

Original Research Article

Effects of pre-germination treatments and storage periods on germination and vigour of seeds of three cassava (*manihot esculenta* crantz) cultivars

ABSTRACT

Seeds of three cultivars of cassava were collected from the CSIR-Crops Research Institute at Fumesua in the Ashanti Region of Ghana to determine the effect of storage periods and seed pre-germination treatments on the germination percentage and vigour of seeds from three varieties of cassava. The experiment was conducted under laboratory conditions and a 3 x 3 x 7 factorial in Completely Randomized Design (CRD) with three replications was used. The first factor was cultivar at three levels (Ahwengyanka-1, Ahwengyanka-2 and Aworowa-3); the second factor was pre-germination treatments at seven levels (hot water, cold water, mechanical scarification, three concentrations of acid scarification and no treatment as the control); the third factor was storage period at three levels (no storage, three months storage and six months storage). The study revealed that seeds of cassava stored for up to three months produced about 40% germination after undergoing the various pre-germination treatments. Seeds of the Aworowa-3 cultivar stored for six months produced the highest germination percentage of 61.0%. Seeds mechanically scarified with sand paper produced the highest germination percentage of 48.9% whereas seeds with no treatment recorded the least percentage of 34.8%. The study concluded that Aworowa-3 seeds without storage produced a higher germination percentage than Ahwengyanka-1 and Ahwengyanka-2 seeds without storage but all the seeds of the three varieties had the highest germination percentage when stored for six months.

Keywords: dormancy, conductivity, scarification, breeding, clones

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the most important tropical root crop in many parts of Africa because of its edible roots [1]. It is also cultivated for its leaves and tender shoots which are rich sources of proteins, vitamins A, B, C, and other minerals are consumed as vegetable [2-3]. In Africa, Ghana ranks third after Nigeria and the Democratic Republic of Congo as the largest cassava producing country [4]. Cassava in Ghana is grown across all the agro-ecological zones and contributes 22% to Ghana's Agricultural Gross Domestic Product (AGDP) [5].

25 However, a major problem with freshly harvested cassava seeds is its characteristic
26 dormancy which is a well-documented occurrence in *Euphorbiaceae* genera [6-9]. The
27 period of dormancy in cassava seeds could last for a minimum period of 6-9 months under
28 ambient temperatures [10] with adverse consequences on breeding programmes. .
29 Dormancy in seeds, therefore is known to inhibit the germination of intact viable seeds under
30 favorable conditions [11-13]. Under cold storage conditions (4°C and 70–80% relative
31 humidity) cassava seeds can remain dormant for up to 7 years with no loss of germination.

32 To overcome this problem so that cassava seed breeding programmes can be undertaken
33 anytime without the time-related dormancy limitation, development of suitable dormancy-
34 breaking techniques are of paramount importance. Generally, treatments used to break seed
35 dormancy may include mechanical scarification, chemical scarification (especially sulfuric
36 acid), cold-wet, hot water, electrasonic waves and stratification [14-15]. Moreover,
37 germination of cassava seeds can be favored by dry heat and complete darkness [16].
38 Genetically, cassava clones are highly heterozygous and sexual propagation (propagation
39 through seeds) results in a wide diversity of phenotypes, which is of interest to breeders [17].
40 This discovery has endeared to breeders who have started to use cassava seeds as the
41 starting material in their breeding programmes. There is however scanty information on how
42 pre-germination treatments and storage duration affect seed quality.

43 This study sought to determine the most suitable pre-germination treatment for high
44 germination in cassava seeds stored for breeding programmes.

45 2. MATERIAL AND METHODS

46

47 2.1 Experimental Site

48 The experiment was conducted at the CSIR-Crops Research Institute (CRI), Fumesua, near
49 Kumasi from June, 2015 to January, 2016. Specific tests and analyses were carried out in
50 laboratories at the CRI, Faculty of Agriculture, KNUST, Kumasi.

51 2.2 Source of Cassava Seeds

52 Cassava seeds were obtained from cassava trial fields at the CSIR-Crops Research Institute
53 (CRI), at Fumesua, near Kumasi. The seeds were harvested from growing cassava plants.
54 The cultivars collected were Ahwengyanka-1, Ahwengyanka-2 and Aworowa-3. The seeds
55 were cleaned, dried and put in paper bags. Seeds were stored under ambient temperature
56 conditions in the cassava seed storage room for periods of three and six months. The
57 storage environment had a temperature range of 25°C – 30°C. Daily monitoring and
58 recording of temperature and relative humidity were done for the entire storage period.

59 2.3 Experimental Design

60 A 3x3x7 factorial arrangement in Completely Randomized Design was used. The
61 experiment was replicated three times. The first factor was variety at three levels
62 (Ahwengyanka-1, Ahwengyanka-2 and Aworowa-3). The second factor was storage period
63 at three levels (0 month, 3 months and 6 months). The third factor was pre-germination
64 treatment at seven levels (Hot water soaking (HW), Cold water soaking (CW), Acid
65 scarification for 5 minutes (AS5), Acid scarification for 10 minutes (AS10), Acid scarification
66 for 30 minutes (AS30), Mechanical scarification (MS), and No treatment as Control). The
67 details of the pre-germination treatments (PGT) were as follows:

68 Hot water bath (HW): Thirty seeds of cassava were placed in conical flasks containing
69 200ml of water and put into a water bath at a temperature of 100°C for 30mins. The seeds
70 were allowed to cool after 30 minutes and they were placed into Petri dishes containing
71 wetted paper towels for the germination test.

72 Cold water (CW): Thirty seeds were soaked in tap water for 24 hours after which they were
73 placed on the paper towels in the Petri dishes and observed for occurrence of germination.

74 Acid scarification (AS): Thirty seeds were soaked in 50 mls of concentrated sulfuric acid for
75 periods of (i) 5 minutes, (ii) 10 minutes and (iii) 30 minutes. After each period, the seeds
76 were thoroughly washed with tap water to remove any acid residue and a litmus paper was
77 used to test for the presence or absence of acid. The seeds were placed in the Petri dishes
78 when the absence of acid was confirmed.

79 Mechanical scarification (MS): The seed coat of thirty seeds was scraped with sand paper
80 to remove some part of the seed coat for easy imbibition of water. The seeds were placed on
81 the paper towels in the Petri dishes and germination was observed.

82 **2.4 Data Collected**

83 **2.4.1 1000 seed weight**

84 The weight of 1000 seeds was determined as recommended by the International Seed
85 Testing Association [18].

86 **2.4.2 Temperature and relative humidity of storage room**

87 The temperature and relative humidity readings of the storage room (ambient) were taken at
88 specific times during the day. An Acurite manufactured indoor digital temperature and
89 humidity monitor (00325) was used in taking the various readings.

90 **2.4.3 Germination percentage**

91 Germination percentage of seeds were determined using procedures recommended by [19].

92 **2.4.4 Determination of seed vigour**

93 Seed vigour was determined using the conductivity test as prescribed by [20].

94 **2.5 Analysis of Data**

95 Data collected were subjected to Analysis of Variance (ANOVA) using STATISTIX version
96 10. Data on germination were square root transformed before analysis. Tukeys Honest
97 Significant Difference (HSD) was used for the separation of treatment means. The
98 probability of declaring significant differences between treatments was set at $P=.01$.
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100

101 **3. RESULTS**

102

103 **3.1 Ambient Temperature and Relative Humidity of Storage Environment**

104 The storage temperature ranged from 26°C to 32°C whereas the relative humidity ranged
105 from 84% to 91%. The minimum temperature was recorded in September, 2015 while the
106 maximum was recorded in November, 2015. The minimum relative humidity was recorded in
107 July, 2015 while the maximum was recorded in August, 2015 (Table 1).

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111 **Table 1: Minimum and maximum temperature and relative humidity of storage**
 112 **environment**

Month	Minimum Temperature (°C)	Minimum Relative humidity (%)	Minimum temperature (°C)	Maximum relative humidity (%)
June	29	88	24	88
July	27	84	23	89
August	28	86	22	91
September	26	89	21	87
October	30	86	24	88
November	32	84	25	86
December	30	85	24	86

113

114 **3.2 Effects of Cultivar and Storage Period on Percentage Germination (%) of**
 115 **Cassava Seeds.**

116 Significant variety x storage period interactions were observed in the percent germination of
 117 cassava seeds (Table 2). Seeds of all three cultivars stored for six months produced
 118 significantly the highest percent germination, while seeds of Ahwengyanka-1 and
 119 Ahwengyanka-2 without storage produced the least germination percentages.

120 Among the cultivars, Aworowa-3 seeds produced significantly higher germination
 121 percentages than Ahwengyanka-1 and Ahwengyanka-2 which produced the least yet similar
 122 germination percentages.

123 Among the storage periods, seeds stored for six months produced significantly the highest
 124 germination percentage, 1.94 times greater than the least obtained from seeds without
 125 storage (Table 2).

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129 **Table 2: Effects of cultivar and storage duration on the germination percentage of**
 130 **cassava seeds**

Storage Duration	Germination Percentage (%)			
	Cultivars			
	Ahwengyanka-1	Ahwengyanka-2	Aworowa-3	Mean
No storage	28.1	27.6	36.2	30.6
Three months	38.6	38.6	41.4	39.5
Six months	58.6	58.6	60.9	59.4
Mean	41.8	41.6	46.2	
HSD (0.01): Cultivar =2.54; Storage =2.54; Cultivar x Storage = 5.40				

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132 **3.2.1 Effects of cultivar and pre-germination treatments on the percent germination of**
 133 **cassava seeds**

134 Significant cultivar x pre-germination treatments interactions were observed for percent
 135 germination (Table 3). Aworowa-3 seeds treated by mechanical scarification with sand paper
 136 produced significantly the highest percent germination although similar to Aworowa-3 seeds
 137 treated with hot water and Aworowa-3 seeds soaked in cold water. The least percent
 138 germination was recorded for Ahwengyanka-2 seeds without any pre-germination treatment.

139 Among the cultivars, Aworowa-3 seeds produced significantly higher percent germination
 140 than Ahwengyanka-1 and Ahwengyanka-2 which produced the least yet similar germination
 141 percentages.

142 Among the pre-germination treatments, seeds treated with mechanical scarification with
 143 sand paper (MS) produced significantly the highest percent germination, 40.4% greater than
 144 the least obtained from seeds without treatment any pre-germination treatments (Control)
 145 (Table 3). The other pre-germination treatment methods also produced germination
 146 percentages which were 28.7% (Hot and cold water) and 23.4% (acid scarifications 5, 10
 147 and 30 mins) greater than the control.

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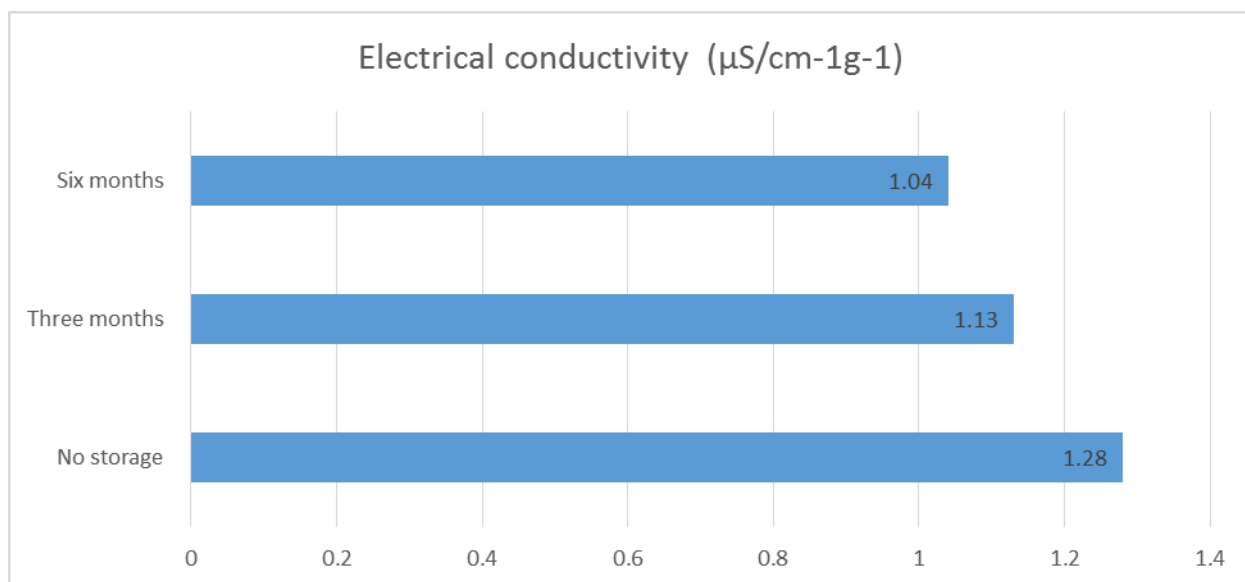
151 **Table 3: Effects of cultivar and pre-germination treatments on percent germination of**
 152 **cassava seeds**

Pre-germination treatments	Germination Percentage (%)			
	Cultivars			Mean
	Ahwengyanka-1	Ahwengyanka-2	Aworowa-3	
Mechanical scarification	45.6	44.4	56.7	48.9
Control	30.0	30.0	44.4	34.8
Cold water	43.3	43.3	47.8	44.8
Hot water	43.3	43.3	47.8	44.8
Acid scarification-5mins	43.3	43.3	42.2	42.9
Acid scarification-10mins	43.3	43.3	42.2	42.9
Acid scarification -30mins	43.3	43.3	42.2	42.9
Mean	41.8	41.6	46.2	
HSD (0.01): Cultivar =2.54; PGT =4.59; Cultivar x PGT = 9.18				

153

154 **3.3 Effect of Storage Periods on Vigour of Cassava Seeds**

155 Significant differences were obtained in the storage periods for the electrical conductivity of
 156 the seeds. Seeds without storage produced significantly the highest electrical conductivity
 157 value ($1.28 \mu\text{S}/\text{cm}^{-1}\text{g}^{-1}$) whereas the least conductivity value was obtained from seeds
 158 stored for six months ($1.04 \mu\text{S}/\text{cm}^{-1}\text{g}^{-1}$) (Fig 1).



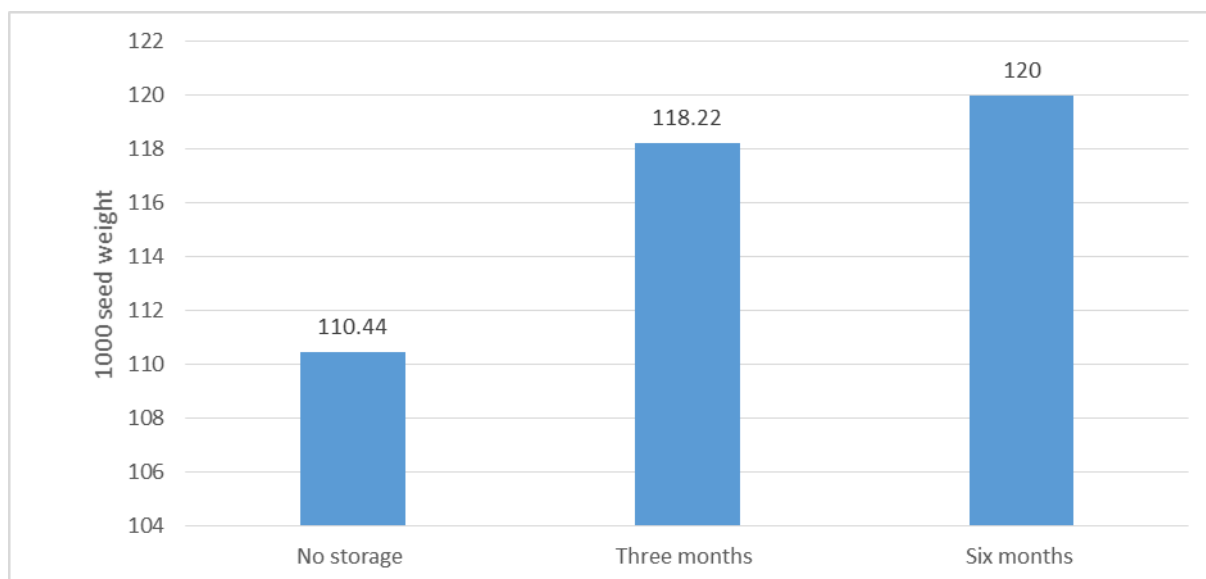
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161 **Fig 1: Effect of storage period on electrical conductivity (vigour) of cassava**
162 **seeds.**

163 **4.5 Effects of Storage Periods on 1000 Seed Weight of Cassava Seeds**

164 Seeds stored for six and three months were significantly heavier than those without storage.
165 Seeds without storage had the least 1000 seed weight, 9.56 % and 7.78 % less in weight
166 than those stored for six months and three months, respectively (Fig 2).

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170 **Fig 2: Effect of storage period on 1000 seed weight of cassava seeds**

4.0 DISCUSSION

In the present study, cassava seeds stored for six months produced the highest 1000 seed weight. Interestingly, increasing seed weight was observed to be directly proportional to the lengthening of the storage period. This observation could be attributed to the high relative humidity recorded during the time of storage which resulted in the imbibition of moisture by the seeds during the period of storage. The 1000 seed weight obtained for the three cultivars of cassava seeds used in this study agrees with the findings of [21] that on the average, the 1000 seed weight of cassava seeds was 100g.

The pre-germination treatment accorded seeds of a cultivar may have an effect on their germination response [22]. This finding was evident in the present study where Aworowa-3 seeds recorded profound differences in germination percentage depending on the pre-germination treatment. When Aworowa-3 seeds were mechanically scarified with sand paper they produced a germination percentage of 56.7% as against when similar Aworowa-3 seeds were acid-treated (42.2%). The seeds that were treated by mechanical scarification using sand paper produced the highest germination rate while the seeds with no treatment produced the least. This observation agrees with [23] who reported that pre-treatment of seeds by mechanical scarification had significant effects on the probability of water imbibition leading to increase in germination by seeds. Mechanical scarification of dormant seeds has also been found to be an efficient method in increasing the rate of germination over time in *Acacia* spp. seeds [24], seeds of *Adesmia* spp. [25] and cassava seeds [26]. Cassava seeds exhibit the exogenous type of dormancy due to the hardness of the seed coat and mechanical scarification is one of the best methods used in breaking physical or exogenous dormancy in cassava seeds. Moreover, [27] indicated that mechanical scarification was an effective method used in breaking physical, mechanical and chemical dormancy in seeds since the impermeability of hard seed coat to water and gases had been found to be part of the many causes of poor germination in seeds [28].

In the present study, seeds treated with sulfuric acid recorded lower germination rates but this contradicts an earlier study by [29], who reported that treating *Peltophorum pterocarpum* seeds with sulfuric acid yielded no germination. He explained that, the acid acted mainly on the micropylar and hilar regions of seeds and higher acid concentrations and long soaking periods not only damaged the soft tissues, but it created cracks on the testa, which allowed the acid to gain access into the embryonic tissue causing the death of the seed. In the present study, the rate of germination of seeds treated with sulfuric acid was about 42 %, an indication that sulfuric acid had a positive effect on the germination of cassava seeds, an observation also found by [30]. Both short (5 minutes) and long (30 minutes) periods of immersion recorded germination. In other studies, the dormancy in *Cassia fistula* seeds was released when the seeds were soaked in concentrated sulfuric acid for 15 minutes [31]. Similarly, [32] obtained significantly higher germination rate of *Peltophorum pterocarpum* seeds treated by soaking in sulfuric acid for 5 – 75 minutes, washed and soaked in distilled water for 8-24 hours. These results however contradict those of [33] and [34] who indicated that prolonged immersion of seeds in sulfuric acid (H_2SO_4) injured the seeds and also destroyed some vital parts of the embryo leading to low or no germination. The occurrence of germination in the seeds pretreated with hot water but not stored could be due to the ability of the hot water to cause rupture of the hard seed coat which allows water imbibition leading to seed germination and further rapid emergence [35-36]. In the present study, the germination percentages recorded for cassava seeds were higher than the 10-40% previously reported [37].

Among all three varieties, Aworowa-3 had the highest mean germination percentage. This could be attributed to several factors such as varietal differences, the oil and protein contents, weight of seed, moisture content, among others. The Aworowa-3 seeds stored for

221 six months produced the highest germination. It proved to be the best of the varieties used in
 222 this study since germination even occurred without storage whereas the remaining two
 223 varieties failed to germinate. From the present study, percentage vigour increased as
 224 storage increased implying that vigour of cassava seeds increases over time; this could be
 225 due to the chemical processes that take place in the seeds during storage such as the
 226 release of dormancy which occurs as storage increases. The increase rate of vigour could
 227 ascertain the reason why germination rates increased with increasing storage. However,
 228 even though the seeds without storage had a significantly high leachate rate which indicates
 229 low percentage vigour [38], they still produced an appreciable rate of germination (about
 230 40%). The pattern of vigour of cassava seeds does not agree with the findings of [39] that
 231 vigour declines in storage as a result of deterioration of seed quality which precedes loss in
 232 standard germination and this could be attributed to the inherent dormancy exhibited by
 233 seeds of cassava.

234 The increasing rate of germination with increasing storage confirms reports of [40] that the
 235 length of storage caused the release of dormancy in seeds of *Peltophorum pterocarpum*.
 236 [41] also observed that an increase in the rate of germination occurred with an increasing
 237 storage period, especially in *Manihot glaziovii* and *Manihot pseudoglaziovii* seeds. Similarly,
 238 [42] reported that there was an increase in the rate of germination of stored seeds of
 239 different plant species and explained that the room temperature adequately attenuated the
 240 intensity of dormancy and caused acceleration in the germination and emergence of
 241 seedlings.

242 Conditions such as the temperature and relative humidity of the storage environment also
 243 play major roles in seed quality maintenance or deterioration. High temperature and relative
 244 humidity (25°C/75%) in storage environments cause seeds to deteriorate at a faster rate
 245 thereby leading to lower seed vigour and germination percentages than storage
 246 environments with low temperature and relative humidity (12°C/60%) [43-44].

247 5. CONCLUSION

248 A rate of 40% germination was obtained from cassava seeds stored up to three months. This
 249 is appreciable since seeds stored up to three months are known to exhibit dormancy which
 250 is usually released after a period of time. Aworowa-3 seeds without storage produced a
 251 higher germination rate than Ahwengyanka-1 and Ahwengyanka-2 seeds without storage but
 252 all the seeds of the three varieties had the highest germination percentage when stored for
 253 six months. Mechanical scarification with sand paper proved to be the best method in
 254 breaking dormancy of cassava seeds especially seeds stored up to three months.

255 COMPETING INTERESTS

256 Authors have declared that no competing exist
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