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Enhancing Germination of Seeds of a Multipurpose Tree Species *Combretum molle*

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Abstract

Combretum molle R. Br. ex G. Don (Combretaceae) is a highly valued indigenous multipurpose tree species that has gained considerable attention in recent times due to its importance as a medicinal plant as well as an alternative species for wood and charcoal production. However, we lack adequate information on simple and appropriate methods that could be used to break its distinct seed dormancy to support domestication of wild populations apparently undergoing over exploitation and the negative impacts of deforestation. Eight pre-treatment methods involving complete removal of wing, complete removal of mesocarp, overnight soaking in cold water (24 °C) and partial removal of wing by scorching with fire, and their combinations were tested against the seed dormancy. Of all, the complete removal of mesocarp and overnight soaking in cold water followed with additional soaking in cold water for 24 h took the shortest imbibition (2 days) and germination (7 days) periods, highest germination value (76) and germination energy (80%). Similarly, the same treatment scored significantly ($P < 0.05$) higher cumulative germination of 72%. To obtain best germination traits, *C. molle* seeds should be pre-treated by removing the mesocarp and overnight soaking in cold water followed with additional soaking in cold water for 24 h, before sowing. These results have wide implications including supporting domestication and forest landscapes restoration in Tanzania.

Keywords: Domestication; Restoration; Indigenous species; Dormancy; Lesser known species

Introduction

Tanzania contains 48.1 million hectares of forest and woodland resources representing about 51% of the total land area (MNRT 2015). Despite its rich natural capital endowments, Tanzania like many other countries in the tropics is experiencing high rate of deforestation. In fact, according to the URT (2017) deforestation rate is 469,420 ha per annum. Deforestation has indeed contributed to a serious threat of extinction to some important indigenous tree species including *Karomia gigas*, *Pterocarpus angolensis*, *Prunus africana*, *Afzelia quanzensis*, *Pericopsis angolensis* and *Milicia excelsa*. This is not acceptable and Tanzania has put in place several mechanisms to reduce deforestation and ensure sustainable forest management. These mechanisms include setting aside almost 35% of total land as protected area, increasing budgetary allocation for restoration, improvements of policies and, adoption and implementation of several regional and global initiatives to combat deforestation including the Paris Agreement, African Forest

Landscape Restoration Initiative (Afr100) and Reducing Emissions from Deforestation and land Degradation (REDD+).

As these high valued indigenous tree species are becoming scarce there is an increase in price of wood and other products from those sources forcing users to look for equal substitutes from lesser known indigenous multipurpose tree species including *Combretum molle* R. Br. ex G. Don. Combretum *molle* (mlama or msana in Swahili) has a wide native distribution range (Mbuya et al. 1994, Orwa et al. 2009), grows relatively fast (Backeus et al. 2006) and coppices easily (Chidumayo 2013) it can therefore be included in afforestation/reforestation programmes as well as in strategies for adaptation and mitigation of the unequivocal negative impacts of climate change. The species is increasingly threatened due to over harvesting for wood (e.g. for making handles, building poles, stools and construction and fence posts), firewood and charcoal production (Luoga et al. 2000, Singo 2007). Most importantly, different parts of the tree are widely used traditionally to treat different human ailments including bacterial, viral and fungal infections as well as general body weaknesses. For example, roots are used to treat fever, constipation, headaches and general swelling and pains (Fyhrquist et al. 2002). The root and leaf together are believed to be antidote for snake bite. Leaves extracts are used to relieve pains from chest and act as anthelminthic against different types of worms (Orwa et al. 2009). Gum from the bark, crushed dried or fresh leaves are used for treating wounds because they contain several different antimicrobial compounds (Mogashoa et al. 2019). There is therefore a need to emphasize domestication of *C. molle* and other important lesser known tree species on the decreasing trend in the same way like the well-known indigenous ones and exotic tree species.

The increased demand for high valued indigenous tree seeds like those of *C. molle* by many individual tree growers, communities, institutions, non-governmental organizations and the government has created a need for critical examination of all channels of seed handling including seed collection, storage and germination to produce best planting materials for domestication and restoration (Msanga et al. 2018, MNRT 2021). Low germination rates and prolonged germination periods are among the important issues to deal with particularly for problematic tree species like *C. molle* with little known best methods to raise massive seedlings for successful large scale planting and domestication. As many other indigenous tree species, the major challenge of this species is its seed dormancy as well as its difficulty in handling (Mbuya et al. 1994, Msanga 1998). Thus, this study was carried out to identify the best simple and appropriate method(s) that would be suitable for optimal production of seedlings through breaking the seed dormancy. Knowledge generated from this study contributes to better understanding of efficient and practical methods for propagation of *C. molle* in large quantities to bolster domestication and forest landscapes restoration programmes.

Materials and Methods

Seed collection and preparation

In this study, *C. molle* fruits were collected from Morogoro Fuelwood Plantation (MFP) in Tanzania. The plantation covers an area of about 500 ha and is found within the latitude 6°40'S and longitude 37°39'E at an altitude of 560 m.a.s.l. The area often experiences annual rainfall of around 800 mm. The plantation is located 15 km north of Morogoro Municipal and about 200 km west of Dar es Salaam. MFR has other indigenous tree species including *C. zeyheri*, *Kigelia africana*, *Xeroderis stuhlmannii* and *Vachellia tortilis* and was a good source of indigenous tree seed collections (Singo 2007). Collection of *C. molle* fruits for this study was done before the short rains in December. Fruits collection was done by climbing matured healthy trees and shaking vigorously to release fruits. At least 15 trees were sampled in the field to obtain fruit lot for the study. Fallen fruits were collected in the tarpaulin to avoid cracking and contamination from the tree litter and soils.

Good fruits (i.e. fruits without damage or malformation) were separated from chaffs and other debris using winnowing techniques to reduce volume and weight. The collected fruits were packed and labelled properly including information on the collector, location and date of collection. These were then transported to the Directorate of Tree Seed Production (DTSP) Laboratories, Tanzania Forest Services Agency in Morogoro, Tanzania.

Fruits were left to dry under the house shade for 14 days to allow natural opening of the capsules. Seeds were extracted by shaking the capsules using hands and cleaned by hands to remove debris. Seed cleaning was done by removing all the impurities manually while ensuring that no damage is done on the seeds. After cleaning, seeds were placed on the sun to dry. Formal seed tests like moisture content, purity test and viable seeds per kg were conducted before taking the required sample for germination experiment (ISTA 2009). Samples for use in moisture content determination were prepared using a grinder then dried in the oven at 103 ± 2 ºC for 17 ± 1 hour. At the end of the prescribed period containers with the samples were placed in desiccators to cool for 30-40 minutes then reweighted. Computation of moisture content was done using the following formula: Mc = (ODW/OW) x 100; where Mc = Moisture content (%), ODW = Oven dry weight (g) and OW = Original weight (g) (ISTA 2009).

Germination experiment

The experiment was laid out in a Randomized Complete Block Design (RCBD) replicated four times with 8 treatments in the laboratory: complete removal of wing (T1), complete removal of wing and mesocarp (T2), complete removal of wing and overnight soaking in cold water (24 °C) followed with additional soaking for 24 h (T3), complete removal of mesocarp and overnight soaking in cold water followed with additional soaking for 24 h (T4), overnight soaking in cold water without the removal of the wing with additional soaking for 24 h (T5), partial removal of wing by scorching with fire (T6), partial removal of wing by scorching with fire and overnight soaking in cold water (T7) and control (T8) (Table 1). These methods have shown to be effective for breaking dormancy in other Combretaceae species in African savanna landscape (Mbuya et al. 1994, Msanga 1998).

Table 1: Overview of pre-treatment methods used in the germination study of *Combretum molle* seeds

Treatments	Complete removal of wing	Complete removal of mesocarp	Overnight soaking in cold water	Additional soaking in cold water for 24 h	Partial removal of wing by scorching with fire
1	√				
2	√	√			
3	√		√	√	
4		√	√	√	
5			√	√	
6					√
7			√		√
8 (control)					

River sand (previously washed to remove silt and organic matter) was moistened and placed on plastic trays each with the size of 15 cm x 15 cm. Twenty seeds were distributed equally on the trays while ensuring that they do not touch each other in order to discourage the spread of fungal mould (Willan 1985). A layer of sand equal to the width of the seed was spread uniformly on top of the seeds. Water was applied

manually to maintain moist medium at all times. Mean temperature and humidity inside the laboratory were 26 ± 0.6 °C and 80 ± 5%, respectively. Daily and cumulative germinations were recorded until no further germination sign could be observed. The procedure for recording germination was to allow viable seed to emerge on the sand surface showing at least 10 mm of its cotyledons and hypocotyls (ISTA 2009). Imbibition period which is the number of days from sowing to commencement of germination was also recorded. In addition, the total germination period defined as number of days from sowing to completion was also recorded.

Germinated seeds were expressed as percentage of all seeds in each tray. Cumulative germination was obtained by summing up all germinated seeds starting from day one to the termination of the experiment. Number of germinated seeds was expressed as percentage of all seeds sown per treatment. Germination energy was determined by counting all germinated seed per treatment after terminating the experiment. Imbibition period was obtained by counting the number of days from sowing to commencement of germination. Total germination period was a period (in days) since when first seedling started to emerge on the surface to the last day when last seedling emerged. Germination value (GV) was calculated in order to obtain value of expected number of seedlings from sowing as

$$GV = \left(\frac{\sum DGS}{N} \right) \frac{GP}{10};$$

where GV = Germination value, GP = Germination percent at the end of the test, DGS = Daily germination speed obtained by dividing cumulative germination percent by the number of days since sowing, $\sum DGS$ = Cumulative daily germination speed, N = Frequency number of DGS calculated during the experiment and 10 = constant (Djavanshir and Pourbeick 1996).

Before data analysis, visual inspection and significance test were conducted to check for standard parametric statistical assumptions. Visual inspection was done by plotting of residuals against normal scores and predicted values whereas significance test was done using Shapiro Wilks's test. All percentage values, e.g. daily germination percentage, cumulative germination percentage and germination energy percentage were arcsine transformed to remove bias, stabilize variance and improve distribution before the analysis (Sokal and Rohlf 1995). However, the actual values are presented in figures for clarity. Means and standard errors for both daily and cumulative percentages were calculated and used to measure the variability between treatments. To compare differences in mean daily germination and mean cumulative germination percentages among different treatments, one-way Analysis of Variance (ANOVA) was used. Significant means between treatments were separated using Duncan's Multiple Range Test (DMRT) at significance level of $P < 0.05$ (Sokal and Rohlf 1995). Statistical analysis was carried out using SAS (Statistical Analysis Systems Institute, Inc. 1999).

Results

Seed moisture content, purity and viability

In this study, the average moisture content of seeds at the time of sowing was 10.7% (±0.12 SD) whereas seed purity was 99.9% (±0.1) and seed viability 89% (±0.13). The seeds exhibited epigeal germination meaning that the radicle emerges from the seed and the hypocotyl elongates raising the cotyledons, epicotyl and remains of the seed coat above the ground. The hypocotyledons were short with two leafy cotyledons. On exposure to light, they changed colour from white to yellowish and then green.

Daily and cumulative germinations

There were significant differences ($P < 0.5$) in mean daily germination percentages among treatments, and the days after sowing (Table 2). The complete removal of mesocarp and overnight soaking in cold water followed with additional soaking in cold water for 24 h (T4) had the shortest imbibition period (2 days), while the longest period (12 days) was attained when seeds were overnight soaked in

cold water without the removal of the wing-T5 (Figure 1). No seeds were germinated following overnight soaking in cold water without the removal of the wings (T5) during the first 10 days (Table 2).

Table 2: Average values for daily germination percentages of *Combretum molle* seeds

Treatments[1]	Number of days after sowing					
	5	6	7	8	9	10
1	0	0	11.7^{a} (8.4)	28.5^{a} (2.3)	12.8^{b} (1.2)	11.7^{a} (4.8)
2	0	0	2.9^{ab} (2.9)	27.9^{a} (1.8)	22.2^{a} (3.4)	2.9^{b} (2.9)
3	0	0	7.0^{ab} (4.2)	12.8^{b} (1.2)	26.3^{a} (2.4)	7.0^{bc} (4.2)
4	17.8 (7.1)	20.6 (7.0)	2.9^{ab} (2.9)	10.1^{c} (5.0)	2.9^{d} (2.9)	2.9^{bc} (2.9)
$\bar{6}$	$\hat{0}$	$\hat{0}$	$\hat{1}1.1^{ab}$ (0.4)	$\hat{5}.1^{bc}$ (5.0)	$\hat{5}.8^{bc}$ (3.3)	$\hat{1}1.1^{a}$ (3.9)
7	0	0	0	0	7.0^{bc} (2.3)	0
8	0	0	8.2^{ab} (4.7)	0	9.9^{c} (2.4)	8.2^{b} (4.5)

[1]Means of four replicates followed by standard deviations in parenthesis; within each category means in the same column followed by the same letters are not statistically different at $P < 0.05$ according to DMRT. Details of methods are shown in the methodology.

The effective germination period for *C. molle* was considered to be 10 days since the commencement of the experiments. After this period, germination was found to be of no economic importance because all emerging seedlings were too weak and ended up dying (Figure 1). The complete removal of wing (T1), complete removal of wing and mesocarp (T1) and the removal of wing and overnight soaking in cold water (T3) all took six days for seeds to start germinating (Figure 1).

There were significant differences ($P < 0.05$) between different treatments in mean cumulative germination percentage within the study period of 17 days (Table 3). Soaking of seeds in cold water without the removal of the wing started germinating from day 12 (Table 3). All treatments attained maximum cumulative germinations within 13 days since the commencement of the experiment. At day 17 un-germinated seeds were found dead and rotten hence the experiments were terminated (Figure 1).

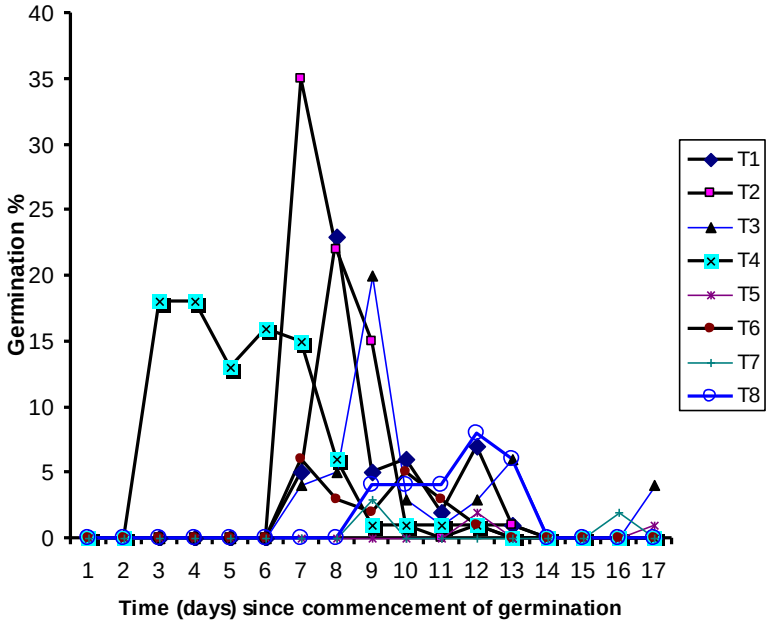

Figure 1: Daily germination percentages of *Combretum molle* seeds subjected to different dormancy breaking methods.

Table 3: Average values for cumulative germination percentages of *Combretum molle* seeds subjected to different dormancy breaking methods

Treatments[1]	Number of days after sowing			
	6	10	13	**17**
1	0	38.0^{c} (1.2)	44.4^{c} (2.4)	44.4^{c} (2.4)
2	0	58.8^{b} (1.7)	60.1^{b} (1.3)	60.1^{b} (1.3)
3	0	34.2^{c} (3.1)	40.4^{c} (2.4)	42.7^{c} (2.0)
4	34.2 (5.3)	70.5^{a} (3.4)	72.1^{a} (2.6)	72.1^{a} (2.6)
5	0	0	5.8^{e} (3.3)	7.0^{e} (4.2)
6	0	23.2^{d} (2.7)	26.0^{d} (3.8)	26.0^{d} (3.8)
7	0	7.0^{e} (4.2)	7.0^{e} (4.2)	11.1^{e} (3.9)
8	0	16.2^{d} (1.8)	30.5^{d} (2.6)	30.5^{d} (2.6)

[1]Means of four replicates followed by standard deviations in parenthesis; within each category means in the same column followed by the same letters are not statistically different at $P < 0.05$ according to DMRT. Details of methods are shown in the methodology.

Complete removal of wing followed with overnight soaking in cold water (T4) and the complete removal of wing and mesocarp (T2) resulted in the highest cumulative germination rates (Figure 2). Germination levelled off after 13 days for T1, T2, T4, T6 and T8, while seeds from other treatments continued to germinate until the end of the experiment although with low numbers (Figure 2). Soaking of seeds in water without the removal of wing (T5) and partial removal of wing by scorching with fire and overnight soaking in cold water (T7) were the least treatments in yielding mean cumulative germination percentages (Figure 2).

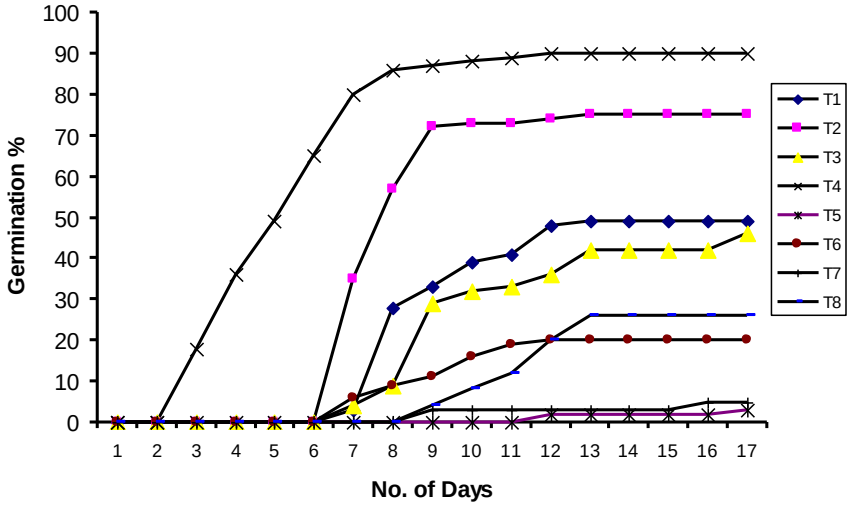

Figure 2: Cumulative germination of *Combretum molle* seeds subjected to different dormancy breaking methods.

Other germination traits

Table 3 presents results of other germination traits whereby mean cumulative germination percentage differed significantly ($P < 0.05$) among the studied treatments. Mean cumulative germination percentage did not differ significantly ($P > 0.05$) between the complete removal of wing (T1) and removal of wing and overnight soaking in cold water followed with additional soaking in water for 24 h (Table 4). Similarly, partial removal of wing by scorching with fire (T6) and the control (T8) were not significantly different ($P > 0.05$) in mean cumulative germination percentage. Complete removal of mesocarp and overnight soaking in cold water (T4) resulted in the highest germination energy of 80%, while complete removal of wing and mesocarp without soaking in cold water (T2) resulted in germination energy of 57% (Table 4). The lowest germination energy was obtained by overnight soaking in cold water without removal of the wing (T5) followed by partial removal of wing by scorching in fire and overnight soaking in cold water (T7) with 2% and 3%, respectively (Table 4). Again, complete removal of mesocarp and overnight soaking in cold water (T4) provided the highest germination value of almost 76 followed by complete removal of wing and mesocarp without soaking (T2) with germination value of 31.4 (Table 4). The longest germination period was observed in untreated seeds (T8) followed by overnight soaking in cold water without the removal of the wing-T5 (Table 4).

Table 4: Other germination traits of *Combretum molle* seeds subjected to different dormancy breaking methods

Treatments[1]	Imbibition period (days)	Cumulative germination (%)	Germination energy (%)	Germination period (days)	Germination value
T1	6	44.4^{c} (2.4)	33^{c} (2.1)	9 (1.1)	10.2 (1.2)
T2	6	60.1^{b} (1.3)	57^{b} (1.5)	8 (1.3)	31.4 (2.3)
T3	6	42.7^{c} (2.0)	32^{c} (1.9)	10 (2.2)	6.6 (1.1)
T4	2	72.1^{a} (2.6)	80^{a} (2.7)	7 (1.3)	75.9 (2.8)
T5	12	7.0^{e} (4.2)	2^{f} (0.1)	12 (2.6)	0.01 (0)
T6	7	26.0^{d} (3.8)	9^{e} (0.7)	8 (1.2)	1.8 (0.4)
T7	7	11.1^{e} (3.9)	3^{f} (0.1)	9 (1.8)	0.04 (0)
T8	8	30.5^{d} (2.6)	26^{d} (2.4)	13 (2.4)	1.4 (0.1)

[1]Means of four replicates followed by standard deviations in parenthesis; within each category means in the same column followed by the same letters are not statistically different at $P < 0.05$ according to DMRT. Details of methods are shown in the methodology.

Discussion

In this study, the seeds examined have shown to have low moisture content and good capacity to maintain viability in laboratory conditions confirming that indeed *C. molle* seeds are orthodox (Katende et al. 1995). Only a few untreated individuals were able to germinate in the control experiment despite the high overall viability of seeds (89%). Failure or low germination rate observed in untreated seeds suggests that *C. molle* has physical seed dormancy caused by seed coat (Msanga 1998). The high viability of seeds confirms also that the low germination in control treatment was not caused by natural mortality but rather the dormancy like in many other indigenous tree species in woody grassland and semi-arid areas of tropical savanna (Chidumayo 1997). Dormancy is a state which allows species to delay germination until when favourable conditions for optimal growth and establishment are available. So, dormancy allows seeds of *C. molle* to pass over the dry conditions often associated with annual wild fires. The dry conditions are usually followed with short rains which then stimulate germination and promotes seedlings emergence (Backeus et al. 2006, Chidumayo 2013).

Seeds of *C. molle* exhibited different germination rates when subjected to different treatments under the laboratory conditions. This is an indication that each treatment has different effect on the study seeds. However, the best germination results were obtained with the complete removal of mesocarp and overnight soaking in cold water followed with additional soaking in cold water for 24 h. Similar results were recorded in *C. zeyheri* (Msanga 1998) confirming that species within the same genus share certain physiological traits (Zobel and Talbert 1984).

Minimum imbibition period observed in this treatment might have been caused by the

rapid absorption of water which resulted into effective absorption of water into the seed embryo hence shortened the imbibition period (Chamshama 2001). Seed coat acts as a physical barrier preventing water penetration to the seed embryo in *C. molle*. So, by complete removal of mesocarp and overnight soaking in cold water the seed coat is softened allowing water to penetrate into the seed embryo and initiate the germination process. The other probable explanations could be that soaking might have also contributed partly to higher germination rate through minimizing chemical inhibitors which can delay or prevent seed germination (Lars 2000, Andrew et al. 2008). The minimum imbibition period observed in the pre-treatment by removal of mesocarp might have also in addition been caused the absence of mesocarp to allow enough absorption of water by seed embryo hence speeding up the germination of *C. molle*.

Low germination rate observed in seeds pre-treated by partial removal of wing through scorching the seed with fire might have been caused by the damage which killed the seed embryo before germination. This is attested by low numbers of regenerants in the Miombo woodland where fire is frequent and common (Chidumayo 1997, Backeus et al. 2006). Fire has been reported to enhance germination of certain Miombo tree species (Dallu 2002). However, results obtained from the pre-treatment of *C. molle* seeds by scorching with fire provided different results. This suggests that some Miombo tree seeds like *C. molle* seeds cannot tolerate certain levels of fire beyond the threshold (Chidumayo 1997). No wonder *C. molle* is often found on termite hills and evergreen forests to avoid harsh conditions like those imposed by relatively high intensity fires and water logging (Orwa et al. 2009). This is also manifested by the ecology of *C. molle* which is a dry season fruiting tree species in Southern Africa. It usually releases its fruits after fire when the ground is more or less bare and is easy for wind dispersal (Chamshama 2001, Dallu 2002).

Poor germination results obtained from overnight soaking of seeds in cold water without the removal of the wing might have been due to the failure of the plumule and radicle to rupture the seed coat fast enough to release the growing parts hence causing premature death of the seedling before emerging from the germination media (Chisha-Kasumu et al. 2007, Andrew et al. 2008). Germination capacity was similar to germination percent because there were no viable seeds found after the germination period. The highest germination value obtained by complete removal of mesocarp and overnight soaking in cold water and by complete removal of wing and mesocarp suggests that mesocarp of *C. molle* seed must be removed in order to obtain good germination responses (Katende et al. 1995, Orwa et al. 2009). It is known that dormancy is controlled by a variety of genes which determine the level it is present in seeds (Carvalho and Nakagawa 2000). Therefore, the few seeds that germinated from the control treatment are likely to possess no or low levels of dormancy in this study.

Conclusions

This study sought to determine simple methods for raising massive seedlings of socio-economically important indigenous multipurpose tree species *C. molle* through breaking the seed dormancy to support domestication and on-going restoration efforts. Results have revealed that indeed C. *molle* seed has a physical dormancy due to seed coat which acts a barrier for water absorption to stimulate germination. The study species has been able to germinate well within a short period of time with minimum efforts, low cost and without specialized training indicating high potential for inclusion in massive seedling production under managed nurseries systems. Germination is an important trait that determines the quality of a propagule, early performance (e.g. in survival and growth) and end product. Of all the treatments, complete removal of mesocarp and overnight soaking in cold water followed with additional soaking in cold water for 24 h provided the best results. This treatment has provided short germination period and high values for

cumulative germination percentage, germination value and germination energy. Therefore, for an efficient germination of *C. molle* seeds, the mesocarp should be completely removed and overnight soaked in cold water followed with additional soaking in cold water for 24 h, before sowing. These results have wide implications including supporting domestication of *C. molle* from the wild and on-going forest landscapes restoration efforts in Tanzania.

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