultivation. There are indications that the position of fruit on the plant affects the germination capacity since seeds extracted from capsules at the top and middle of the stem are better than those from the base[4,5]. Considerable diversity of *Corchorus* species can be found in markets, which reflects the diversity found in the field. Such observations can partly be explained by the phenomenon of volunteer crops caused by dormancy and irregular germination

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patterns. Viable seeds remain in the soil until their dormancy is broken, which could be a long time. Over-ripe, black capsules collected from dry plants at the end of the season are reported to contain more seeds with dormancy symptoms than yellow or brown capsules[4,5]. The objective of this study was therefore, to investigate the effect of sulphuric acid, nitric acid, hot water, gibberellic acid (GA_3) and 2-chloroethyl phosphoric acid (ethephon) on the germination capacity of one-and-2-year old *Corchorus tridens* (jute mallow) seeds.

MATERIALS AND METHODS

Experimental site: Trials were carried out in the Plant Physiology Laboratory in the Crop Science and Production Department, at the Botswana College of Agriculture, Gaborone, Botswana. The Botswana College of Agriculture is on latitude 24°34'S and longitude 25°57' with an altitude of 994 m above sea level, located at Sebele, 10 km from Gaborone, along Gaborone-to-Francis Town highway. Seeds were obtained from the Department of Agriculture Research, Ministry of Agriculture at Sebele, Gaborone, Botswana.

Experimental design and procedure: The experimental design in all the four trials was completely randomized design with four replicates. A total of 400 seeds were used per treatment. Before all the experiments were done the seeds were floated in the beaker in order to remove those seed that were not viable. The seed that floated were assumed not to be viable. Only the seed that sunk and settled at the bottom of the beaker were used for the study.

Trial 1: The treatments in trial 1 were control (distilled water), hot water (simmering hot water at 98.5°C), sulphuric acid 98% (10, 20 and 30 min) and nitric acid 65% (10, 20 and 30 min). This trial was done with one-year-old seeds of *Corchorus tridens* cultivar, with short fruits with 3 spikes and seeds were of medium size. The control seeds were soaked in distilled water for 24 h and then sown in moist germination paper in petri-dishes and placed under favourable conditions for germination. In the hot water treatment, seeds were soaked in simmering hot water (98.5°C) for 24 h and sown immediately to petri-dishes containing moistened germination paper.

In the sulphuric acid (98%) treatment, seeds were divided and put into three 100 mL heat resistant non-corrosive glass beakers and sulphuric acid (H_2SO_4) was poured slowly on the side of the beaker to a level where all seeds were covered (50 mL). The three seed lots in beakers were left for different times, one for 10, 20 and 30 min, after which the seeds were removed, and the acid drained off into another beaker. The seeds were thoroughly washed and rinsed to remove all the acid. The seeds were then soaked in distilled water to be ready for germination after 24 h. After 24 h, the seeds were germinated in the petri-dishes (100 seeds per petri-dish) per replicate containing moist germination paper.

In the nitric acid (65%) treatment same procedure was undertaken as for sulphuric acid. Seeds in three beakers were treated with nitric acid (65%) and left for 10, 20 and 30 min in acid. Acid was drained off and seeds rinsed with distilled water to wash off the acid then soaked for 24 h and germinated in petri-dishes containing moist germination paper.

Trial 2: Trial 2 was a repeat of trial 1, except that two-year-old seed was used in the trial.

Trial 3: The treatments in experiment 3 were distilled water, concentrated sulphuric acid (98%) (10, 20 and 30 min), gibberellic acid (GA_3) at 50, 100 and 150 mg L^{-1} and 2-chloroethylphosphoric acid (ethephon) at 2000, 4000 and 6000 mg L^{-1}. One-year old seeds of *Corchorus tridens* were used. The control seeds were soaked in distilled water for 24 h, before they were placed in petri-dishes containing germination paper. The germination paper was maintained moist throughout the study period.

The seeds for gibberellic acid treatment were soaked in 100 mL of GA_3 at 0 (distilled water), 50, 100 and 150 mg L^{-1} for 24 h before they were placed in petri-dishes containing germination paper. Similarly the seeds for ethephon treatment were soaked in 100 mL of ethephon at 0 (distilled water), 2000, 4000 or 6000 mg L^{-1} for 24 h before they were placed in petri-dishes containing germination paper. The pH of ethephon was neutralized to pH 8 using sodium hydroxide. The seeds for sulphuric acid treatment were soaked in 98% sulphuric acid for 10, 20 or 30 min as in trial 1.

Trial 4: Trial 4 was a repetition of trial 3, except hot water treatment was introduced and two-year old *Corchorus tridens* seeds were used. Corchorus seeds were soaked in simmering hot water at 98.5°C for 24 h as in trials 1 and 2. In all the trials seeds were kept at room temperature 26±5°C and relative humidity of 35-50%. In trials 1 and 2, seeds were observed every two days for a period of 8 days. In trials 3 and 4, seeds were observed for 6 days. The cumulative number of seeds that germinated was recorded. The germination paper was moistened with distilled water every 48 h. Data collected

was number of seeds that germinated after every 1 to 2 days for a period of 6 to 8 days. Germination here refers to the protrusion of the radicle.

Data analysis: Analysis of variance was performed on the data collected using the general linear models (Proc GLM) procedure of the statistical analysis system program package. Treatment means were separated using Tukey's Studentized Range Test (p=0.05). Procunivariate procedure was carried out on the residuals to support the assumptions of normality made by the researchers.

RESULTS

Treating Corchorus seeds with concentrated sulphuric acid (98%) for 10, 20 or 30 min significantly increased the germination of the seeds compared to the control (distilled water), hot water, nitric acid, gibberellic acid and ethephon (Table 1-4). In trial 1, treating one-year-old Corchorus seed with concentrated nitric acid (65%) for either 10, 20 or 30 min had no effect on seed germination after 2 days compared to control (Table 1). Sulphuric acid treatment after 2 days, significantly increased seed germination compared to control, hot water, nitric acid (Table 1), GA_3 and ethephon (Table 3 and 4). However, one-year-old seed soaked for 10 min in sulphuric acid significantly germinated more than those soaked in sulphuric acid for 20 or 30 min (Table 1 and 3). In trial 1, the seeds soaked in sulphuric acid for 20 min significantly germinated more than those in sulphuric acid for 30 min after 2, 4, 6 and 8 days, respectively (Table 1).

Gibberellic acid and ethephon had no effect on the germination of Corchorus seeds (Table 3 and 4). In trial 3, 1.5 days after treatment, sulphuric acid significantly increased the germination of Corchorus seed compared to control, gibberellic acid and ethephon (Table 3). There

Table 1: Effect of hot water, sulphuric acid and nitric acid on the germination of one-year-old *Corchorus tridens* seed (Trial 1)

Treatments	2 Days	4 Days	6 Days	8 Days
Distilled water	0.0e	0.3e	0.3e	0.3d
Hot water (98.5°c)	29.0c	42.0c	53.5c	58.0c
Sulphuric acid (98%):				
10 min	75.5a	98.3a	99.8a	99.8a
20 min	45.5b	80.8b	88.8b	88.5b
30 min	19.5b	33.8d	42.0d	53.0c
Nitric acid (65%)				
10 min	0.0e	0.3e	0.05e	0.8d
20 min	0.0e	0.0e	0.0e	0.3d
30 min	0.0e	0.5e	0.5e	0.5d
Significance	****	****	****	****
HSD	7.73	7.65	7.02	7.52

****; Significant at p=0.0001

Means separated by Tukey's studentized range (HSD) Test at p=0.05; means within columns followed by the same letter are not significantly different.

Table 2: Effect of hot water, sulphuric acid and nitric acid on germination of two-year-old *Corchorns tridcus* seed (Trial 2)

Treatments	2 Days	4 Days	6 Days	8 Days
Distilled water	1.0b	1.3b	1.3c	1.3c
Hot water (98.5°c)	33.5a	43.8a	46.8ab	47.8ab
Sulphuric acid (98%):				
10 min	41.3a	45.5a	49.8ab	51.0a
20 min	36.8a	39.8a	41.5ab	44.0b
30 min	30.5a	34.3a	34.8a	37.3b
Nitric acid (65%)				
10 min	0.0b	0.0b	0.0c	0.0c
20 min	0.5b	0.8b	0.8c	0.8c
30 min	0.0b	0.0b	0.0c	0.0c
Significance	****	****	****	****
HSD	11.4	12.1	13.2	12.8

****; Significant at p=0.0001

Means separated by Tukey's Studentized Range (HSD) Test at p=0.05; means within columns followed by the same letter(s) are not significantly different.

Table 3: Effect of various treatments on the germination of one-year-old Corchorus seed (Trial 3)

	Mean number of seeds that germinated out of 400 seeds			
Treatments	1.5 days	3.0 days	5.0 days	6.0 days
Distilled water	0.00b	0.75c	1.00c	1.25b
98% H_2SO_4 (min)				
10	27.75a	63.25a	96.75a	98.25a
20	24.25a	55.75ab	91.25b	96.75a
30	19.50a	49.50b	91.00b	96.75a
GA_3(mg L^{-1})				
50	0.00b	0.00c	1.00c	1.75b
100	0.00b	0.00c	1.50c	2.25b
150	0.00b	0.75c	0.75c	1.00b
Ethephon (mg L^{-1})				
2000	0.00b	0.00c	1.50c	1.75b
4000	0.50b	1.00c	0.75c	1.00b
6000	0.00b	0.25c	1.00c	1.25b
Significance	****	****	****	****
HSD	11.66	8.06	4.91	3.50

**** Significant at p=0.0001.

Means separated using Turkey's studentized range (HSD) test at p=0.05; means followed by the same letter(s) within columns are not significantly different.

Table 4: Effect of various treatments on the germination of two-year-old Corchorus seed (Trial 4)

	Mean number of seeds that germinated				
Treatments	2.0 days	3.0 days	4 days	5 days	6 days
0 (Water)	0.00d	0.00d	0.00c	0.00c	0.00c
Hot water	2.25cd	2.25cd	2.50c	2.50c	3.50c
98% H_2SO_4 (min)					
10	6.5ab	7.25ab	9.50b	10.75ab	13.00ab
20	4.75bc	5.50bc	6.00bc	6.00bc	7.50bc
30	9.50a	10.25a	16.25a	16.25a	18.75a
GA_3(mg L^{-1})					
50	0.00d	0.00d	0.00c	0.00c	0.00c
100	0.25d	0.25d	0.25c	0.25c	0.25c
150	0.00d	0.00d	0.00c	0.00c	0.00c
Ethephon (mg L^{-1})					
2000	0.00d	0.00d	0.00c	0.00c	0.00c
4000	0.25d	0.25d	0.25c	0.25c	0.25c
6000	0.25d	0.50d	0.50c	0.50c	0.50c
Significance	****	****	****	****	****
HSD	4.03	3.82	6.02	6.04	7.59

**** Significant at p=0.0001.

Means separated using Turkey's studentized range (HSD) Test at p=0.05; means followed by the same letter(s) within columns are not significantly different.

was no significant difference between seeds treated with sulphuric acid for 10, 20 or 30 min. After 3 days, still the seeds treated with sulphuric acid had significantly higher germination percentage compared to the control, gibberellic acid and ethephon (Table 3). However, after 3 days seeds treated with sulphuric acid for 10 min significantly increased the germination of Corchorus seeds compared to those treated with sulphuric acid for 30 min (Table 3). There was no significant difference between seeds treated with sulphuric acid for 10 and 20 min as well as those for 20 and 30 min, respectively, in trial 3 (Table 3). After 5 days, still the seeds treated with sulphuric acid had significant higher germination percentage compared to control, gibberellic acid and ethephon (Table 3). After 5 days, in experiment 3, seeds treated with sulphuric acid for 10 min significantly increased the germination of corchorus seeds compared with those treated with sulphuric acid for 20 or 30 min (Table 3). After 6 days, in experiment 3, seeds treated with sulphuric acid had high germination percentage compared to control, gibberellic acid and ethephon (Table 3). There was no statistical difference between seeds treated with gibberellic acid and ethephon (Table 3 and 4).

Corchorus seeds stored for 2 years resulted in a significant decrease in seed viability (Table 2 and 4). In trial 2, treating two-year-old Corchorus seeds with sulphuric acid and hot water significantly increased seed germination compared to distilled water and nitric acid in all the days of study (Table 2). Treating two-year-old Corchorus seeds with concentrated nitric acid (65%) for either 10, 20 or 30 min had no effect on seed germination compared to control (Table 2). Sulphuric acid and hot water treatment, after 2 days significantly increased seed germination compared to control and nitric acid, but there was no significant difference between these two treatments. There was no significant difference between two-year-old seeds soaked for 10 min in sulphuric acid and the ones soaked for 20 and 30 min after 2, 4, 6 and 8 days (Table 2). After 8 days, the seed treated with sulphuric acid for 10 min had significantly higher germination percentage than those treated with sulphuric acid for 30 min (Table 2).

In trial, sulphuric acid significantly increased the germination of 2 year old Corchorus seeds compared to control, hot water treatment, ethephon and gibberellic acid (Table 4). There was a significant difference between seeds treated with sulphuric acid for 20 and 30 min, respectively (Table 4). Hot water significantly increased seed germination compared to the control seeds, but lesser than sulphuric acid, except for seeds treated with sulphuric acid for 30 min (Table 4). After 3 days, still the seeds treated with sulphuric acid had significantly higher germination percentage compared to control, hot water, ethephon and gibberellic acid (Table 4). There were no significant differences between seeds treated with sulphuric acid for 10 and 20 min, but there were differences between seeds treated with sulphuric acid for 20 and 30 min (Table 4). After 4, 5 and 6 days, the seeds treated with sulphuric acid had significantly higher germination percentage compared to control, hot water treatment, ethephon and gibberellic acid (Table 4). There was no significant difference between 2 year old seeds treated with sulphuric acid for 10 and 20 min.

DISCUSSION

Dormancy is a condition where seeds will not germinate even when the environmental conditions (water, temperature and aeration) are permissive for germination[6]. Seed dormancy prevents immediate germination but also regulates the time, condition and place that germination will occur. In nature, different kinds of primary dormancy have evolved to aid the survival of the species by programming the time of germination for particular favorable times in the annual cycle[7,8]. In Botswana, due to the erratic, unreliable, semi-arid and desert conditions, most seeds of plant species develop one or two or more forms of dormancy as survival mechanism to preserve the plant species. Seed dormancy is an evolutionary adaptation to delay germination after the seed has been shed from the plant.

The results of the present study showed that treating Corchorus seed with concentrated sulphuric acid for 10, 20 or 30 min broke the type of seed dormancy in Corchorus seed. The results also showed that treating *Corchorus tridens* seed for more than 30 min significantly decreased the germination capacity of the seed. Hot water treatment also enhanced the germination of corchorus seed. This results showed that the type of seed dormancy in *Corchorus tridens* in Botswana is physical dormancy (seed coat dormancy). Seeds with physical dormancy fail to germinate because the seed is impermeable to water[6]. Physical dormancy is most often caused by a modification of seed covering, especially the outer integument layer of the seed, that may become hard, fibrous, or mucilaginous during dehydration and ripening. The seed coat hardens and becomes impervious to water. Germination can be induced by any method that can soften or scarify the seed coat. This implies that concentrated sulphuric acid and simmering hot water scarified the Corchorus seed and promoted water imbibition by the seed, hence the increased seed germination. Gibberellic acid and ethephon did not have an effect on germination capacity of Corchorus seed, implying that the type of seed dormancy

in *Corchorus tridens*, from Botswana is not physiological dormancy but physical dormancy.

Exposing the seeds for more than 10 min to 98% sulphuric acid, decreased significantly the germination capacity because sulphuric acid being corrosive might have damaged the embryos of some seeds suggesting that corchorus seeds should only be exposed to sulphuric acid for no more than 10 min. Nitric acid had no effect on seed germination of Corchorus seed, suggesting that nitric acid concentration used was not corrosive enough to break the hard seed coat dormancy.

These results showed that *Corchorus tridens* seeds stored for two years or more loose their viability by almost 50 to 91.5%, unless the 2 year-old seed required more time in acid. Experiments in Zimbabwe with *Corchorus tridens*, showed that 25-year old seeds had a germination percentage of 75%, whereas fresh seeds did not germinate well because of dormancy problem[4]. The results from Zimbabwe are contradictory with the present results, because the seeds stored for two years significantly lost their viability.

From the results of this study it can be concluded that sulphuric acid treatment and hot water treatment overcame the seed dormancy in *Corchorus tridens*. The results also showed that the type of seed dormancy in *Corchorus tridens* in Botswana is physical dormancy (seed coat dormancy) or impervious seed coat dormancy. The results further showed that Corchorus seed stored for two years or more loose their viability by 50 to 91.5%. Although sulphuric acid treatment significantly increased seed germination, the time for which seed should be soaked in the acid is important. Seed soaked for more than 10 min may lead to a decrease in germination because of embryo damage by the acid. Based on these results the authors recommend the use of sulphuric acid for breaking dormancy of *Corchorus tridens* seed in Botswana and the time for soaking the seed in sulphuric acid should be 10 min. The authors also recommend the use of hot water, as some farmers may not have access to or do not know how to handle sulphuric acid.

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