

The quality of jute mallow (*Corchorusolitorius* L.) seeds exposed to different hot water-steeping and cooling protocols

Absract

Steeping of jute mallow (*Corchorusolitorius*) 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 WAS. Steeping of seeds at 80 and 97 °C for 5 seconds significantly enhanced germination. Cultivar ‘Amugbadu’ seeds steeped in cold water immediately after steeping in water at 97 °C survived better during storage than those that were simply left to gradual cooling in ambient condition. ‘Oniyaya’ seeds exhibited no differential response to cooling protocol. Unsteeped (control) seeds and those steeped at 40 and 60 °C prior to storage, had greater longevity 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. Generally ‘Oniyaya’ seeds were of higher quality than those of ‘Amugbadu’.

Key words: *Corchorus olitorius*, seed dormancy, steeping, storage, germination rate and synchronization

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. Oladiran (1986) reported the effectiveness of hot water-steeping in this respect which has been confirmed by other reports (Ayanlaja *etal.*, 2001; Velempini, *etal.*, 2003; Emongor *etal.*, 2004). Nkomo and Kambizi (2009) recorded best germination of *Corchorusolitorius* seeds when they were first prechilled 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 Denton *etal.* (2013). According to Halford (1993), 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. Emongor *etal.* (2004) and Chauhan and Johnson (2008) 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 (Ayanlaja *etal.*, 2001). 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. Ajayi and Fakorede (2001) 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. 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.

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55 **Materials and Method**

56 Samples of *Corchorus olitorius* seeds of cultivars ‘Oniyaya’ and ‘Amugbadu’ harvested in
 57 November, 2012 were subjected to nine treatments: St1) seeds steeped in hot water at 97 °C for 5
 58 seconds as recommended by Oladiran (1986) and then spread out on absorbent paper to cool
 59 down and dry in ambient condition(temperature of about 30 °C and relative humidity of about
 60 30%); St2) seeds steeped as in St1 followed immediately by steeping in cold water; St3) seeds
 61 steeped as in St1 followed immediately by steeping in hot water at 80 °C; St4) seeds steeped as
 62 in St1 followed immediately by steeping in hot water at 60 °C; St5) seeds steeped as in St1
 63 followed immediately by steeping in warm water at 40 °C; St6) seeds steeped in hot water at 80
 64 °C followed immediately by steeping in cold water; St7) seeds steeped in hot water at 60 °C
 65 followed immediately by steeping in cold water; St8) seeds steeped in warm water at 40 °C
 66 followed immediately by steeping in cold water; St9) control (non-treated). Seeds of the different
 67 treatments were left to dry on absorbent paper in ambient condition for seven days. The moisture
 68 content at which seedequilibrated with the ambient condition was then determined using the high
 69 constant temperature oven method (ISTA, 2005). Samples of each of the treatments were thinly
 70 spread in glass Petri dishes and placed in an incubator at 33 ± 0.5 °C and at $83 \pm 2.5\%$ for a
 71 period of 20 weeks. Seed samples were drawn for germination test at two-weekly interval. In
 72 addition to the nine treatments listed above, samples of the untreated (St9-control) seeds were
 73 steeped as in St2 above following storage for 12-20 weeksto obtainSt10. This was to ascertain
 74 the viability of the untreated seeds at these points. Samples of St7 and 8 were similarly tested at
 75 18 and 20 WAS to obtain St11 and 12 respectively. On each of the testing days, four replicates of
 76 50 seeds each were counted and spread on distilled water-moistened filter paper and incubated at
 77 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, germination rate was determined using the expression recommended by Labouriau (1983) while germination synchronization was determined using the formula adopted by Ranal and Santana (2006) based on an idea expressed by Primack (1980). 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 (SAS, 1999). The least significant difference method (LSD) and Duncan Multiple Range Test were used for mean separation where significant differences were obtained.

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. 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 were still low. Seed germination behaviour in the two cultivars varied with storage period. There was no significant interaction between steeping treatment and cultivar within the first six weeks of storage. Table 2 shows that seed germination was significantly higher in ‘Amugbadu’ than in ‘Oniyaya’ across steeping treatments at 0WAS (Table 2). By 2WAS the germination of

103 'Oniyaya' seeds had improved and was statistically similar to that of 'Amugbadu' up to 6WAS.
 104 The interaction between steeping treatment and cultivar on seed germination was significant
 105 from 8WAS (Table 3). It is obvious that seeds of 'Oniyaya' steeped at 80 and 97 °C germinated
 106 significantly higher than those of 'Amugbadu' from 8 to 20WAS. Germination percentages
 107 remained at fairly high levels in 'Oniyaya' for about 12/14WAS in St1-6 (Table 3) as against
 108 about 6WAS in 'Amugbadu' (data not shown); generally, no significant differences were
 109 recorded between cultivars in St7-9. Table 3 shows further that the cooling protocol adopted
 110 following steeping of seeds in hot water at 97 °C affected the longevity of seeds of the two
 111 cultivars differently. 'Amugbadu' seeds that were steeped in cold water immediately after hot
 112 water exposure (St2) germinated significantly higher at 8 to 12WAS than seeds that were left to
 113 cool gradually under ambient condition (St1). Seeds that were steeped at 80, 60 and 40 °C
 114 following steeping at 97 °C before cold water-steeping (St3-5) were only significantly lower in
 115 germination than St1 seeds at 8WAS. Seeds steeped at 80 °C only, followed immediately by cold
 116 water-steeping (St6) survived better than St1 seeds during 8-20WAS. In cultivar 'Oniyaya' there
 117 was generally no differential response to cooling protocol in seeds steeped at 97 °C. Seeds
 118 steeped at 80 °C only (St6) germinated significantly higher than St1 at 16-20WAS. Table 4 shows
 119 that germination was greatly enhanced (*ca* 78-89%) when seeds of both varieties from the
 120 control treatment (St9) were steeped in hot water at 97 °C for 5 seconds (St10) following 12-20
 121 weeks of storage. Also, seeds of the two varieties previously subjected to 40 and 60 °C steeping
 122 prior to storage (St8 and 7 respectively in Tables 3) and which germinated poorly throughout the
 123 storage period, had improved germination when subjected to hot water steeping at 97 °C for 5
 124 seconds at 18 and 20WAS (St12 and 11 respectively in Table 4). However, "Oniyaya" seeds that
 125 were previously subjected to 60 °C before they were again steeped at 97 °C (i.e. St12),

126 germinated significantly lower (59 and 39% at 18 and 20WAS respectively) than seed of St10
127 and 11.

128 Germination was significantly slower at the onset of storage in the unsteeped seeds (St9) than in
129 those steeped in hot water while all hot water-treated seeds germinated at similar rates
130 irrespective of cooling protocol (Figure 1). As from 2WAS, germination rate of St9 (control)
131 seeds generally improved till the end of storage. This trend was also recorded for St5 seeds from
132 14-20WAS. For all the seeds steeped at 97 °C (irrespective of cooling protocol), as well as those
133 steeped at 80 °C, germination was consistently fast up to 4WAS followed by a steady decline as
134 from 6WAS. Figure 2 reveals that seeds of the two varieties were statistically similar in
135 germination rate within the first 10 weeks of storage. As from 12WAS however, the superiority
136 of “Oniyaya” over “Amugbadu” became significant. Figure 3 shows that prior to storage (i.e. at
137 0WAS), seeds generally germinated more uniformly when steeped in hot water at 97 °C
138 irrespective of the cooling protocol adopted (St1-5) and when steeped at 80 °C (St6) than when
139 they were steeped at 40 and 60°C (St7 and 8) or when unsteeped (St9). As storage progressed
140 however, whereas germination became more non-uniform in St1-6, there were gradual
141 improvements in St7-9. Germination was better synchronized in “Oniyaya” than in
142 “Amugbadu” at 12, 14 and 16WAS (Figure 4).

143 144 **Discussion**

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146 The considerable increase in the moisture content of seeds of St1-6 during storage at high
147 relative humidity as recorded in this study is an indication of improved permeability due to the
148 softening of the coats by hot water. It is this change in permeability that must have been
149 responsible for the loss of dormancy (and therefore, enhanced germination), a confirmation that
150 dormancy in this species is physical caused by water-impermeable seed coat (Baskin *etal.*, 2000).

151 Oladiran (1986) had hypothesized that hot water may be alleviating dormancy in *Corchorus* by
 152 weakening the seed coat. Ayanlaja *etal.* (2001) postulated that some chemicals secreted by
 153 earthworm are also capable of weakening woody seedcoat of jute seed. Steeping in water at 40
 154 and 60 °C did not result in substantial seed coat permeability and therefore the seed remained
 155 relatively dry (with moisture content of an average of about 5-6%) and retained high level of
 156 dormancy. Denton *etal.* (2013) have also reported the ineffectiveness of dry heat of 40-60 °C
 157 compared to the effectiveness of 90 °C in breaking *Corchorus olitorius* seed dormancy. The
 158 effectiveness of temperatures of 80 and 97 °C in overcoming seed dormancy as recorded here,
 159 agrees with that reported by Halford (1993) for *C. cunninghamii*. Based on Halford's report,
 160 NSW Department of Environment and Conservation (2004) asserted that temperature of 60-70
 161 °C which may be produced by low intensity forest fire may not be sufficient to stimulate
 162 *C. cunninghamii* seed germination. However, the ability of some seeds exposed to 60 °C to
 163 significantly germinate (though low) better than the untreated ones, whereas most seeds
 164 germinated well following exposure to 80-97 °C in the current study, is indicative of variability
 165 in the depth of dormancy of the individual *C. olitorius* seeds. This view agrees with that
 166 expressed by Madeiras *etal.* (2007) for *Phlox pilosa* seeds. The existence of differential rates of
 167 seed dormancy release in *C. cunninghamii* was alluded to in the report by NSW Department of
 168 Environment and Conservation (2004). Variability of dormancy depth is seen as a survival
 169 strategy (Stewart and McGary, 2010) which ensures the perpetuation of species. The poorer
 170 longevity of 'Amugbadu' seeds that were subjected to gradual cooling in ambient condition
 171 following hot water-steeping compared with those that were steeped in cold water immediately,
 172 indicates that hot water steeping at 97 °C must have resulted in embryo damage and that the
 173 extent of such damage could be reduced by immediately steeping treated seeds in cold water.

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175 The poor germination of ‘Oniyaya’ seeds steeped in hot water at 60 °C compared with the values
 176 obtained for the untreated seeds following exposure to steeping at 97 °C at 18 and 20WAS, is an
 177 indication that the earlier exposure to 60 °C(despite immediate steeping in cold water) damaged
 178 the seed embryo even when the treatment did not result in considerable dormancy alleviation.
 179 The greatest damaged to seed longevity was occasioned by steeping of seed at 80-97 °C. The
 180 poorer storability of hot water-steeped seeds as recorded in this study is in agreement with recent
 181 report by Tolorunse *etal.* (2013)which revealed that potential seed longevity wasadversely
 182 affected even by 2-second hot water-steeping.Ibrahim *etal.* (2013)also reported lower
 183 germination value for seeds stored following hot water steeping in comparison to unsteeped
 184 seeds, though the difference between the two seed lots was insignificant.That treated ‘Oniyaya’
 185 and ‘Amugbadu’ seeds still gave acceptable germination percentages at 12/14 and 6WAS
 186 respectively despite the adverse effect of hot water steeping on potential seed longevity in the
 187 two cultivars used in this study is note worthy.This result runs contrary to the report by
 188 Schippers (2000) that hot water steeped seeds cannot be stored. Better longevity of such seeds
 189 will even be attained if storage is in more conducive environment of lower humidity and
 190 temperature.The significant variation in seed longevity of the two jute varieties in this study
 191 agrees with the view expressed by McDonald (1999) that this trait may be species or variety
 192 specific. Differences in seed longevity among varieties have also been reported in other crops
 193 (Kamaswara and Jackson, 1996; Mrda *etal.*, 2010; Adebisi *etal.*, 2012).The poorer germination
 194 recorded as from 8WAS for ‘Amugbadu’ seeds that were previously steeped at 97 °C compared
 195 with ‘Oniyaya’ is an indication of faster deterioration in the former.

196 The enhanced total germination, germination rate and synchronization before storage due to hot
 197 water-steeping can be attributed to improved seed coat permeability. Katzman *etal.* (2001) stated
 198 that seed treatments may overcome dormancy and enhance germination by altering the physical
 199 integrity of seed coverings. This alteration could also constitute damage to the seed coat. Damage
 200 to seed coat has been reported to result in seed deterioration in soybean and also reducing storage
 201 ability and germination rate (Okabe, 1996). Subsequent decline in performances in respect of
 202 total germination, germination rate and synchronization is indicative of decline in seed vigour.
 203 However, whereas a decline in seed vigour is normally known to occur before loss of
 204 germination (Demir and Mavi, 2008), the two occurred around the same time (*ca* 8WAS) in this
 205 study for seeds exposed to 80-97 °C.

206 According to Koornneef *etal.* (2002) both dormancy and germination are influenced by the
 207 combined effects of the potential growth of the embryo and the resistance of the surrounding
 208 tissues. The significantly better germination of seeds of ‘Amugbadu’ than ‘Oniyaya’ at 0WAS
 209 following steeping at 80 and 90 °C, with substantial improvement in the latter as storage
 210 progressed as recorded in this study may therefore, suggest that the coats of ‘Oniyaya’ seed still
 211 placed significant restraint on the embryo even after steeping and that this was relaxed by
 212 2WAS. The subsequent decline in the percentage germination, germination rate and
 213 synchronization of the seeds of both cultivars could be adduced to decline in embryo potential
 214 growth. Aging has also been reported to result in delayed and decreased germination in other
 215 crops (Hacisalihoglu *etal.*, 1999). Contrary to the trend above, the improvement in seed
 216 germination rates and synchronization recorded for untreated seeds and those exposed to only 40
 217 and 60 °C steeping as seeds aged could be assumed to be due to the weakening of the seed
 218 coverings as storage progressed. According to Welbaum *etal.* (1998) germination speed is

219 influenced by a co-action of the embryo's growth potential and a reduction in the physical
220 strength of surrounding seed coverings.

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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

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375 Table 2. Mean effect of cultivar on seed germination percentage at 0-6WAS

Cultivar	Storage period (weeks)			
	0	2	4	6
‘Amugbadu’	62a	62a	60a	59a
‘Oniyaya’	51b	60a	58a	61a

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377 Means followed by the same letter in a column are not significantly different at $P \geq 0.05$ using the
 378 Duncan’s Multiple Range test.

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398 Table 3. Interaction effect of cultivar and steeping treatment on the germination percentage of
399 *Corchorusolitorius* seeds at 8-20WAS

Cultivar	Steeping treatment	Storage period (weeks)						
		8	10	12	14	16	18	20
‘Amugbadu’	St1	57de	40c	29c	13de	5def	3d	1ef
	St2	65bc	57b	46b	12de	11cde	7d	3def
	St3	52e	54bc	37bc	10de	8cdef	5d	1ef
	St4	52e	49bc	38bc	12de	15cd	5d	4def
	St5	49e	51bc	39bc	13de	7cdef	2d	1ef
	St6	72abc	58b	46b	32c	18c	16c	7cd
	St7	15f	16d	9d	24cd	5def	4d	6cd
	St8	4g	1f	4de	4ef	1f	1e	3def
	St9	5g	6ef	4de	1fg	1f	4d	4def
‘Oniyaya’	St1	84a	79a	75a	66ab	38b	40b	35b

St2	89a	84a	75a	63b	47ab	45ab	61a
St3	71abc	85a	71a	66ab	33b	52a	34b
St4	86a	88a	72a	77a	50a	57a	52a
St5	84a	85a	74a	79a	49a	41b	33b
St6	81a	83a	74a	77a	53a	50a	56a
St7	6fg	8de	6de	13de	1f	2e	13c
St8	2g	1f	3e	0g	1f	2d	1f
St9	2g	5ef	3e	6ef	3ef	4d	3def

Means followed by the same letter in a column are not significantly different at $P \geq 0.05$ 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; St3 = steeped at 97 °C for 5 seconds followed by 5 seconds steeping at 80 °C, followed by steeping in cold water; St4 = steeped at 97 °C for 5 seconds followed by 5 second steeping at 60 °C followed by steeping in cold water; St5 = steeped at 97 °C for 5 seconds followed by 5 seconds steeping at 40 °C followed by steeping in cold water; St6 = steeped at 80 °C for 5 seconds followed by steeping in cold water; St7 = steeped a 60 °C and immediately steeped in cold water; St8 = steeped at 40 °C for 5 seconds and immediately steeped in cold water; St9 = control (no hot water steeping).

Table 4. 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 $P \geq 0.05$ using the Duncan's Multiple Range test.

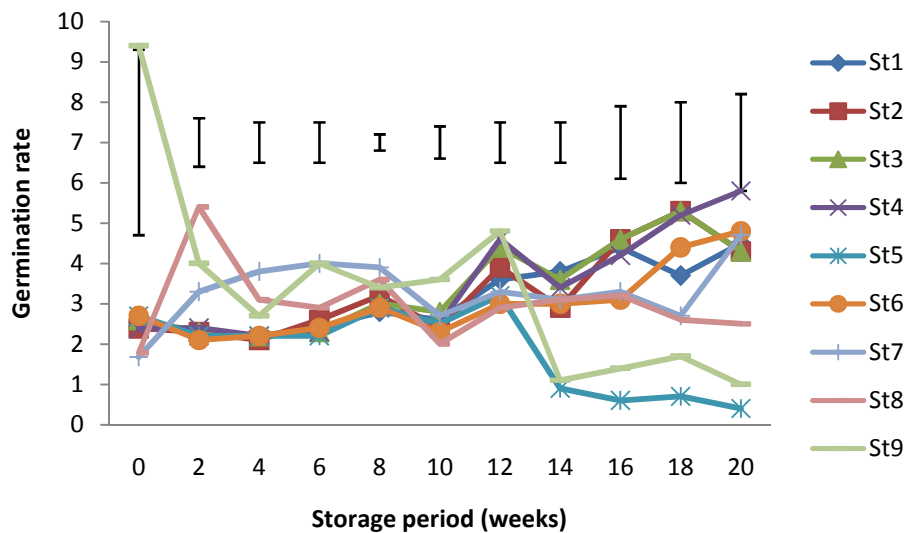


Fig. 1 Mean effect of steeping treatment on seed germination rate following storage

I: LSD bar at P=0.05

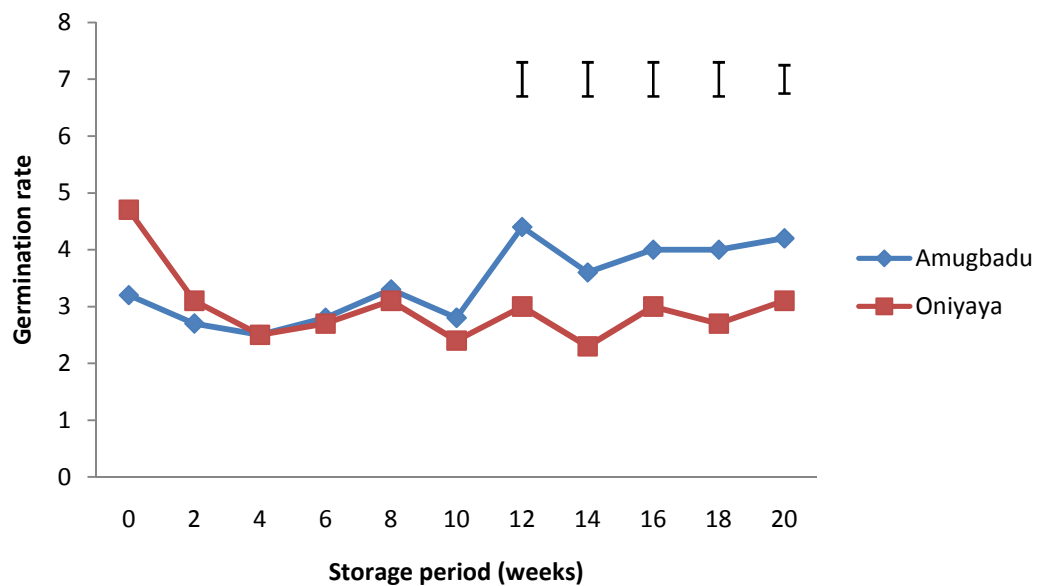
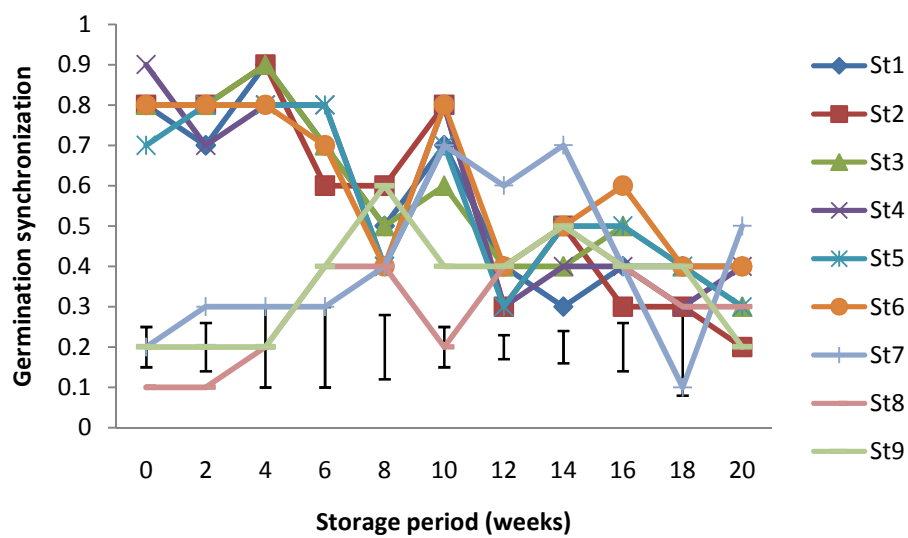


Fig. 2 Effect of cultivar on seed germination rate following storage

I: LSD bar at P=0.05



458 **Fig. 3**Effect of steeping treatment on seed germination synchronization following storage

459 **I: LSD bar at P=0.05**

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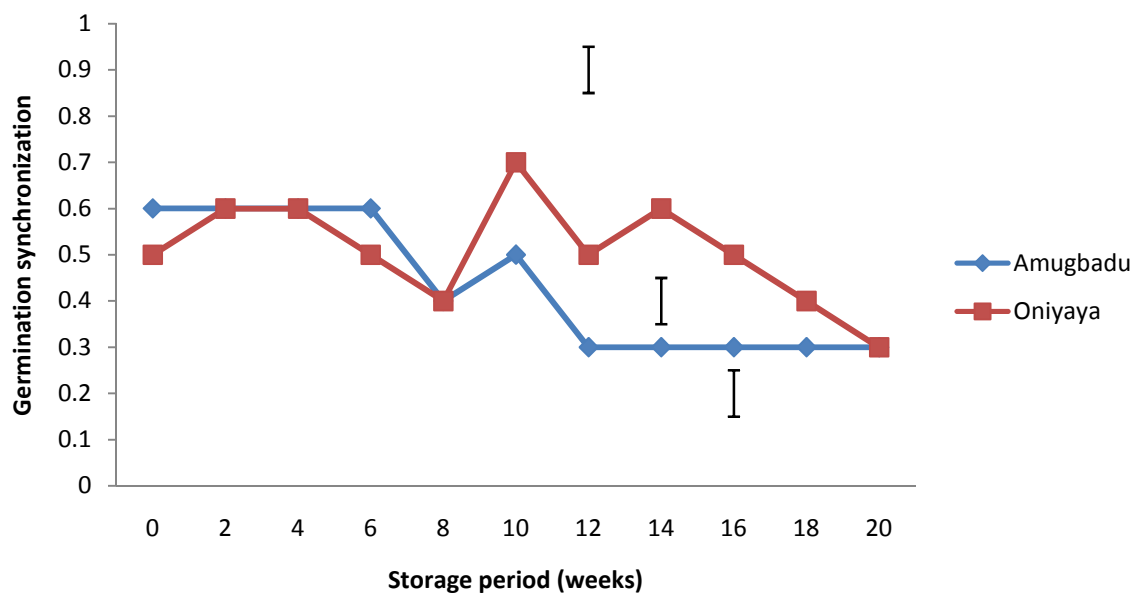


Fig.

4Effect of cultivar on seed germination synchronization following storage

I: LSD bar at P=0.05