

Variations in *Cupressus lusitanica* Mill. Seed Yield and Quality under Different Extraction Conditions in Kenya

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Abstract: Seed demand for *Cupressus lusitanica* seed has been increasing to meet the myriad uses for the species. It is grown in Kenya majorly as a commercial plantation species. This necessitated the need to enhance seed extraction for sustainable availability of quality seed. Therefore this study's objective was understanding the variations of *C. lusitanica* seed yield and quality under different extraction conditions. Five hundred and forty mature cones were collected and subjected to different soaking treatments (SH-soaked in hot water and SC-soaked in cold water) to mimic the current practice (NS-not soaked) and suggest ways for improvement. Seed yield performance was compared across different extraction temperatures current practice-drying bed (DB), greenhouse(GH) against extraction temperatures 40°C, 50°C, 65°C, and 85°C, within a 48 hour period. Germination tests were conducted to validate seed quality under different extraction treatments. The key finding from this study was that cones pretreated by soaking in cold water and exposed to 85°C for 48 hours yielded a high number of seeds (189±9.3) and with the highest germination percent (15%, $p < 0.05$). This finding allows for the design of a low energy-intensive method using cold water and higher temperatures for a short duration for increased seed yield while maintaining quality.

Keywords: cone pretreatment, *Cupressus lusitanica*, extraction temperature, germination, seed yield

1. INTRODUCTION

Globally, *Cupressus lusitanica* is one of the important fast-growing plantation species, highly preferred for biomass, fuelwood, timber, and medicinal uses (1–5). In the tropics and subtropics, this species has been identified and prioritized for planting to support commercial forestry, afforestation, and conservation (6–10). In Kenya this is an exotic species planted commercially for the production of saw timber, plywood, and poles for building and construction; and for services such as live fence, shade, and ornamental (11–14). These uses have led to increasing demand for *C. lusitanica* seed for ease of propagation (15,16). Seeds have been the traditional and primary source of *C. lusitanica* propagules (17–20).

In order to meet the global seed demand, *C. lusitanica* seeds are extracted from cones using myriad ways (21,22). There is scanty literature focusing on cone gathering and seed harvesting practices targeting cypress, whereby, seed release and seed germination are greatly influenced by the environment (temperature and moisture) (23–25). Cypress cones sizes have been previously shown to have a positive correlation with seed yield but a gap exists in the influence of moisture and temperature on seed extraction (23,26,27). Seed counting has been used conventionally as a method of quantifying seed yield in various coniferous species (25,27–29). Improvement of seed extraction techniques in conifers has contributed to the changes in seed handling processes and the overall seed quality assessed by germination capacity (30–33).

The present study seeks to analyze the effects of extraction pretreatments, site conditions, and temperature on seed yield and quality of *C. lusitanica*. The specific objectives were (i) To determine the effect of soaking of cones and temperature duration on seed release, (ii) To determine the effects of extraction temperature and duration on seed release (iii) To determine the interactions between soaking pretreatments, extraction temperature and duration on seed release and (iv) To determine the interactions between soaking pretreatments, extraction temperature and duration on germination of *C. lusitanica* seeds

2. MATERIALS AND METHODS

Cupressus lusitanica cones were collected from a clonal seed orchard in Londiani within the Rift Valley region of Kenya. The area is located at latitude 0° 10' South and longitude 35° 36' E and also at an elevation of 2,320 to 2,500 m above sea level. The annual precipitation ranges from 1,000 to 1,500 mm. The temperature ranges between 14°C and 17°C with an average of 15.7°C. This area has a cool and moist climate, which is suitable for *C. lusitanica* seed production (34).

Mature cones were collected in June 2020, from a 14-year-old *C. lusitanica* seed orchard. The cones were closed at that time (35). The cones were packed in gunny bags and were brought to the Rift Valley Eco-region Research Programme laboratory, Kenya Forestry Research Institute (KEFRI).

Five hundred and forty cones were randomly selected as described by Aniszewska, 2013, and grouped in batches three groups of 180 each. The first group of cones not to be soaked (NS) was used as the control. The second group (180 cones) consisted of cones to be subjected to soaking in cold water (SC) while the third group (180 cones) was of cones to be subjected to soaking in hot water (SH). Further, in setting up for the temperature extraction experiment, the three groups (NS, SC, and SH) of cones were subdivided into 6 temperature categories (GH, DB, 40°C, 50°C, 65°C, and 85°C) of 30 cones each then labeled (Table 1) (37). Greenhouse (GH) and seed drying bed (DB) are infrastructures currently used for seed extraction in Londiani, Kenya. The temperature and humidity during this study were recorded using a data logger. The other experimental temperatures (40°C, 50°C, 65°C, and 85°C) were achieved using an oven (YAMATO DS411). Coldwater soaking involved immersion of cones in a water bath for 10 minutes while soaking in hot water involved immersion of cones for 10 minutes in water that had been heated to boiling point 100°C (38).

Table 1. Seed extraction by temperature regimes (GH, DB, 40°C, 50°C, 65°C, and 85°C), cone pretreatment (NS, SH & SC) and the number of cones per treatment.

S/No	Temperature	Treatment	No of cones
1.	DB (49±3.7°C)	NS	30
2.		SH	30
3.		SC	30
4.	GH (34.9±12.0°C)	NS	30
5.		SH	30
6.		SC	30
7.	40°C	NS	30
8.		SH	30
9.		SC	30
10.	50°C	NS	30
11.		SH	30
12.		SC	30
13.	65°C	NS	30
14.		SH	30
15.		SC	30
16.	85°C	NS	30
17.		SH	30
18.		SC	30
Total			540

DB= Drying bed, GH= Greenhouse, NS=Not soaked, SC=Soaked in cold water, SH = soaked in hot water.

After pretreatment (NS, SH, and SC), cones were put in marked glass Petri dishes and subjected to extraction under the different conditions (DB, GH, 40 °C, 50 °C, 65 °C, 85 °C) for up to 48 hours. Observations for seed release and seed counts per cone were done at 24 hours and at 48 hours. Seeds that had not been released from open cones were forcefully removed manually using forceps to account for seeds that were retained in a cone and labeled as 48F (48hours forced).

Germination tests were conducted for seed released after 24 hr and seeds released after 48 hr under the 6 temperature conditions (DB, GH, 40 °C, 50 °C, 65 °C, 85 °C). Four replicates of 50 seeds each from each of the 18 categories were placed in Petri dishes lined with wet cotton wool (distilled water) and placed in the cultivation chamber (TOMY CFH-415) at 27°C and 70%RH. The number of germinated

seeds was recorded daily for up to 30 days. Radicle emergence was taken as the criterion for germinability (39). Seed released were presented as mean counts in tabular and graphical forms by soaking conditions (NS, SC, SH) and extraction temperature (DB, GH, 40 °C, 50 °C, 65 °C, 85 °C). A comparison of performance in seed extraction was done statistically by probability (R software) and Tukey HSD performed to determine the soaking conditions and temperature ideal for most seed yields. Germination percentage was done for different soaking and extraction temperatures to determine the treatment with the highest performance in quality ($p < 0.05$).

3. RESULTS AND DISCUSSION

Within the first 24 hours, cones that were soaked in cold water had released up to 77% of seeds from the cone. After 48 hours this study observed that cones soaked in cold water (SC) had the highest mean number of seeds released (278 ± 2.7 , $p < 0.05$) accounting for 90% of the seed in a cone with only 10% retained in the cone. The current practice of *C. lusitanica* seed extraction without soaking the cones yields 61% (55 ± 2.1 , $p < 0.05$) with 39% being retained in the cone after 48 hours (Table 2). In this study, soaking of the cones and extracting seeds after 48 hours showed an increased yield of 90% (229 ± 2.2 , $p < 0.05$). The mean number of seeds released from the 540 cones after the soaking treatment was 171 ± 2.1 ($p < 0.05$) and the mean number of seeds retained in a cone was 29 ± 1.2 ($p < 0.05$) (Table 2). Studies on cypress cone processing have focused on soaking and non-soaking practices. The key observation in regard to the soaking effect was that soaking in cold water (SC) in this study, had a higher seed yield than cones that were not soaked (18,40,41). This finding refined Belcher and Karrfalt, (1977) study whose observations on cypress cone opening suggested 10 seconds soaking in hot water and soaking in cool water improved seed yield by 20-50% without specifying the exposure. This study showed that the combined effect of soaking cypress cones (SC + SH), prior to extraction resulted in 90% seed yield compared to the current practice of not soaking (NS) which yielded 61% (Table 2). Soaking has been shown to have an effect on the serotiny of cypress cones and the retention of seeds (41). This study observed that the cones that were not soaked (NS) retained the highest number of seeds after 48 hours (39%) that had to be forced out. This study, therefore, agrees that soaking had a positive effect on seed release from *C. lusitanica* cones. Thus, soaking as a pretreatment reduces the extraction time while maintaining a higher seed yield.

Table 2. Mean seed counts after exposure 24 hours, 48 hours, and forcing after 48 hours (48F), from each treatment (NS-not soaked, SH-soaked in hot water and SC-soaked in cold water) with standard error, and percent of total seed released by exposure and cumulative percent of seed released at the end of 48 hours including the forced out seeds.

Treatment	Duration (hrs)	Mean cumulative number of seeds	SE	Percent of seed released (%)	Percent of cumulative seed released (%)
NS	24	19	1.7	21	
SH	24	136	2.0	67	
SC	24	236	2.5	77	
NS	48	55	2.1	39	61
SH	48	180	1.6	22	90
SC	48	278	2.7	14	90
NS	48F	91	1.1	39	39
SH	48F	201	0.9	10	10
SC	48F	307	1.5	10	10

The totals for each treatment (NS, SH, and SC) in the percent columns = 100% per treatment per column ($n=540$)

This study observed that the lower temperature for extraction (DB, GH, and 40 °C) yielded 58% of seed at the end of 24 hours. The higher extraction temperatures (50 °C, 65 °C, and 85 °C) had the highest mean cumulative seed yield of 153 ± 10.1 , 149 ± 10.6 and 145 ± 9.6 respectively at 24 hours. This accounted for 71% and 73% of the available seed from the cone (Table 3). After 48 hours the same higher extraction temperatures maintained the highest cumulative seed yield of 202 ± 10.2 , 192 ± 10.6 , and 189 ± 9.3 accounting for 93% and 95% of the available seed from the cones. The seeds that were retained and had to be forced out from the cones ranged from 5% to 27% for all the temperature treatments. The lower extraction temperatures (DB, GH, and 40 °C) showed above 23% seed retention after 48 hours (Table 3). Higher temperatures have been shown to have an effect on serotiny of cypress cones and the retention of seeds though lacking information on exact temperatures preferred (41,42).

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Table 3. Mean seed counts after exposure 24 hours, 48 hours and forcing after 48 hours (48F), from each temperature treatment (DB-Drying bed, GH-Green house, 40°C, 50°C, 65°C, and 85°C) with standard error, and percent of total seed released by exposure and cumulative percent of seed released at the end of 48 hours including the forced out seeds.

Treatment	Duration (hrs)	Mean cumulative number of seeds	SE	Percent of seed released (%)	Percent of cumulative seed released (%)
DB	24	103	8.8	58	
GH	24	120	9.7	58	
40°C	24	111	8.6	58	
50°C	24	153	10.1	71	
65°C	24	149	10.6	73	
85°C	24	145	9.6	73	
DB	48	130	9.2	15	73
GH	48	159	9.5	19	77
40°C	48	155	9.7	23	81
50°C	48	202	10.2	22	93
65°C	48	192	10.6	21	93
85°C	48	189	9.3	22	95
DB	48F	179	9.0	27	27
GH	48F	206	8.7	23	23
40°C	48F	191	9.5	19	19
50°C	48F	218	10.0	7	7
65°C	48F	206	10.0	7	7
85°C	48F	199	9.2	5	5

The totals for each temperature treatment (DB, GH, 40°C, 50°C, 65°C, and 85°C) and exposure (24hrs, 48hrs, and 48F in the percent columns = 100% per treatment per column (n=540)

This study similarly observed that higher temperatures (50°C, 65°C, and 85°C) resulted in the highest seed yield at both 24 and 48 hours of exposure. The same higher temperatures caused the cones to only retain 5% to 7% of seed that had to be forced out after 48 hours. The present study observed no significant differences ($P < 0.05$) in seed yield extracted at 50°C, 65°C, and 85°C for the *C. lusitanica* cones. Observations from this study showed that for the higher temperatures, the first 24 hours had significantly higher seed yield than the next 24 hours (48 hours) which agreed with other studies (37,43).

The cones that were not soaked prior to extraction (NS) and subjected to lower extraction temperatures (DB, GH, and 40°C) yielded between 0 to 9 mean cumulative seed released after 24 hours (Table 4). The highest performing cones at 24 hours came from cones subjected to 50°C and 65°C after being soaked in cold water (SC) that yielded 264 ± 4.4 and 269 ± 5.2 respectively. These temperatures of 50°C and 65°C similarly performed best on cumulative seed released after 48 hours; 317 ± 1.3 and 316 ± 0.2 respectively, ($p < 0.05$). Cones that were soaked in cold water and exposed to 65°C had released 98.2% of the available seed in the cone (Table 4). In relation to seed retention in the cones, the lower extraction temperatures yielded a high variability ranging from 11.4% to 75.5%, contrasting with the lower variability of 1.8% to 21.4% observed in the higher extraction temperatures (50°C, 65°C, and 85°C) (Table 4). There are studies that have shown a combination of environmental factors could lead to increased seed yield from cypress cones (37,43). This study showed that the combined effect of soaking *C. lusitanica* cones in cold water and exposing it to a high temperature of 65°C for 48 hours yielded 98% of the seeds in a cone, refining the environmental factors required to attain this yield. This is important to seed production and designing the mechanism for this production to be achieved.

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Table4. Mean seed counts after treatment (NS-not soaked, SH-soaked in hot water and SC-soaked in cold water), each temperature treatment (DB-Drying bed, GH-Glass house, 40°C, 50°C, 65°C, and 85°C), and exposure 24 hours, 48 hours and forcing after 48 hours (48F), with standard error, and percent of total seed released by treatment, by temperature, by exposure and cumulative percent of seed released at the end of 48 hours including the forced out seeds.

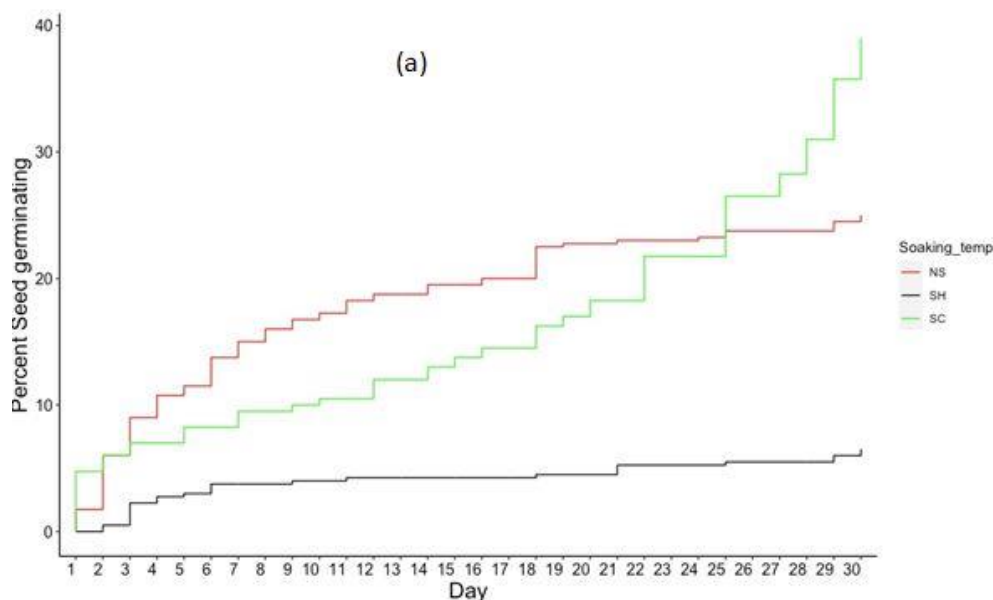
Treatment	Temperature	Duration (hrs)	Cumulative number of seed	SE	Percent number of seed released (%)	Percent cumulative number of seed released (%)
NS	DB	24	0	0.1	0.6	
SH	DB	24	106	0.7	56.5	
SC	DB	24	204	0.1	73.2	
NS	GH	24	0	0.0	0.1	
SH	GH	24	138	2.6	64.7	
SC	GH	24	221	0.0	73.1	
NS	40°C	24	9	0.6	10.8	
SH	40°C	24	119	2.9	63.6	
SC	40°C	24	205	1.1	67.6	
NS	50°C	24	37	3.9	36.0	
SH	50°C	24	159	5.0	73.7	
SC	50°C	24	264	4.4	79.2	
NS	65°C	24	32	3.5	35.3	
SH	65°C	24	148	4.5	72.1	
SC	65°C	24	269	5.2	83.5	
NS	85°C	24	38	4.3	41.3	
SH	85°C	24	145	5.0	72.7	
SC	85°C	24	252	5.0	82.8	
NS	DB	48	18	2.0	24.0	24.5
SH	DB	48	146	3.1	21.4	77.9
SC	DB	48	227	2.4	8.3	81.5
NS	GH	48	42	5.0	40.5	40.6
SH	GH	48	182	2.1	20.6	85.3
SC	GH	48	253	3.1	10.6	83.8
NS	40°C	48	40	3.2	36.9	47.7
SH	40°C	48	166	1.5	25.0	88.6
SC	40°C	48	259	4.3	18.0	85.6
NS	50°C	48	81	1.0	42.6	78.6
SH	50°C	48	207	0.2	22.2	95.8
SC	50°C	48	317	1.3	15.8	95.0
NS	65°C	48	71	0.6	42.4	77.7
SH	65°C	48	190	0.3	20.5	92.6
SC	65°C	48	316	0.2	14.8	98.2
NS	85°C	48	81	0.0	46.0	87.3
SH	85°C	48	192	0.1	23.6	96.3
SC	85°C	48	295	0.2	14.1	96.8
NS	DB	48F	73	3.3	75.5	75.5
SH	DB	48F	187	1.6	22.1	22.1
SC	DB	48F	278	3.0	18.5	18.5
NS	GH	48F	102	1.4	59.4	59.4
SH	GH	48F	213	1.1	14.7	14.7
SC	GH	48F	302	2.6	16.2	16.2
NS	40°C	48F	84	1.5	52.3	52.3
SH	40°C	48F	188	1.0	11.4	11.4
SC	40°C	48F	303	1.3	14.4	14.4
NS	50°C	48F	103	1.1	21.4	21.4

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SH	50°C	48F	216	0.7	4.2	4.2
SC	50°C	48F	333	0.8	5.0	5.0
NS	65°C	48F	91	1.5	22.3	22.3
SH	65°C	48F	205	1.1	7.4	7.4
SC	65°C	48F	322	0.6	1.8	1.8
NS	85°C	48F	92	1.1	12.7	12.7
SH	85°C	48F	199	0.5	3.7	3.7
SC	85°C	48F	304	0.9	3.2	3.2

The totals for each treatment (NS, SH, and SC), temperature (DB, GH, 40°C, 50°C, 65°C, and 85°C) and extraction period (24 hours, 48 hours and 48F) in the percent columns = 100% per treatment per temperature per column (n=540).

Assessment of germination performance over a period of 30 days showed that seeds extracted from cones that were soaked in hot water (SH) had the lowest cumulative germination percentage (Figure 1a). Germination performance ranged between 0 to 15% for soaking and extraction temperature interaction. The differences were observed in seeds that were extracted after 48 hours subjected to 85C for cones that were soaked in cold water (SC) prior to extraction (>15%, p<0.05). The worst performing were seeds extracted from cones subjected to soaking in hot water (SH) whether exposed for 24 or 48 hours (Figure 1b). This study observed similar germination performance of seeds from cones that were not soaked as compared to Sfairi *et al.*, (2012) study who observed germination performance of <20% to about 40%. The present study noted that *C. lusitanica* seeds from cones that were soaked in hot water before extraction performed poorly (Figure 1a). Many studies have focused on the effect of other soaking media on seed germination, while the present study focused on the soaking of cones in cold and hot water, and their effect on germination (45–47). Studies have shown that the seed derived from seed processing can be no better than the speed with which you start (27,40,44,46,48,49). The same studies have shown that to upgrade seed quality substantially, it would be at the expense of decreasing the seed volume by removing the empty and damaged seed for which this study did not do. Hence, the germination performance in this study varied from 0 to 15%. This study observed that seeds extracted from cones soaked in cold water and extracted after 48 hours at 85°C had the highest germination performance (Figure 1b).



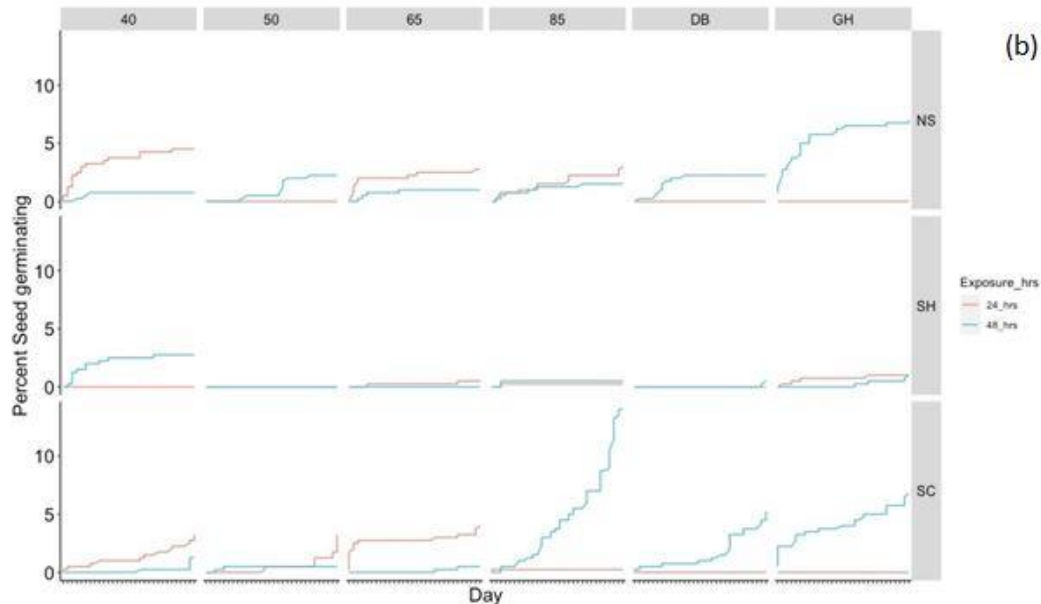


Figure 1. a) shows a 30-day cumulative percent germination performance based on soaking treatment (NS-not soaked-red, SH-soaked in hot water-black, and SC-soaked in cold water-green), b) shows a 30-day cumulative percent germination performance for the combined interaction of soaking (NS, SH, and SC), extraction temperature (DB-Drying bed, GH-Glass house, 40°C, 50°C, 65°C, and 85°C), and exposure period of seeds released at 24 (red) and 48 hours (blue) intervals.

4. CONCLUSIONS

This study has shown that soaking *C. lusitanica* cones in cold water prior to extraction has a positive effect on seed release. Higher extraction temperatures also favor seed release both independently and combined with the soaking effect. This study has shown that the seeds extracted via a combination of soaking of *C. lusitanica* cones and extracting at a temperature of 85°C for 48 hours have the highest germination performance.

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