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Physiological Dormancy in Mature Cassava (*Manihot esculenta* var Brakpo) Seeds

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Abstract Brakpo is a popular cassava variety in Ogoni land, Nigeria whose mature seeds express low and irregular percentage seed germination. This attribute, which is common in many cassava types, limits its use in seedling production and breeding activities. Although seed dormancy is the well-known cause, the exact form of dormancy involved and its control is unclear. The objective of this study was to determine the effect of seed treatments that regulate embryo growth (two alternating cycles of cold (4 ℃) and warm (26-30 ℃) temperatures (CWCW), warm temperature at 40 ℃ and 10 µM Fluridone) and seed coat hardness (scarification and no-scarification) on germination of mature cassava 'Brakpo' seeds. The seeds were collected at brown dry fruit stage and subjected to the treatments in a 2x4 factorial experiment arranged as completely randomized design. Treatment included two alternating cycles of cold (4 ℃) and warm (26-30 ℃) temperatures (CWCW), warm temperature at 40 ℃ and 10 µM Fluridone) and seed coat hardness (scarification and no-scarification). Scarification alone (control) increased percent germination by 20% compared to zero in the untreated control. Embryo growth treatment were more effective when combined with scarification than without. Among the scarified seeds, embryo treatments increased percent germination in the order CWCW (64%)> FLU (4%)> 40 ℃ (2%). Only CWCW induced germination (7%) in non-scarified seeds. Emergence speed index (ESI) and plumule/radicle lengths followed the same trend. This study suggests that scarification plus two cycles of cold and warm temperature can increase germination to acceptable values. Also, dormancy in Brako may be due more to physiological dormancy (inability of viable embryo to grow) (44%) than hardness of seed coat (20%) or structure of seed coat (7%).

Keywords Cassava, Dormancy, Germination, Breeding pace, Seed

1. Introduction

Cassava (*Manihot esculenta Crantz*) is a member of the family Euphorbiaceae. Globally, it is the fourth most important crop being consumed by about a billion people [1]. In Africa, cassava is a dietary staple and it is highly important in food security because the edible root tuber can store long underground, the plant can thrive under poor environmental and soil conditions and it can grow well under mixed cropping systems [2,3]. The tuber is rich in carbohydrates, calcium, vitamin B and C and essential minerals such as potassium, calcium, zinc, iron, phosphorous. Apart from food, cassava starch is applicable in many types of products such as sweeteners, glues, textiles, paper, biodegradable products, monosodium glutamate, and drugs [4]. Cassava chips and pellets are used in animal feed and alcohol production [4]. The peel and leaves are also used in the production of biofuel and so important as alternative energy source. Cassava yield in Nigeria is lower than the world average (10.6 tons per ha in 2002) and much lower than the yield from many countries cultivating far less area of land to cassava. Barbados for example produces 27.3 tons per hectare in [5]. Thus, the high production values from Nigeria [6,1] are largely due to the cultivation of larger land area than most other countries. To meet the demand for cassava in Nigeria, therefore, productivity must increase well above the world average. A major contributing factor to low cassava yield is the widespread use of low yielding (local) disease susceptible varieties among farmers in Nigeria [7,8]. Profitable commercial cassava production depends on the supply of quality stem cuttings that are high yielding, resistant to prevalent pests and diseases such as cassava mosaic disease (CMD) and unfavorable environmental conditions such as drought, *etc*., [9]. It is for this reason that cassava breeders constantly breed (through hybridization) to obtain seedlings with superior qualities. The challenge, however, is that the pace at which breeders obtain seed stock for national service cassava multiplication program is slow [10,8]. This has been attributed to the presence of low

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percentage seed germination [11,7,6] that can be as low as 33% or less when the seeds are collected and planted at fruit maturity stage [6].

Identifying the likely cause(s) of poor seed germination in cassava is key to increasing seed germination. Cassava seeds are commonly collected, in Nigeria, between the months of December and February from stands planted earlier in the year (February-April). These seeds may be stored for up to one year before they are germinated. Viable seeds collected at fruit maturity (*i.e.*, at brown dry fruit stage) are known to stay non-growing for 3 to 6 months while viable seeds that are ready to germination require only about 16 to 40 days to germinate [12]. This observation validates that cassava seed express dormancy.

Dormancy is the phenomenon that prevents seeds of many plants from germinating over a long period of time and keeps seeds at different depths of dormancy, which leads to undesirable staggered germination. Primary seed dormancy is caused by conditions either external to the embryo (exogenous dormancy) or within the embryo (endogenous dormancy). While exogenous dormancy is due to: 1.) physical factors of the seed coat that prevent water or gas intake, 2.) mechanical factors such as the hardness of seed coat, 3,) chemical factors such as the presence of chemical inhibitors, or 4.) all the above [13], endogenous dormancy is due to: 1.) physiological factors that relate to the nature of the embryo, 2.) morphological factors of the embryo (underdeveloped or poorly differentiated embryo), or 3.) a combination of both physiological and morphological factors [14]. Some treatments have been shown to improve seed germination. These include scarification, the application of dry heat (at 60 ℃ for 7 or 14 days or 100 ℃ for a few seconds) that mimic bush burning effect and their combinations [15,16,17,8,18]. Also, acid and low temperature stratification have been shown to promote cassava seed germination [9,7]. Scarification and dry heat treatments are thought to act by breaking hard seed coat inhibition, which in turn promotes water/gas uptake and embryo expansion and hence germination while stratification is believed to enhance germination by triggering embryo activity [19] perhaps through the control of abscisic acid [20]. The effect of cold stratification in the absence of scarification is, however, not well known in cassava. Also, although alternating cold (4 ℃) and warm (26-30 ℃) cycles are reported to increase the permeability of some seed coats to water and gas and increasing embryo growth, the effects of two and three cold and warm cycles on germination of cassava seeds is hardly reported. Although the role of abscisic acid (ABA) in seed germination is well documented, and ABA is thought to control embryo growth potential, the role of abscisic acid (ABA) and the effect of Fluridone on germination of mature cassava seeds is not widely reported. Fluridone is a herbicide that is also known to inhibit a critical step (i.e., the inhibition of the action of the enzyme required for the conversion of phytoene to the ABA precursor carotenes) in the biosynthesis of abscisic acid (ABA). In view of the above and the fact that cassava seeds contain mature viable embryos even before fruit maturity [21,22], this study proposes that low germination among cassava seeds collected at fruit maturity is controlled by a combination of physical factors of the seed coat and physiological factors of the embryo.

Therefore, the objective of this study was to determine the effect of embryo growth regulating treatments (two alternating cold (4 ℃) and warm (26-30 ℃) cycles, warm temperature at 40 ℃ and 10 µM Fluridone) and seed coat treatment (scarification or no-scarification) on germination of cassava seeds collected at seed maturity.

2. Materials and Methods

Experimental location

The experiment was conducted in the Department of Crop and Soil Science, Faculty of Agriculture University of Port Harcourt, Nigeria to determine to effects of various physical and chemical treatments on germination of cassava seeds collected after fruit maturity. The University of Port Harcourt is located between latitude 04 °31' to 05 °00N and longitude 006 °45' 007 °00'E. A propagator was used to store treated seeds under optimum temperature and humidity. The propagator was 2m x 1m x 1m (length, width and height) in dimension with the sides covered with transparent polythene film. Temperature and relative humidity in the propagator were monitored using digital meters.

Seed collection and preparation

Seeds of the local cassava variety var. Brakpo were collected from a farm in Eleme, Rivers State, Nigeria in January 2015 at brown dry fruit stage, which occurs well after seed and fruit maturity. The collection site is located in humid tropical region, characterized by inherently low fertility soils [23]. Fruit maturity usually occurs between October and December of the preceding year depending on variety.

The fruits were wrapped up in a bag, exposed to sunlight and allowed to dehisce. Brakpo was evaluated in this study because it is a popular cassava variety in the area that some farmers in the area described as 'difficult to germinate' and so, brought their travail to the attention of the authors. Samples of the seeds were taken for determination of seed weight and moisture content. The seeds were either scarified (by mechanically thinning down the seed coat) or not scarified following the experimental design. Flotation test was then conducted to separate pseudo seed from true seeds and only true seeds were subjected to the experimental treatments. Surface sterilization of good seeds was done using 1.5% sodium hypochlorite solution containing two drops of liquid soup for 5 minutes. All materials used in the study such as forceps, cotton wool *etc*., were surface sterilized for 10 minutes in 5% sodium hypochlorite solution.

Surface sterilized seeds were rinsed in distilled water and

then placed in a sterile petri dishes lined with moist cotton wool. Each petri dish was sealed with masking tape to reduce desiccation and then each petri dish was wrapped in a black polythene bag to prevent light and then, they were placed in the propagator. Dark storage is known to promote germination in cassava [17].

Experimental Treatments

Treatments were:

1. Non scarified + no treatment (Non scarified control)
2. Non scarified + soak in warm water at 40 ℃ for 6 hr
3. Non scarified + two alternating cycles of moist cold temperature (4 ℃) for 2 hr followed by moist warm temperature (26-33 ℃) for 2 hrs,
4. Non scarified + 10 µM Fluridone for 6 hr
5. Scarified + no treatment (control)
6. Scarified + soak in warm water at 40 ℃ for 6 hr
7. Scarified + two alternating cycles of moist cold temperature (4 ℃) for 2 hr followed by moist warm temperature (26-33 ℃) for 2 hrs, (CWCW)
8. Scarified + 10 µM Fluridone for 6 hrs

Two alternating cycles of cold and warm temperature treatment

This treatment was chosen because alternating freezing and thawing technique is known to improve seed coat permeability to water and gases while moist cold temperature promotes embryo growth potential. However, the effects of alternating freezing and thawing temperatures on germination of cassava seed is not well documented. In this treatment, seeds on moistened cotton wool were exposed to low temperature at 4-5 ℃ for 2 hrs after which the petri dishes were transferred onto a raised concrete floor directly intercepting the sunlight and heat energy. Temperature on the surface of the peri dishes ranged from 26 to 33 ℃ depending on time of day. The cycle was repeated after which, the petri dishes were transferred to the propagator.

Experimental design and data collection

There were two levels of seed thinning treatment and embryo growth enhance treatment at four levels germination inducing treatments. Therefore, the experiment was arranged as a 2 x 4 factorial with 8 treatment combinations, replicated three times and 15 seeds per replicate. Seeds were observed daily, over 30 d from treatment day, for date of radicle and plumule emergence. Data on number of leaves and length of plumule and radicle were recorded.

Data analysis

Percentage germination

Percentage emergence was calculated by dividing the number of germinating seeds per treatment by the total number of treated seeds per treatment and multiplying the result by 100.

Emergence Speed Index (ESI)

Emergence Speed Index (ESI) was calculated using the equation below:

$$ESI = EI/NI + E2/N2 + ... + Ei/Ni \quad (1)$$

Where; ESI = emergence speed index; E1, E2…..Ei = number of seedling at the first, second…..and last count; N1, N2….Nn = number of days after the sowing until the first, second….and last count [25].

Mean Time (days) Emergence (MTE)

Mean Time to Emergence (MTE) was calculated using the equation below:

$$MTE = (E1*NI) + (E2*N2) + ... + (En*Nn)/Etotal \quad (2)$$

Where: MTE = Mean time (days) for emergence of seedlings; E1, E2...En = number of seedlings that have emerged at the first, second...and last day; Nn = number of days taken to emergence; Etotal = total of seedling that have emerged per treatment [26].

Data derived from Emergence Speed index (ESI), Mean Time (days) Emergence (MTE) and seedling growth was run using General ANOVA, two-way anova program on GENSTAT Discovery Edition 4 software. Means were separated using standard error of difference (s.e.d) at 5% probability level.

3. Results and Discussion

Temperature and relative humidity in the propagator and seed parameters

Average temperature in the propagator during the morning, afternoon and evening hours were 20.9 ℃, 26.5 ℃ and 22.9 ℃ respectively while the relative humidity values were 75, 65% and 86% respectively. Thus, generally, the air temperature in propagator was within favorable ranges for germination. In this study, the average fresh weight of 100 seeds was 8.12 g.

Effect of seed treatment on percentage germination

Table 1. Effect of treatments on % seed germination

	Scarified	Non-scarified
Control	20	0
CWCW	64	7
10 µM FLU	4	0
40 ℃	2	0

CWCW= two alternating cycles of cold (4 ℃) and warm temperatures (26 ℃)

In the controls, percentage seed germination moved from zero under non-scarified treatment (experimental control) to 20% when seeds were scarified (treatment control) (Table 1). A zero seed germination in the treatment control shows that seeds of Brakpo cassava cultivar express dormancy as observed by the farmers in its growing area. It also indicates that its dormancy can be slightly managed by using scarification. In this study, the effect of scarification on germination was similar to that reported for other cassava species [7] and seeds of other plant species [27]. The small effect of scarification on percentage seed germination

suggests that the dormancy expressed by Brakpo is due only to a small extent by the presence of hard seed coat. Dormancy caused by the presence of hard seed coat has also been reported in over 15 angiosperm families [14,12] but not in the cassava cultivar Brakpo.

By applying two cycles of cold and warm temperatures (CWCW) on moist imbibed seeds, percentage germination was increased by 44% among scarified seeds compared to that in experimental control. Among non-scarified seeds, CWCW increased percent germination by only 7% compared to the non-scarified control. The 64% seed germination recorded by applying CWCW on scarified seeds, was the highest observed in this study and highly remarkable when compared with the data in the controls or existing reports. The ability of CWCW to highly increase the number of germinating scarified seeds while only slightly increasing the number of germinating non-scarified seeds suggests that dormancy in cassava cv. Brakpo is strongly related to factors internal to the embryo then factors external to it (such as low embryo growth potential and impermeable seed coat). This finding suggests a strong role of embryo dormancy on cassava (Brakpo) seed germination and the effect of CWCW on cassava seed germination is not common. Although it is not how CWCW worked to induce germination on scarified seeds and slightly on non-scarified seeds, it may have been related to the capacity of freezing-low temperature to induce embryo growth as well as the capacity of alternating temperatures to solubilize numerical apertures associated with impermeability of seed coats to water and gas [13,19]. In nature, the development of the specialized structures are environment sensitive; coinciding with the approach of the last phase of seed development when desiccation begins, seed coats harden and dormancy deepens, and their dysfunction coincide with the perception of favourable environmental cues (such as temperature) at the start of the growing period [24,22]. The caruncle, which has been suggested to play possible roles in the perception of environmental changes and water uptake was unharmed during seed treatments. The effect of two alternating cold and warm temperatures (CWCW) on percentage germination also suggests a likely role of gibberellic acid and abscisic acid (ABA) in the control of embryo growth of some seed since cold temperature treatment have been shown to elicit ABA induced germination responses [20].

The insignificant effects of 10 µM FLU treatment on the percentage of germinating scarified seeds indicate that abscisic acid (ABA; a growth inhibiting hormone whose concentration increases during the last phase of seed development and decreases as seeds approach their natural germinating time) may play a role in determining the rate of germination in mature dormant cassava 'Brakpo' seeds. A similar lack of effect of FLU in the enhancement of germination of dormant immature seeds (with mature embryos) of two varieties of cassava was reported by others [21,22]. The finding in this study shows that FLU does not promote germination of mature cassava seed. It also suggests a possible role of ABA in embryo growth as shown in coffee [20] or a role of ABA in yam tuber dormancy [28,29].

The insignificant effects of 40 ℃ treatments on the percentage of germinating scarified cassava seeds indicates that warm temperature (at 40 ℃) alone may not play significant role in determining the rate of germination in cassava 'Brakpo' seeds. Although it is not clear whether temperature in the seed was lower or higher than 40 ℃, the immersion of cassava seeds in warm water at 80 and 90 ℃ for two mins has also been reported not to induce germination [7].

Treatment effects on Emergence Speed Index (ESI)

Radicle ESI was significantly ($P<0.05$) affected by seed coat thinning treatment (scarification), embryo growth enhancing treatments, and their interaction (Table 2). The trend in Table 2 is same as that presented for Table 1 above. Therefore, the significantly faster ESI under CWCW treatment explains why percentage germination was higher under CWCW treatment. Embryo growth potential (as discussed by Royal Tasmanian Botanical Garden, 2015) may clearly higher in the CWCW treatment than in the control other treatments and appears to have contributed significantly to the higher percentage of germinating seeds.

Table 2. Effect of seed treatments on radicle Emergence Speed Index (ESI)

	Control	CWCW	FLU	40 ℃
Scarified	0.403	1.126	0.186	0.075
Non-scarified	0	0.094	0	0
SED_{Int} (P<0.05)	0.1445			

Effect of seed treatments on average days from treatment to radicle emergence

Throughout this study, no germination was observed amongst the non-scarified seeds except in the CWCW treatment where low percentage (7%) germination was observed at 13 + 0 (SD) d after treatment (Table 3). Thus, the non-scarified cassava seeds expressed long dormancy that was as long as at least 30 days (*i.e.*, the duration of observation). Among the scarified seed lot, the first signs of germination occurred at 6 days after treatment especially under the CWCW treatment. Average duration to germination was 8 or 9 days depending on treatment. Thus, while the treatments did not significantly affect the duration to germination when the seed coat was thinned, the duration to germination was highly delayed by not thinning down seed coats.

These finding indicates that dormant viable cassava seeds collected at brown dry fruit stage can commence growth and emerge out of seed coats (germination) within the same time range as viable non dormant seeds if the seeds are scarified (seed coat thinned down) and then given favorable growing conditions such as CWCW. According to a report [12], viable seeds that are ready to germination require only about 16 to 40 days to germinate while the dormant viable seeds refuse to grow until 3 to 6 months after their collection at brown fruit stage.

Table 3. Effect of seed coat treatment and embryo treatment on average days from treatment to radicle emergence

	Control	CWCW	FLU	40 ℃
Scarified	9.1 ±3.2	9.2 ±2.8	9.1 ±3.2	9.1 ±3.2
Non-scarified	0	13.0 ±0	0	0

Values after the ± sign are standard deviation (SD)

Effect of seed treatments on early seedling growth

Due to absence of or presence of poor germination, the effect of some treatments were are assessed here. At the end of the study period, both plumule and radicle lengths were significantly longer when the seeds were giving two alternate cycles of cold and warm temperatures preceded by scarification than when scarified alone (Table 4). Similarly, the lengths were about two times longer when the seeds were scarified than non- scarified. This trend was similar to that observed for the effect of the treatments on percentage germination. Thus, these results show that the application of measures that enhance embryo growth while removing growth restrictions caused by seed coat promote early seedling growth better than removing seed coat restrictions alone.

Table 4. Effect of treatments on plumule and radicle length

	Control	CWCW
	Plumule length	
Scarified	3.85	5.73
Non-scarified	1.08	0
Sed (P<0.05)	1.19	
	Radicle length	
Scarified	1.8	2.12
Non-scarified	0.9	0
Sed (P<0.05)	0.553	

4. Conclusions

This study has shown that scarification (thinning down of seed coat) needs to be combine with treatments that enhance embryo growth to obtain high germination rate in cassava (Brakpo). The combination of scarification and two cycles of cold (4 ℃) and warm temperature (26-30 ℃) for 6 hrs each time can increase radicle and plumule germination of mature cassava seeds to as high as 64%. This combined treatment also increased radicle emergence speed index (ESI) and enhanced early seeding growth (radicle length). Also, it showed that warm temperature alone was not enough to stimulate embryo growth in scarified seeds, and ABA may play a role in the control of embryo growth. This imply that dormancy in Brako is determined by physiological dormancy (inability of viable embryo to grow) (44%), followed by hardness of seed coat (20%) and structure of seed coat (7%). The findings from this study could help improve the selection process in cassava breeding. Further studies that compared CWCW and cold temperature treatments may be investigated as well as studies that test the effect of a combination of cold temperature and FLU, FLU alone and ABA alone.

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