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Full Length Research Paper

Comparative evaluation of the germination capability of three morphologically different wild genotypes of *Corchorus olitorius* L. from Northern KwaZulu-Natal, South Africa

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***Corchorus olitorius* L. is a wild plant utilised as a vegetable in many parts of Africa. Wild indigenous vegetables have been recommended to alleviate nutritional deficiencies and household food insecurity. Efforts to domesticate and cultivate wild *C. olitorius* and other wild vegetables are being hampered by seed dormancy. The main cause of dormancy in *C. olitorius* has been suggested to be an impermeable seed coat. This study evaluates the response of wild genotypes *of C. olitorius* with different seed sizes to various dry heat and hot water treatments. Steeping seeds in boiling water (95°C) for 10 s and soaking seeds in a hot water bath at 80°C for 10 min resulted in a highest response to germination in the tested genotypes of this species. The study also recorded significant interactions between heat treatment and seed sizes. It was therefore concluded that genotypes of *C. olitorius* with different sizes require diverse durations of exposure to heat treatment methods to break dormancy caused by an impermeable seed coat.**

Key words: *Corchorus olitorius,* genotypes, germination, heat treatment, wild vegetables species.

INTRODUCTION

Wild *Corchorus olitorius* is an indigenous species in Africa and it is utilised as a vegetable in many parts of the continent. It is known by many different names but the most common are ‘Jews mallow’ and wild/bush okra (Palada and Chang, 2003). The species has been domesticated in West Africa for several decades and it is most popular among the western population of Nigeria as weaning soup for children and good delicacy for adults. It is mostly known as a wild vegetable in southern Africa; still mainly collected from the wild and eaten or preserved by sun drying and eaten as off-season vegetable during food scarcity in northern parts of South Africa. The increased utilisation of wild vegetables like wild *C. olitorius* in South Africa has been suggested as one of the ways to alleviate nutritional deficiencies and household food insecurity especially among populations with marginal or no income (Lewu and Mavengahama, 2010; Bharucha and Pretty, 2010; Ohdav *et al*., 2007; Jansen van Rensburg *et al*., 2007; Modi *et al*., 2006). Any effort to domesticate, commercialise and increase the yield of wild vegetables will depend on increasing the germination capabilities of the vegetables; as well as developing local seed supply systems in order to ensure reliable supplies of highly viable seed; as is the case with other domesticated crops. Uncertain availability of seed,

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variation in the quality of seed, lack of variety selection for uniformity of desired agronomic traits are some of the identified constraints to the cultivation of wild vegetables (Smith and Ezyaguirre, 2007).

Other studies indicated that several indigenous vegetable species have sub-optimal germination capabilities; thus hindering cultivation efforts (Velempini *et al*., 2003; Emongor *et al*., 2004; Modi, 2007). If the recent calls to promote the consumption of wild vegetables due to their superior nutritional content are not matched with increased propagation and cultivation, this could lead to an unsustainable increase in harvesting from the wild; an occurrence common with most species collected from wild population (Lewu *et al.,* 2007). Previous reports on the nutritional analysis of wild population of the species indicated that *C. olitorius* contained more nutritional qualities in terms of crude protein, iron, calcium and magnesium than cabbage and spinach purchased from public vegetable stores in South Africa (Ndlovu and Afolayan, 2008). There appear to be an upsurge in the collection of the species in the wild; and the influence of increased wild harvest is yet to be documented in South Africa. If the increase in consumption of this species is not matched with propagation and or cultivation, *C. olitorius* could end up with very low and unsustainable wild population or even go to extinction in South Africa. The cause of seed dormancy in *C. olitorius* has been suggested to be an impermeable seed coat (Emongor *et al*., 2004; Velempini *et al*., 2003). This might pose a challenge to propagation and cultivation initiatives. To achieve a successful commercial production of the species, there are several effective methods for breaking the type of dormancy (impermeable seed coat) in wild *C. olitorius*. Early reports documented very high germination percentages (between 80 and 90%) after subjecting seeds to sulphuric acid treatment (Velempini *et al*., 2003; Emongor *et al*., 2004) and variation in temperature (Nkomo and Kambizi, 2009). These studies may be more appropriate for large scale seed production systems where farmers can afford the cost of chemicals for scarification. However, few studies have documented the potential of solving hard seed coat dormancy in wild genotypes of *C. olitorius* especially among smallholder farmers in South Africa with limited financial resources and rudimentary knowledge of handling dangerous chemicals. With increasing population and rising level of poverty among this marginal income population, unsustainable harvesting of wild population of *C. olitorius* is inevitable; especially when the nutritional benefit of the species becomes increasingly known among the people.

Several methods, including heat treatment, chemical (acid) and mechanical scarification can be used to open the seed coat. For smallholder rural farmers, scarification by acids is inappropriate due to cost, stringent handling and sale policies/requirements of the chemicals due to their hazardous nature. Mechanical scarification like puncturing of seed coat with a needle is possible for varieties with large seeds; but is still tedious and impracticable for small seeded varieties like *C. olitorius* and large seed-lots. Heat treatment was selected as an appropriate method for evaluation in this research. The choice of heat treatment became necessary due to the need to identify a suitable method that can easily be used at a rural household system where laboratory heaters with controllable temperature baths and chemicals such as acids are not available. In this study heat treatment was separated into dry heat and hot water treatment. Therefore, the objective of this study was to identify the best and easiest heat treatment method that can be used by rural households of South Africa.

Materials and methods

The experimental design was a completely randomised design (CRD) with five replications. Fifty seeds were used per experimental unit and placed in Petri dishes lined with Whatman No 1 filter papers and moistened with sterile distilled water for the duration of the experiment. The Petri dishes were placed on the laboratory workbench at room temperature (25-28°C) for the duration of the study. Two factors were considered in the study which were; wild genotypes of *C. olitorius* (designated Co1, Co2 and Co3; Co being *Corchorus olitorius*) and heat treatment (T1 to T6) in a factorial combination, giving a total of 18 treatments as follows (Co1 xT1; Co1 x T2; Co1 x T3; Co1 x T4; Co1 x T5; Co1 x T6; Co2 x T1; Co2 x T2; Co2 x T3; Co2 x T4; Co2 x T5; Co2 x T6; Co3 x T1; Co3 x T2; Co3 x T3; Co3 x T4; Co3 x T5; Co3 x T6). Wild genotypes of *C. olitorius* were differentiated by visual assessment. Each genotype had distinct morphological differences with reference to leaf area, pod and seed sizes. Co1 had very small seeds, leaf area and pods. Co2 had medium pods, seed sizes and leaf area; while Co3 had large seeds, pod sizes and large leaf area. The levels of heat treatments include: T1 – seeds placed on filter paper moistened with distilled water in a Petri dish (control); T2 – seeds tied inside nylon cloth and steeped in boiling water for ten seconds and dried, prior to germination. T3 - seeds placed on a hot aluminium lid and placed over a bath of boiling water for 5 seconds. T4 - seed placed on an aluminium lid over a bath of boiling water for 10 seconds. T5 – seed placed on an aluminium lid over a bath of boiling water for 15 seconds. For each of the aluminium treatments, the seeds did not have direct contact with the hot water but were allowed to absorb heat while the aluminium lid serves as heat transfer medium at the stated time periods respectively. T6 – seed tied in nylon cloth and placed in a water bath maintained at 80°C for ten minutes. All treatments were placed on filter paper moistened with distilled water in a Petri dish for the duration of the experiment.

Measurements taken were germination percentage, number of days to final germination and the temperature for each heat treatment. The criterion for germination was a visible radicle emergence from the seed coat. Germination was recorded daily for 14 days and germinated seed was removed from the Petri dish.

Data collected were subjected to analysis of variance (ANOVA) using the Genstat Statistical package to test for significant treatment effects. Means were separated using Duncan's multiple range test (DMRT) (Steel *et al*., 1997). Before analysis, count data were converted to percentages. The percentage data ranged from 0 to 82 and were, therefore, arc sine transformed (Gomez and Gomez, 1984). Prior to transformation, zeros were replaced by (1/4*n*) where *n* is the number of units upon which the percentage

Table 1. Main effect of heat treatment on germination percentage of *Corchorus olitorius* from northern KwaZulu-Natal.

Heat treatment	Germination percentage (%) (arcsine transformed)	Germination percentage (%) (actual)
T1	12.04^b	6.13^b
T2	38.32^a	42.53^a
T3	2.87^c	0.80^c
T4	1.95^c	0.40^c
T5	2.69^c	0.67^c
T6	38.71^a	42.93^a

T1 – Seed placed on filter paper moistened with distilled water in a Petri dish (control); T2 – seed tied in nylon cloth and steeped in boiling water for 10 s and dried; T3- seed placed on a hot aluminium lid placed over a bath of boiling water for 5 s. T4 seed placed on the lid over a bath of boiling water for 10 s; T5 seed placed on aluminium lid over a bath of boiling water for 15 s; T6- seed tied in nylon cloth and placed in a water bath at 80℃ for 10 min. Means within the same column with different letters are significantly different at 5% level by Duncan's multiple range test (DMRT).

data was based. A denominator of 50 was used for computing the percentage in the present study (Gomez and Gomez, 1984).

RESULTS

There was a significant difference ($p<0.01$) in the germination percentage of the three genotypes, with genotype Co2 having the highest response to germination, whereas Co1 recorded the lowest germination percent. Heat treatment resulted in statistically significant ($p<0.01$) differences in germination (Table 1). The three dry heat treatments (T3, T4 and T5) resulted in lowest germination, dropping lower than the control. Steeping seeds for 10 min in water maintained at 80℃ (T6) resulted in numerically highest germination, although this was not statistically different from T2.

This study also showed significant interactions between heat treatments and the genotypes (Figure 1). The two hot water treatments T2 and T6 resulted in similar germination trends across all genotypes. Genotype Co2 recorded the highest level of germination, followed by Co3, with the lowest response to germination being Co1. The highest germination percentage for Co1 (20%) was achieved with the control. The three dry heat treatments resulted in significantly low percentage (<10%) germination for the three genotypes.

DISCUSSION

The three genotypes evaluated in this study were collected from the same natural population. The mother plants possess distinct morphological difference with reference to leaf area, fruit and seed sizes. Our findings suggest that wild *C. olitorius* seeds with different sizes may require diverse durations of exposure to hard seed coat dormancy-breaking treatments. Previous reports appeared to miss this important morphological seed difference when giving production guidelines and advice (Velempini et al., 2003; Palada and Chang, 2003; Emongor et al., 2004). The present result is supported by early observation that multiplication and propagation of *C. olitorius* is constrained by high level of morphotypes (Adebooye and Opabode, 2004). Similar result was also reported on agronomic requirements of *Cleome gynandra* "cat's whiskers" (Chigumira, 1995). There is need for morpho-genetic characterisation of wild populations of *C. olitorius* so as to develop distinct varieties. This will help to reduce the challenge of varietal differences and the mixing of genotypes during cultivation and propagation programmes.

Although treatments of wild *C. olitorius* seeds with acids like nitric and sulphuric acids resulted in very high germination percentages, however, these acids are very dangerous, require training in handling and may not be recommended in the smallholder farming conditions. Therefore, there is the need to develop other appropriate, simple and effective ways of breaking seed coat induced dormancy in wild *C. olitorius*. Heat treatment becomes an appropriate and handy method to circumvent hard seed-coat-induced dormancy in traditional households with limited skill in handling more advanced and dangerous scarification technologies. Steeping seeds in hot boiling water for 10 s achieved about 60% germination for the medium sized (Co2) seeds. Other studies on wild *C. olitorius* reported higher germination percentages (Velempini et al., 2003; Emongor et al., 2004) without reference to possible germination differences due to seed sizes. The current study emphasised this difference and further reinforce the importance of morpho-genetic variation in seed germination studies. Dry heat probably killed the seeds of genotype Co1 as there was almost zero percent germination for all the three dry heat treatments. For genotypes Co2 and Co3, dry heat may possibly not have been effective in breaking the dormancy as limited germination was observed. Genotype Co1 achieved the highest germination (20 %) without heat treatment, yet when hot water treatments (T2 and

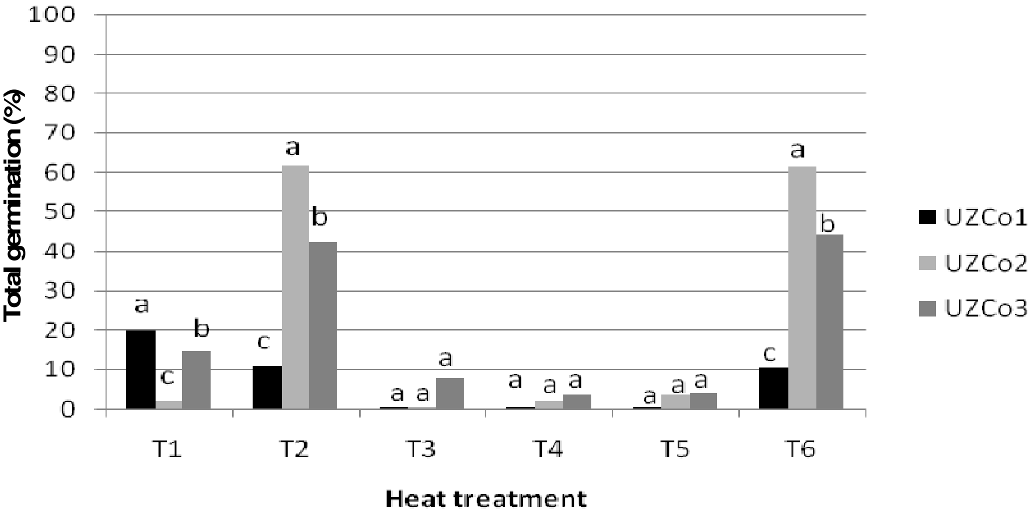

Figure 1. Interaction effects of heat treatment methods and genotypes on germination of *Corchorus olitorius* seed. T1 – Seed placed on filter paper moistened with distilled water in a Petri dish (control); T2 – seed tied in nylon cloth and steeped in boiling water for 10 s and dried; T3- seed placed on a hot aluminium lid placed over a bath of boiling water for 5 s; T4- seed placed on the lid over a bath of boiling water for 10 s; T5- seed placed on aluminium lid over a bath of boiling water for 15 s. T6 seed tied in nylon cloth and placed in a water bath at 80°C for 10 min. Co1- *C. olitorius* accession 1; Co2- *C. olitorius* accession 2; Co3- *C. olitorius* accession 3. Means at each heat treatment were separated by DMRT at 5% level of significance. Bars with different letters within the same cluster (T1 - T6) are significantly different at 5% level of significance.

T6), were applied, the germination percentage dropped by 50% (10%) and almost to zero percent for all the three dry heat treatments. Following the results of the current study, it is highly suggestive that heat treatment killed the very small seeds of genotype Co1.

Moreover, that some seeds of the three genotypes still germinated without heat treatment suggests that some portion of wild *C. olitorius* seeds were able to overcome hard seed coat dormancy without any treatment. The few seeds that are able to overcome imposed seed dormancy in wild populations of the species could be responsible for the natural regeneration of wild *C. olitorius* in its natural habitat. This observation also suggests that the natural regeneration capability of wild *C. olitorius* in its natural habitat is very low and may not support unsustainable harvest. In circumstances where the harvesting of the species from the wild increases, there will be need for farmers to collect seed and propagate the vegetables in home gardens or fields to prevent genetic erosion. The observation that the hot water treatments (T2 and T6) caused about 60% germination for the medium sized seeds and about 40% germination for the larger sized seeds indicates that more exposure period to hot water could increase the germination percentage of both the medium and large sized seeds. Without further empirical evidence, it would be difficult to draw any meaningful explanations for the extremely low germination of the medium and large sized seed with reference to the three dry heat treatments. However, the result indicates that the dry heat treatment was not effective against hard seed dormancy and may have actually killed the seeds.

Conclusion

Steeping medium to large sized seeds of wild *C. olitorius* in 80°C hot water for 10 min and in boiling water for 10 s resulted in the highest germination percent. Genotypes of wild *C. olitorius* with different seed sizes require different exposure time to heat treatment methods in order to break impermeable seed coat dormancy. However, there is a need for the characterisation of different wild *C. olitorius* varieties for further differentiation into their morphological classes.

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