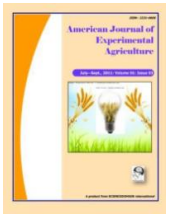

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The Quality of Jute Mallow Seeds Exposed to Different Hot Water-Steeping and Cooling Protocols

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Authors' contributions

This work was carried out in collaboration between all authors. Author KDT design the study, wrote the protocol and wrote the first draft of the manuscript. Author JAO reviewed the experimental design and all drafts of the manuscript. Authors HI and NCA managed the analyses of the study. Authors HI and NCA identified the seeds. Authors KDT and JAO performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Steeping of dormant jute mallow (*Corchorus olitorius* L.) seed in hot water at high temperature for enhanced germination, seems to be the most favoured of all other methods. Literature however, appears to be silent on the cooling protocol to adopt to ensure high quality after a seed lot may have been steeped in hot water. Seeds of two cultivars of this crop were subjected to nine hot water/cooling treatments and then dried back. They were then thinly spread and stored in open glass dishes at 83% relative humidity and 33°C. The moisture content of seeds steeped in water at 80 and 97°C increased from about 5-6% prior to storage to about 10-11% after 6-18 weeks after storage (WAS). Steeping of seeds at 80 and 97°C for 5 seconds significantly enhanced germination to about 88% and 77% in 'Amugbadu' and 'Oniyaya' respectively compared to about 8% in the control (unsteeped) seed. Cultivar 'Amugbadu' seeds steeped in cold water (*ca* 27°C) immediately after steeping in water at 97°C recorded higher germination percentage of 90% - 46% within 0-12 weeks of storage compared to the range of 88 to 29% recorded within the same period in seeds that

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were simply left to gradual cooling in ambient condition. 'Oniyaya' seeds exhibited no differential response to cooling protocol. Unsteeped (control) seed of both cultivars recorded higher germination of 82/85% at 20 WAS than seeds steeped at 97°C (irrespective of cooling protocol) and 80°C prior to storage. Furthermore, whereas seeds of the latter group germinated slower and less uniformly as storage progressed, a gradual increase in the values of these vigour indices were recorded in the former group of seeds. Across steeping treatments the germination percentage of 'Oniyaya' seed declined less rapidly than that of 'Amugbadu' seeds during storage from *ca* 61% to 32% in the former compared with about 62% to 3% in the latter.

Keywords: Corchorus olitorius; seed dormancy; steeping; storage; germination rate and synchronization.

1. INTRODUCTION

Dormancy poses a serious problem to successful seed germination and seedling establishment in jute mallow and different methods have been employed to alleviate the problem. [1] reported the effectiveness of hot water-steeping in this respect which has been confirmed by other reports [2,3,4]. [5] recorded best germination when seeds of *Corchorus olitorius* were first pre-chilled at 6°C for 3½ days before they were incubated at 35°C. Exposure of the seed of this crop to dry heat at 90°C for 5 or ten minutes has also been reported to break dormancy by [6]. According to [7], the temperature required to break seed dormancy in *C. cunninghamii*in Australia ranged between 80-100°C. It was therefore concluded that soil heating from bush fire would promote the germination of seeds of this species. [4,8] reported the effectiveness of sulphuric acid and seed coat scarification respectively. Earthworm cast leachate has also been found effective in dormancy breaking in *C. olitorius* seeds [2]. Of all the methods stated above, though not an exhaustive list, the use of hot water appears to be the most favoured as water is cheaply available and can be more safely handled by all users.

The usual practice when hot water is used to break seed dormancy in this crop is to immediately steep treated seeds in cold water which is assumed to bring down seed temperature and so reduce damages that may occur to the embryo by the treatment at high temperature. [9] recorded better germination and seedling growth when maize seeds that had been stored in deep freezer and refrigerator were first left to equilibrate in ambient condition for some days before they were tested at a temperature higher than that in which they were stored. The usual practice when hot water is used to break seed dormancy in this crop is to immediately steep treated seeds in cold water to bring down seed temperature. It is of the opinion that this would reduce damages that may occur to the embryo by the treatment at high temperature. It is possible that exposure of jute mallow seeds to extreme temperatures in quick successions may damage the embryo. This study aimed at determining the effect hot water-steeping/cooling protocol may have on seed quality in two different cultivars of *C. olitorius* in a bid to enhance seed germination.

2. MATERIALS AND METHODS

Samples of *Corchorus olitorius* seeds of cultivars 'Oniyaya' and 'Amugbadu' harvested in November, 2012 were subjected to nine treatments: St1) seeds steeped in hot water at 97°C for 5 seconds and immediately spread out on absorbent paper to cool down and dry in ambient condition (temperature of about 30°C and relative humidity of about 30%); St2) seeds steeped as in St1 followed immediately by steeping in cold water as recommended by [1] as being optimum for obtaining high germination of dormant *Corchorus olitorius* seeds as well as an improvement in seedling emergence; St3) seeds steeped as in St1 followed immediately by steeping in hot water at 80°C; St4) seeds steeped as in St1 followed immediately by steeping in hot water at 60°C; St5) seeds steeped as in St1 followed immediately by steeping in warm water at 40°C; St6) seeds steeped in hot water at 80°C followed immediately by steeping in cold water; St7) seeds steeped in hot water at 60°C followed immediately by steeping in cold water; St8) seeds steeped in warm water at 40°C followed immediately by steeping in cold water; St9) control (non-treated).

Seeds of the different treatments were left to dry on absorbent paper in ambient condition for seven days. The moisture content at which seed equilibrated with the ambient condition was then

determined using the high constant temperature oven method [10]. This involved the drying of two replicates of seeds of about 1g each in the oven at 130°C for 1hr. After this period seeds and container were cooled over silica gel in a desiccators for 1hr. The weight of dry seeds was subsequently taken and the moisture content was determined on wet weight basis. Samples of each of the treatments were thinly spread in glass Petri dishes and placed in an incubator at 33±0.5°C and at 83±2.5% for a period of 20 weeks. Seed samples were drawn for germination test at two-weekly interval. In addition to the nine treatments listed above, samples of the untreated (St9-control) seeds were steeped as in St2 above following storage for 12-20 weeks to obtain St10. This was to ascertain the viability of the untreated seeds at these points. Samples of St7 and 8 were similarly tested at 18 and 20 WAS to obtain St11 and 12 respectively. On each of the testing days, four replicates of 50 seeds each were counted and spread on distilled water-moistened filter paper and incubated at 30°C for 16 days. Germination counts were taken every-other-day and the final cumulative figures were expressed as a percentage of the total seed incubated for each treatment. To further index seed quality, mean germination time was determined using the expression recommended by [11]:

$$t=\frac{\sum ni \times ti}{\sum n} \quad \text{(days)}$$

Where:

- t- Mean germination time,
- *ti*- Given time interval,
- *ni*- Number of germinated seeds during a given time interval
- *n*- Total number of germinated seeds.

Germination synchronization was determined using the formula adopted by [12]: $Z = \sum C_{ni,\,2} / N$, with $C_{ni,\,2} = n_i (n_i-1)/2$ and $N = \sum n_i (\sum n_i-1)/2$

Where $Cn_{i,\,2}$: combination of the seeds germinated in the time *i* and n_i: number of seeds germinated in the time *i*. Z = 1 when the germination of all seeds occur at the same time and Z = 0 when at least two seeds could germinate, one at each time. The higher the value obtained, the better synchronised the germination.

Seed moisture content for St1-9 were again tested at 6 and 18 weeks of storage to determine if there had been changes to the moisture levels compared to what was recorded at the beginning of storage.

Seed germination data (in percentages) were transformed to arcsin values before they were statistically analysed. All data were analysed using the SAS statistical package [13]. The least significant difference method (LSD) and Duncan Multiple Range Test were used for mean separation where significant differences were obtained.

3. RESULTS

Prior to storage (0WAS), moisture contents of St1-9 ranged between 4.8-6.1 and 4.6-6.4% for “Amugbadu” and “Oniyaya” respectively (Table 1) and by six weeks after storage (6WAS), the values had risen considerably to about 10.5% in all seeds steeped in hot water at 97 and 80°C (i.e. St1-6) with little or no changes at 18WAS. Relatively slight moisture increases were recorded in untreated seeds and in those steeped at 40 and 60°C. Steeping of seeds at 97°C (irrespective of cooling protocol) and at 80°C (St1-6) significantly enhanced germination from about 8% in the control to about 68-88% across variety prior to storage i.e at 0WAS (Table 2). Though seeds steeped in hot water at 60°C generally germinated higher than those steeped at 40°C and the control (especially for ‘Amugbadu’ from 0-14WAS), the maximum values of 27% and 13% for Amugbadu at 2WAS and ‘Oniyaya’ at 14WAS respectively were still low. Fig. 1 shows that seed germination was significantly higher in ‘Amugbadu’ (62%) than in ‘Oniyaya” (51%) across steeping treatments at 0WAS. By 2WAS the germination of ‘Oniyaya’ seeds had improved to about 60% and was statistically similar to that of ‘Amugbadu’ up to 6WAS. The interaction between steeping treatment and cultivar on seed germination was only significant from 8WAS (Table 2). It is obvious that seeds of ‘Oniyaya’ steeped at 80 and 97°C germinated significantly higher than those of ‘Amugbadu’ from 8 to 20WAS. For example, whereas ‘Oniyaya’ seeds of St1-6 recorded germination values of 71% - 89% at 8WAS, those of ‘Amugbadu’ had 49% - 72%. At 20WAS ranges of 36% - 56% and 1% - 7% were recorded for ‘Oniyaya’ and ‘Amugbadu’ respectively. Germination percentages remained at fairly high levels (63%-79%) in ‘Oniyaya’ for about 12/14WAS in St1-6 (Table 2) whereas this level of germination was not obtained beyond about 6/8WAS in ‘Amugbadu’; generally, no significant differences were recorded between

cultivars in St7-9. Table 2 shows further that the cooling protocol adopted following steeping of seeds in hot water at 97°C affected the longevity of seeds of the two cultivars differently. 'Amugbadu' seeds that were steeped in cold water immediately after hot water exposure (St2) germinated significantly higher (65% - 46%) at 8 to 12WAS than seeds that were left to cool gradually under ambient condition (St1) with 57% - 29% germination. Seeds that were steeped at 80, 60 and 40°C following steeping at 97°C before cold water-steeping (St3-5) were only significantly lower in germination than St1 seeds at 8WAS. Seeds steeped at 80°C only, followed immediately by cold water-steeping (St6) survived better (72%-7%) than St1 seeds (with 57% - 1% germination) during 8-20WAS. In cultivar 'Oniyaya' there was generally no differential response to cooling protocol in seeds steeped at 97°C. However, seeds of this cultivar that were steeped at 80°C only (St6) germinated significantly higher (56% - 63%) than St1 (40%-35%) at 16-20WAS. When seeds of both varieties from the control treatment (St9) were steeped in hot water at 97°C for 5 seconds (St10) following 12-20 weeks of storage germination was greatly enhanced to about 78-89% (Table 3). Also, 'Amugbadu' seeds previously subjected to 40 and 60°C steeping prior to storage (St8 and 7 respectively in Table 3) and which germinated poorly (1%-16%) throughout the storage period, had improved germination of 72% - 84% when subjected to hot water steeping at 97°C for 5 seconds at 18 and 20WAS (St12 and 11 respectively in Table 3). However, "Oniyaya" seeds that were previously subjected to 60°C before they were again steeped at 97°C (i.e. St12), germinated significantly lower (59 and 39% at 18 and 20WAS respectively) than seed of St 10 and 11.

Germination took significantly longer (*ca* 9.5 days) at the onset of storage in the unsteeped seeds (St9) than in those steeped in hot water (*ca* 1.6–2.7days) while all hot water-treated seeds germinated at statistically similar rates irrespective of cooling protocol (Fig. 1). As from 2WAS, germination rate of St9 (control) seeds generally improved till the end of storage (from about 9.5 days to about 1.0 day). This trend was also recorded for St5 seeds from 14-20WAS. The effect of cultivar on the germination percentage was significant all through the storage period except at 4 and 6 WAS. Seed of cultivar Oniyaya germinated significantly higher than those of Amugbadu except at 0 and 2 WAS (Fig. 2). Fig. 3 shows that for all the seeds steeped at 97°C (irrespective of cooling protocol), as well as those steeped at 80°C, germination was consistently fast up to 4WAS taking only about 2-2.7 days, followed by a steady decline as from 6WAS. Fig. 4 reveals that seeds of the two varieties were statistically similar in germination rate within the first 10 weeks of storage. As from 12WAS however, the superiority of "Oniyaya" over "Amugbadu" became significant; while the mean germination time for former ranged between about 2.5 – 3.0 days that of the former was about 3.8 – 4.5days. Fig. 5 shows that prior to storage (i.e. at 0WAS), seeds generally germinated more uniformly when steeped in hot water at 97°C irrespective of the cooling protocol adopted (St1-5) and when steeped at 80°C (St6) with values ranging from about 0.7 to 0.9 than when they were steeped at 40 and 60°C (St7 and 8 with value of about 0.2) or when unsteeped (St9 with about 0.1 synchronization value). As storage progressed however, whereas germination became more non-uniform in St1-6, there were gradual improvements in St7-9. Germination was better synchronized in "Oniyaya" with value of about 0.3 than in "Amugbadu" with values of about 0.5 – 0.6 at 12, 14 and 16WAS (Fig. 6).

Table 1. Percentage moisture content (on wet weight basis) of seeds of the various treatments at the onset and after 6 and 18 weeks of storage at 83% relative humidity and 33°C

Cultivar	Steeping treatment	Storage period (weeks)		
		0	6	18
'Amugbadu'	St1	5.5	9.9	9.4
	St2	6.0	10.4	10.4
	St3	5.8	10.6	9.5
	St4	5.6	9.5	10.0
	St5	6.1	10.4	10.0
	St6	5.9	10.0	10.0
	St7	5.1	7.0	6.1
	St8	5.3	5.6	5.1
	St9	4.8	5.4	5.5
'Oniyaya'	St1	4.6	10.5	11.3
	St2	5.0	10.6	11.0
	St3	5.9	10.6	10.7
	St4	6.3	10.1	10.3
	St5	6.1	9.7	10.4
	St6	6.4	10.2	9.8
	St7	4.7	5.4	5.0
	St8	5.3	5.5	5.7
	St9	5.8	4.9	5.6

Table 2. Interaction effect of cultivar and steeping treatment on the germination percentage of *Corchorus olitorius* seeds at 8-20WAS

Cultivar	Steeping treatment	Storage period (weeks)										
		0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**
'Amugbadu'	St1	86	82	82	85	57de	40c	29c	13de	5def	3d	1ef
	St2	86	90	87	89	65bc	57b	46b	12de	11cde	7d	3def
	St3	87	83	84	90	52e	54bc	37bc	10de	8cdef	5d	1ef
	St4	88	89	84	86	52e	49bc	38bc	12de	15cd	5d	4def
	St5	88	91	88	84	49e	51bc	39bc	13de	7cdef	2d	1ef
	St6	84	87	89	84	72ab	58b	46b	32c	18c	16c	7cd
	St7	19	27	15	12	15f	16d	9d	24cd	5def	4d	6cd
	St8	8	8.5	3	7	4g	1f	4de	4ef	1f	1e	3def
	St9	8.5	6	7	4	5g	6ef	4de	1fg	1f	4d	4def
'Oniyaya'	St1	68	86	84	87	84a	79a	75a	66ab	38b	40b	35b
	St2	77	88	86	88	89a	84a	75a	63b	47ab	45ab	61a
	St3	73	87	88	90	71abc	85a	71a	66ab	33b	52a	34b
	St4	76	88	80	87	86a	88a	72a	77a	50a	57a	52a
	St5	70	90	87	93	84a	85a	74a	79a	49a	41b	33b
	St6	77	82	86	84	81a	83a	74a	77a	53a	50a	56a
	St7	8	12	7	10	6fg	8de	6de	13de	1f	2e	13c
	St8	3	6	5	3	2g	1f	3e	0g	1f	2d	1f
	St9	8	4	3	7	2g	5ef	3e	6ef	3ef	4d	3def
	Significance	Ns	ns	ns	ns	*	*	*	*	*	*	*

Means followed by the same letter in a column are not significantly different at P≥ 0.05 using the Duncan's Multiple Range test.

St1 = steeped at 97°C for 5 seconds and air-cooled at room temperature; St2 = steeped at 97°C for 5 seconds and immediately steeped in cold water(27 oC); St3 = steeped at 97°C for 5 seconds followed by 5 seconds steeping at 80°C, followed by steeping in cold water(27 oC); St4 = steeped at 97°C for 5 seconds followed by 5 second steeping at 60°C followed by steeping in cold water(27 oC); St5 = steeped at 97°C for 5 seconds followed by 5 seconds steeping at 40°C followed by steeping in cold water(27 oC); St6 = steeped at 80°C for 5 seconds followed by steeping in cold water (27 oC); St7 = steeped a 60°C and immediately steeped in cold water(27 oC); St8 = steeped at 40°C for 5 seconds and immediately steeped in cold water(27 oC); St9 = control (no hot water steeping)

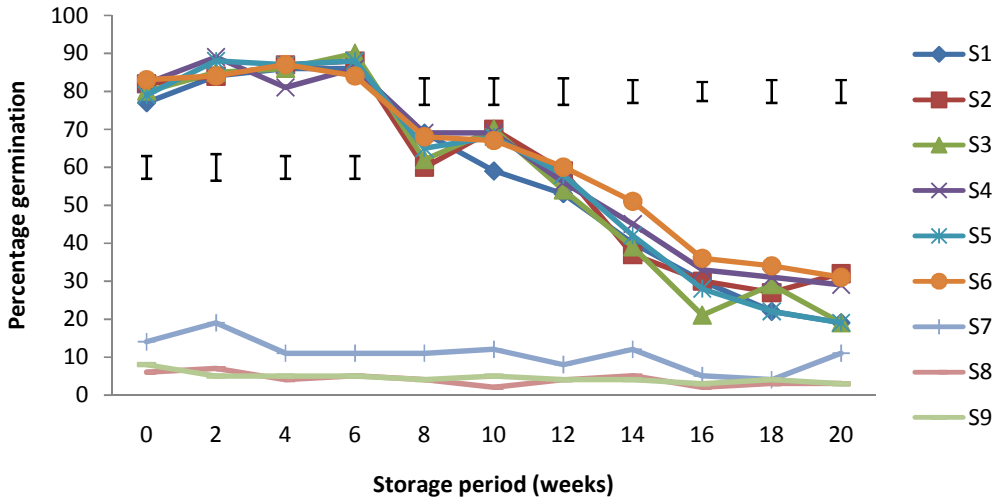

Fig. 1. Effect of steeping treatment on seed germination percentage following storage
I: LSD bar at P=0.05

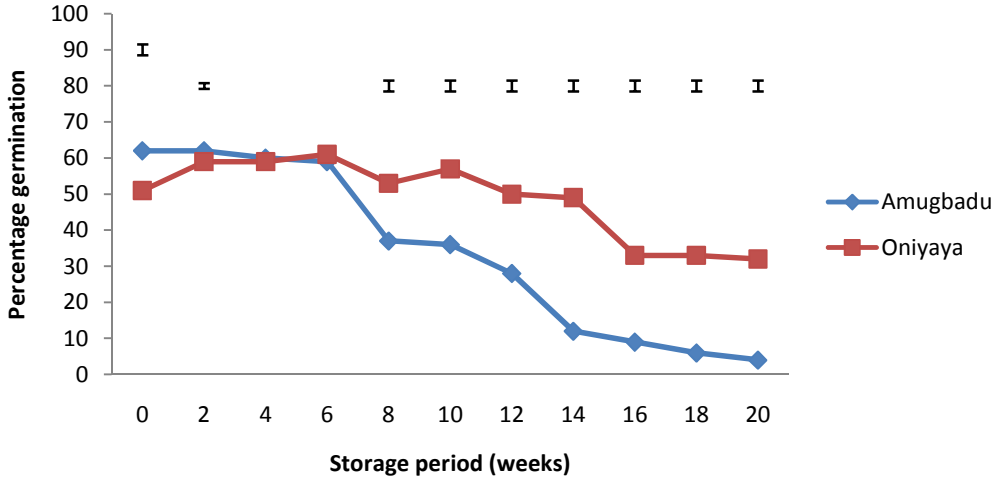

Fig. 2. Effect of variety on seed germination percentage following storage
I: LSD bar at P=0.05

Table 3. Germination percentages of previously untreated seeds that were subsequently steeped at 97°C for 5 sec. (St10) and those previously steeped at 40 and 60°C prior to storage that were subsequently steeped at 97°C for 5 sec. (St11 and 12 respectively) following storage for the periods indicated

Cultivar	Steeping treatment	Storage period (weeks)				
		12	14	16	18	20
'Amugbadu'	St10	84a	86a	78a	83ab	85a
	St11	-	-	-	84ab	72b
	St12	-	-	-	72b	77ab
'Oniyaya'	St10	89a	82a	80a	82ab	82a
	St11	-	-	-	90a	84a
	St12	-	-	-	59c	39c

Means followed by the same letter in a column are not significantly different at P≤ 0.05 using the Duncan's Multiple Range test

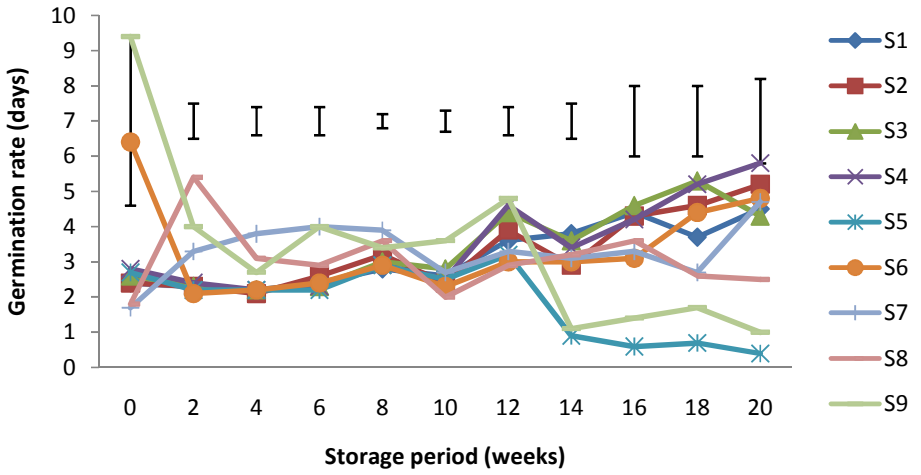

Fig. 3. Effect of steeping treatment on seed germination rate (days) following storage
I: LSD bar at P=0.05

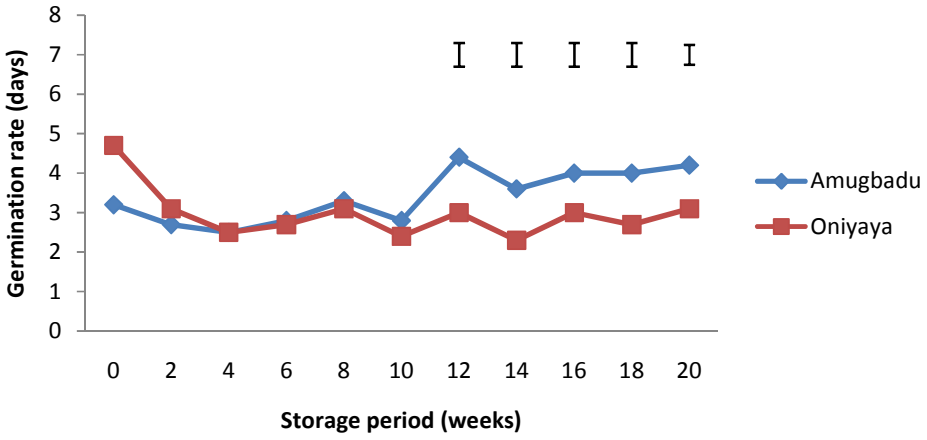

Fig. 4. Effect of variety on seed germination rate (days) following storage
I: LSD bar at P=0.05

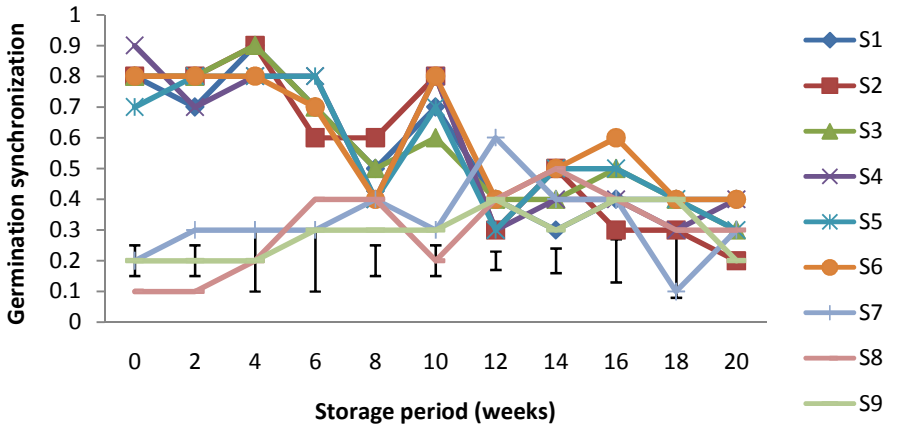

Fig. 5. Effect of steeping treatment on seed germination synchronization following storage
I: LSD bar at P=0.05

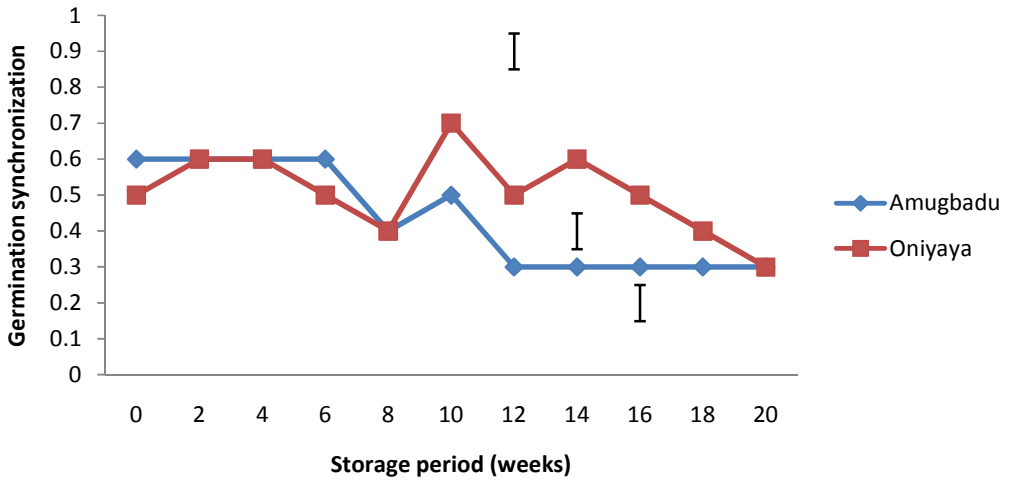

Fig. 6. Effect of variety on seed germination synchronization following storage
I: LSD bar at P=0.05

4. DISCUSSION

The considerable increase in the moisture content of seeds of St1-6 during storage at high relative humidity as recorded in this study is an indication of improved permeability due to the softening of the coats by hot water. It is this change in permeability that must have been responsible for the loss of dormancy (and therefore, enhanced germination), a confirmation that dormancy in this species is physical caused by water-impermeable seed coat [14]. [1] had hypothesized that hot water may be alleviating dormancy in *Corchorus* by weakening the seed coat. [2] postulated that some chemicals secreted by earthworm are also capable of weakening woody seed coat of jute seed. Steeping in water at 40 and 60°C did not result in substantial seed coat permeability and therefore the seed remained relatively dry (with moisture content of an average of about 5-6%) and retained high level of dormancy. [6] have also reported the ineffectiveness of dry heat of 40-60°C compared to the effectiveness of 90°C in breaking *Corchorus olitorius* seed dormancy. The effectiveness of temperatures of 80 and 97°C in overcoming seed dormancy as recorded here, agrees with that reported by [7] for *C. cunninghamii*. Based on [7] report, [15] asserted that temperature of 60-70°C which may be produced by low intensity forest fire may not be sufficient to stimulate *C. cunninghamii* seed germination. However, the ability of some seeds exposed to 60°C to significantly germinate (though low) better than the untreated ones, whereas most seeds germinated well following exposure to 80-97°C in the current study, is indicative of variability in the depth of dormancy of the individual *C. olitorius* seeds. This view agrees with that expressed by [16] for *Phloxpilosa* seeds. The existence of differential rates of seed dormancy release in *C. cunninghamii* was alluded to in the report by [14]. Variability of dormancy depth is seen as a survival strategy [17] which ensures the perpetuation of species. The poorer longevity of 'Amugbadu' seeds that were subjected to gradual cooling in ambient condition following hot water-steeping compared with those that were steeped in cold water immediately, indicates that hot water steeping at 97°C must have resulted in embryo damage and that the extent of such damage could be reduced by immediately steeping treated seeds in cold water.

The poor germination of 'Oniyaya' seeds steeped in hot water at 60°C compared with the values obtained for the untreated seeds that were subsequently subjected to steeping at 97°C at 18 and 20WAS, is an indication that the earlier exposure to 60°C (despite immediate steeping in cold water) damaged the seed embryo even when the treatment did not result in considerable dormancy alleviation. The greatest damaged to seed longevity was occasioned by steeping of seed at 80-97°C. The poorer storability of hot water-steeped seeds as recorded in this study is in agreement with recent report by [18] which revealed that potential seed longevity was adversely affected even by 2-second hot water-steeping. [19] also reported lower germination value for seeds stored following hot water

steeping in comparison to unsteeped seeds, though the difference between the two seed lots was insignificant. That treated 'Oniyaya' and 'Amugbadu' seeds still gave acceptable germination percentages at 12/14 (up to 79%) and 6WAS (up to 90%) respectively despite the adverse effect of hot water steeping on potential seed longevity in the two cultivars used in this study is note worthy. This result runs contrary to the report by [20] that hot water steeped seeds cannot be stored. Better longevity of such seeds will even be attained if storage is in more conducive environment of lower humidity and temperature. The significant variation in seed longevity of the two jute varieties in this study agrees with the view expressed [21] that this trait may be species or variety specific. Differences in seed longevity among varieties have also been reported in other crops [22,23,24]. The poorer germination of 49 – 65% recorded as from 8WAS for 'Amugbadu' seeds that were previously steeped at 97°C compared with 71–89% for 'Oniyaya' is an indication of faster deterioration in the former. Differences in genotype may also explain the significant steeping treatment x cultivars interaction effect on germination percentage recorded from 8WAS. It has been established from this study that Amugbadu seeds are poor storers. Their poor vigour which became evident from 8 WAS must have been responsible for their inability to withstand hot water steeping stress compared to Oniyaya seeds. Ability to tolerate stressful condition is known to vary with cultivars [25].

The enhanced total germination, germination rate and synchronization before storage due to hot water-steeping can be attributed to improved seed coat permeability. [26] stated that seed treatments may overcome dormancy and enhance germination by altering the physical integrity of seed coverings. This alteration could also constitute damage to the seed coat. Damage to seed coat has been reported to result in seed deterioration in soybean and also reducing storage ability and germination rate [27]. Subsequent decline in performances in respect of total germination, germination rate and synchronization as from about 8WAS is indicative of decline in seed vigour. However, whereas a decline in seed vigour is normally known to occur before loss of germination [28], the two occurred around the same time (*ca* 8WAS) in this study for seeds exposed to 80-97°C.

According to [29] both dormancy and germination are influenced by the combined effects of the potential growth of the embryo and the resistance of the surrounding tissues. The significantly better germination of seeds of 'Amugbadu' (84–88%) than 'Oniyaya' (68–77%) at 0WAS following steeping at 80 and 90°C, with substantial improvement to about 90% in the latter as storage progressed as recorded in this study may therefore, suggest that the coats of 'Oniyaya' seed still placed significant restraint on the embryo even after steeping and that this was relaxed by 2WAS. The subsequent decline in the percentage germination and vigour, indexed using germination rate and synchronization of the seeds of both cultivars could be adduced to decline in embryo potential growth. Aging has also been reported to result in delayed and decreased germination in other crops [30]. Contrary to the trend above, the improvement in seed germination rates and synchronization recorded for untreated seeds and those exposed to only 40 and 60°C steeping as seeds aged could be assumed to be due to the weakening of the seed coverings as storage progressed. According to [31] germination speed is influenced by a co-action of the embryo's growth potential and a reduction in the physical strength of surrounding seed coverings.

5. CONCLUSION

It is concluded that seeds of the two cultivars used in this study possessed dormancy which was broken by steeping in water at 80–97°C for five seconds. The treatment also resulted in faster and more synchronized germination compared with the control. The study also revealed that it may be necessary to immediately steep hot water treated seeds in cold water at 27°C for better longevity. Seeds of cv 'Oniyaya' retained higher germination for longer (ca 12/24 WAS) than those of cv 'Amugbadu' (about 6WAS). Seed germination percentage, germination rate and synchronization declined from about 8WAS to the end of the storage period.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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