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Genetic Variability in Seed Dormancy, Germination and Germination Enhancement of Some Cassava (*Manihot esculenta* Crantz) Genotypes

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ABSTRACT

The sustainability of cassava production for food security and agro-industrial demands amidst the growing population and changing global environmental conditions is an interest for breeding programs. The response of cassava seeds to dormancy and germination is a prerequisite for the choice of parents for eventual hybridization. A screen-house experiment was carried out to investigate variations in the dormancy and germination of cassava seeds at the International Institute of Tropical Agriculture (IITA) Ibadan. The study involved a preliminary germination test of seeds of 30 cassava genotypes. Four genotypes (TMe359', 'TMe1700', 'TMe1747' and 'TMe1945') were selected for a test of probable differential responses to two temperature regimes of (45 and 55°C) at three continuous intervals of 5, 10 and 15 days. There were significant differences in the genotypes ($P \leq 0.05$) in days to germination and percentage of germination. Significant differences ($P \leq 0.05$) also existed among the genotypes at different temperatures for days after planting. Each of the four genotypes demonstrated unique quadratic trend response to imbibition in hot water. Wet heat treatment appropriately aided imbibition in the four genotypes studied. Genotypic variation in germination as observed in this study could be a guide for cassava breeders for selection of genotypes with low dormancy as a maternal parent for crosses.

Keywords: clones, dormancy release, germination, genotypes, imbibition
Abbreviations: COSCA, Collaborative study of Cassava in Africa; **FAO**, Food and Agricultural Organization; **IITA**, International Institute of Tropical Agriculture

INTRODUCTION

Cassava (*Manihot esculenta* Crantz), a member of the family *Euphorbiaceae*, and the major cultivated species of the genus *Manihot*, is native to the Amazon in South America (Olsen and Schaal 1999; Nassar and Ortiz 2009), where the American Indians have cultivated it for more than 5000 years. It is a perennial woody shrub whose starchy root is widely consumed daily by more than 800 million people worldwide especially in Africa, Asia and Latin America. Cassava production has since shifted from being a mere subsistence crop to being a commercial crop in sub-Saharan Africa (COSCA 1994). The crop has a potential of a famine crop where other crops have failed (Hahn *et al.* 1979). Cassava has played a vital role in the food security of sub-Saharan Africa. Its ever increasing importance is linked to its tolerance to environmental stresses such as drought. It is proposed that the crop will play a greater role in hunger alleviation and continental food and energy security in the coming decades (FAO, 2008). It is highly rich in carbohydrates, being the third source of calories in the tropics after rice and corn (FAO 2002). Most recently, cassava is increasingly gaining attention in the developing world as an attractive biofuel crop; the ethanol from it has a strong energy balance which can contribute to agro-industrial development in the tropics (African Agriculture 2007).

Cassava (*Manihot esculenta*), also called manioc, tapioca or yuca, is one of the most important food crops in the humid tropics, being particularly suited to conditions of low nutrient availability and able to survive drought (Burrell 2003). More than two-thirds of the total production of cassava is used as food for humans, with lesser amounts being used for animal feed (Nwokoro *et al.* 2002) and industrial purposes. Cassava is the sixth most important staple food crop after wheat, rice, maize, potato and barley, feeding more than 800 million people in the poorest tropical countries world-wide (Srinivas and Anantharuman 2000). Among the starchy staples, it has 40% more carbohydrate than rice and 25% more than maize, making it the cheapest source of calories for human nutrition and animal feeding. The part most commonly used for food is the starchy root, but the leaves and shoots are high in protein and are also consumed by humans and used as animal feed. Although a native of the Neotropics (Olsen and Schaal 2001), cassava is cultivated in the tropical and subtropical regions of Africa, Asia and Latin America. Africa alone produces over 50% of the world's cassava (FAO 2009), and is used by industries for starch-based products, such as alcohol (Sriroth *et al.* 2000; Tonukari 2004).

Cassava is a monoecious plant which produces ovoid or globular trilocular capsule by open-pollination. The resultant viable seeds are mostly heterozygous. When they get established in farmer's field, they introduce variability into the otherwise uniform clonal materials. Cassava breeders have observed for many years that true-seeds derived plants can be highly productive under good management conditions (Belenos 1987). Seeds generated from natural hybridization may have combined genes suitable for higher productivities, resistance, and adaptability than clones. The wide genetic diversity in cassava ensues from indigenous

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Table 1 Identity of thirty cassava genotypes.

Genotypes	Origin	Breeding status	Latitude	Longitude
TMe 359	Nigeria	F1 (58308 X Isunikankiyan)	Unknown	Unknown
TMe 1700	Nigeria	Traditional/Landraces	8.3000002	4.3000002
TMe 2122	Benin	Traditional/Landraces	8.2333002	2.5999999
TMe 3126	Benin	Traditional/Landraces	6.7333298	2.1333301
TMe 1945	Benin	Traditional/Landraces	Unknown	Unknown
TMe 435	Benin	Traditional/Landraces	Unknown	Unknown
TMe 1559	Nigeria	Traditional/Landraces	Unknown	Unknown
TMe1884	Benin	Traditional/Landraces	Unknown	Unknown
TMe1882	Benin	Traditional/Landraces	Unknown	Unknown
TMe 2974	Benin	Traditional/Landraces	6.7333298	2.1333301
TMe 2980	Benin	Traditional/Landraces	6.7333298	2.1333301
TMe 2052	Benin	Traditional/Landraces	Unknown	Unknown
TMe 2098	Benin	Traditional/Landraces	11.0167	2.25
TMe 2063	Benin	Traditional/Landraces	Unknown	Unknown
TMe 1953	Benin	Traditional/Landraces	Unknown	Unknown
TMe 903	Nigeria	Traditional/Landraces	Unknown	Unknown
TMe 2109	Benin	Traditional/Landraces	7.633299	2.2167001
TMe 2064	Benin	Traditional/Landraces	Unknown	Unknown
TMe 1696	Nigeria	Traditional/Landraces	7.9000001	3.9000001
TMe 3142	Benin	Traditional/Landraces	6.7333298	2.1333301
TMe 1053	Nigeria	Traditional/Landraces	Unknown	Unknown
TMe 1668	Nigeria	Traditional/Landraces	4.9000001	6.6999998
TMe 663	Nigeria	Traditional/Landraces	Unknown	Unknown
TMe 551	Nigeria	Traditional/Landraces	Unknown	Unknown
TMe 483	Nigeria	Traditional/Landraces	Unknown	Unknown
TMe 1673	Nigeria	Traditional/Landraces	4.9000001	6.9000001
TMe 537	Nigeria	Traditional/Landraces	Unknown	Unknown
TMe 407	Nigeria	Traditional/Landraces	Unknown	Unknown
TMe 864	Nigeria	Traditional/Landraces	Unknown	Unknown
TMe1747	Nigeria	Traditional/Landraces	7.5	11.1

selection and breeding despite the preponderance of vegetative stem propagation of the crop (Kensinger 1975; Boster 1984; Alves 2002). Rajendran *et al.* (2000) remarked that the true seeds from cassava have been used for propagation in commercial scales.

Seed dormancy is a well documented occurrence in *Euphorbiaceae*; in the genera: *Aleurites* (Eakle and Garcia 1977), *Hevea* (Keleny and Van Haaren 1967), *Manihot* (Nassar and Teixeira 1983) and *Ricinus* (Lago *et al*. 1978). Seed storage behaviour in *Euphorbiaceae* is generally orthodox; however there are exceptions. For example, seeds of *Hevea brasiliensis* are recalcitrant (Handbook of Seed Technology for Genebanks 1985). Dormancy which blocks germination evolved differently across species through adaptation to the prevailing environment so that germination occurs when conditions for establishing a new plant generation are likely to be suitable (Finch-Savage and Leubner-Metzger 2006). In cassava, newly harvested seeds are usually dormant for about 3–6 months of storage at ambient temperature (Ellis *et al.* 1982). Salick *et al.* (1997) however remarked that they could lie dormant for an unknown length of time. The wild species can exhibit extreme dormancy (Nassar and Teixeira 1983).

Seed dormancy can stall crop breeding progress (Morris *et al.* 1989; Morris and DeMacon 1994; Maiti *et al.* 2006). Germination have been observed from dormant seeds by their exposure to high temperatures (40–60°C) for several days to months or to extremely high temperatures (80–120°C) for minutes to several days (Tiue *et al.* 2001). The optimum high temperature depends on the species; there may be variations within species too (Harrington 1923; Thompson 1974). The time required for dormancy to break at high temperatures ranges from several weeks to many months, depending on the species (Baskin and Baskin 1984). The multiplication rate of cassava is very low compared to most seed-propagated crops. While this may not be a major constraint where stable and good stem management practices are available for planting, it could be a major constraint when a new variety is to be developed. Low seed dormancy or efficient dormancy-breaking protocol is required in cassava breeding research where valuable hybrid seed may be lost due to non-germination. This need has necessitated the study. Therefore, the objective of this study was to determine the variability among seeds of cassava genotypes to various treatments for initiation of germination of dormant seeds.

MATERIALS AND METHODS

Preliminary germination test

Seeds of 30 cassava genotypes, 15 each from Nigeria and Republic of Benin respectively (**Table 1**) were collected from the medium term storage (5°C) of the Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria for a two-phase experiment. These cassava seeds have been in storage for over two years. The first experiment entails a preliminary germination test of seeds of the 30 genotypes. 4 genotypes were selected from the preliminary germination test based on their germination percentages comprising (TMe 1747) with a high germination percentage of 80% (TMe 1700 and TMe 1945) having low germination percentages of 10% and TMe 359) with germination percentage of 3.3%.

A seed each was sown at 1.5 cm depth into sterilized top soil per pot. Ten individual pots were arranged in a row of three replicates for each genotype in an insect free screen house at IITA, Ibadan.

Oven-dried heat treatment on seeds

The second phase involved three factors including four selected genotypes from the preliminary germination test ('TMe359', 'TMe1700', 'TMe1747' and 'TMe1945'), two temperature regimes (45°C and 55°C) and three time-lag of continuous oven-drying (5, 10 and 15 days). These 24 treatment combinations were laid out as a factorial experiment in completely randomized design (CRD). Three open aluminum cans were labeled: A, B and C to signify the 3 continuous time-lags of 5, 10 and 15 days, respectively. 30 seeds of each of the 4 genotypes were placed inside a labeled open aluminum can. These were subjected to dry heat treatment inside a preset oven at the temperature regimes of 45°C and 55°C, respectively. The seeds were allowed to cool at room temperature.

Table 2 Days after planting (DAP) and germination percentage of seeds of 30 cassava genotypes.

Genotypes	DAP	Germination percentage (%)
TMe 359	1	3.3
TMe 1700	3	10
TMe 2122	3	10
TMe 3126	3	10
TMe 1945	3	10
TMe 435	4	13.3
TMe 1559	5	16.6
TMe1884	5	16.6
TMe1882	6	20
TMe 2974	7	23.3
TMe 2980	7	23.3
TMe 2052	8	26.6
TMe 2098	9	30
TMe 2063	9	30
TMe 1953	11	36.6
TMe 903	12	40
TMe 2109	12	40
TMe 2064	12	40
TMe 1696	13	43.3
TMe 3142	13	43.3
TMe 1053	14	46.6
TMe 1668	15	50
TMe 663	16	53.3
TMe 551	20	66.6
TMe 483	20	66.6
TMe 1673	20	66.6
TMe 537	21	70
TMe 407	21	70
TMe 864	24	80
TMe1747	24	80

Twenty-five seeds from each treatment were planted at the rate of one seed per pot at the depth of 1.5 cm. Each treatment was arranged in a row of five individual pots with five replicates in the screen house.

Wet heat treatment on seeds

The rate of imbibition for water was determined following the procedure of Bansal *et al.* (1980). Five out of the 30 seeds in each treatment were placed on moist filter paper at room temperature and the seeds weights at time zero (0) taken. Subsequent weights of the seeds were done at six hours interval. The rates of water imbibitions by the seeds were estimated. Initial weights of 100 seeds of the four clones were taken. The 100 seeds were then placed in a tied cloth bag and submerged in the water bath at 100°C for 1, 2 and 3 min, respectively. The seeds were removed after treatment from the cloth bags, blot dry and the final weights were taken. The weight difference was determined by subtracting the initial weight from the final weight. After cooling, 20 randomly selected seeds were planted per treatment. The lay out contains five pots per row in four replicates. The rate of sowing was one seed per hole at the depth of 1.5 cm.

Data collection and statistical analysis

Data was generated on the following: days to emergence, percentage of germination, the differences between the initial and the final weight of seeds presented for imbibition and percentage increase in weight after periodic imbibition. Data on the counting for emergence and percentages were transformed by Log10 and Arc sine, respectively to meet the normality assumption of the analysis of variance (ANOVA). ANOVA was carried out using PROC GLM procedure of Statistical Analysis Systems, SAS package (version 9.1, SAS Institute Inc., Cary, NC, USA). The main effects and their interactions were statistically investigated. Mean separation by Duncan's multiple range test procedures was employed to compare the means of the statistically significant effects. Broad sense heritability was estimated following the approach of Toker (2004) and Turk *et al*. (2006).

RESULTS AND DISCUSSION

A wide variation among the cassava genotypes for the days to germination and the percentage germination was observed (**Table 2**). The relationship between the two traits was significant ($P < 0.0001$) 'TMe359' germinated earliest (the next day after planting) with the least percentage of germination; it took 'TMe864' and 'TMe1747' twenty-four days to germinate after planting with 80% germination percentage. None of the genotypes from Republic of Benin had a germination percentage up to 50%. 60% of the genotypes from Nigeria had germination percentage ≥50% (**Table 2**). The genotypes with early germination days may be said to have no problem of seed dormancy. The characteristic behaviour of the seeds of these evaluated genotypes corroborates the assertion of Salick *et al*. (1997) that seeds could lie dormant for unknown length of time. The results obtained showed cassava genotypes with long dormancy period to have a promising and higher germination percentage. The four genotypes selected for this treatment varied significantly ($(P \leq 0.001)$) for days after planting and germination percentage. The interaction between the genotypes and temperature equally varied significantly at ($P < 0.05$) (**Table 3**). Temperature treatment at 45-55°C was found to influence the rate of imbibition for water in some genotypes. The imbibition rate in 'TMe359' improved and seed weight increased by 0.1g when the temperature was 55°C at the same duration of soaking for five days. 'TMe1747' added 0.3 g to seed weight by the temperature differential of 10°C between 45–55°C under 10 days of soaking in water (**Table 4**). All other interactions did not produce any significance increase in seed weight due to imbibition. Dry heat has been reported to favor seed germination especially when the temperature is above 30°C (Halsey *et al.* 2008). The wet heat treatments of the seeds of the four genotypes show that each of the genotypes had a quadratic response. The response of 'TMe1700' and 'TMe1747' was positive while that of 'TMe359' and 'TMe1945' was negative (**Table 5**). The introduction of high wet temperature lowered the rate of imbibition in

Table 3 Estimates of variability among the traits.

Sources of variation	Days after planting	% Germination
Genotypes	17.02**	5408.87***
Temperature	0.96^{NS}	205.38^{NS}
Days	4.46^{NS}	262.50^{NS}
GxT	10.61*	170.74^{NS}
GxD	4.19^{NS}	52.11^{NS}
TxD	0.57^{NS}	10.95^{NS}
GxTxD	3.90^{NS}	120.78^{NS}
Heritability (%)	38.26	85.89

NS – not significant, * - Significant (P≤0.05), ** - Very significant (P≤0.001), *** - Highly significant (P≤0.0001)

Table 4 Increase in seed weight (g) due to water imbibition of four cassava genotypes at different temperatures and duration of soaking in water.

Temperatures	45°C			55°C		
Duration of soaking (days)	5	10	15	5	10	15
Genotypes						
TMe1747	0.2	0.1	0.1	0.2	0.4	0.1
TMe1700	0.6	0.2	0.1	0.1	0.2	0.2
TMe359	0.5	0.1	0.2	0.6	0.1	0.1
TMe1945	0.1	0.1	0.1	0.1	0.1	0.1

Table 5 Quadratic response of four genotypes.

Genotype	Quadratic response
TMe1700	$Y=-4.9x^2+19.8x-5.9$
TMe1747	$Y=-0.595x^2+3.185x+1.41$
TMe359	$Y=0.8x^2-2.7x+6.2$
TMe1945	$Y=1.7x^2-6.5x+8.9$

'TMe359' and 'TMe1945' initially but continuity in the environment led to the increase in the rate of imbibition. However, the response was different in 'TMe1700' and 'TMe1747'. 'TMe1700' increased in the rate of 5.1 (between 1-2 min in hot water) initially and latter decreased in the rate of 4.7 (between 2-3 min in hot water). The response of the seeds of the four genotypes to dry and wet heat treatment is very similar for 'TMe359', 'TMe1700' and 'TMe1745' (**Table 4; Table 5**) except for 'TMe1945' whose response seem recalcitrant to dry heat treatment (**Table 4**). The genotypes responded differently to seed dormancy and germination inducement; this is in agreement to the results of Morris *et al*. (1989); Morris and DeMacon (1994). Genotypic variation in germination as observed in this study could be a guide for cassava breeders to selecting for genotypes with low dormancy as a maternal parent for crosses.

CONCLUSION

Selection for genotypes with low dormancy for maternal parents for crosses will be necessary for successful breeding activity in cassava. Heat treatment will enhance water imbibitions and consequently increased rate of seed germination in cassava.

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