

## Genotypic variations in physiological deterioration of cassava (*Manihot esculenta* Crantz) storage roots under inland valley conditions

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### Abstract

Cassava (*Manihot esculenta* Crantz) is an important starchy food crop in the inland valley ecosystem areas in West Africa where harvesting and marketing infrastructure is not optimal and postharvest losses are high. An attempt was therefore made to assess the genotypic variability of physiological deterioration (PD) of storage roots obtained from plants grown under inland valley field conditions. Cassava storage roots showed rapid PD within 48 hours after harvesting due to wounding related discoloration. After 6-months field growth under hydro-morphic conditions during the dry season, roots were harvested and the root PD scores obtained visually at 24 and 48 h after harvest was compared among 60 genotypes. The differences in root PD reaction between these genotypes were highly significant ( $P < 0.001$ ) at 24 h after harvest. Genotypic differences in root PD (at 24 h after harvest) were expressed more in the distal than the proximal region of roots. At 48 h after harvest, however the genotypic differences became apparent only at proximal ends of roots. Location of the field with respect to water regime significantly ( $P < 0.001$ ) affected the root PD response 24 h after harvest. Rapid PD test was appropriate to differentiate the genotypic differences 24 and 48 h after root harvest. This study provides an early visual scoring method on cassava root quality and thereby aids to detect a key part of postharvest deterioration losses incurred.

**Key words:** *Manihot esculenta* Crantz, visual assays, wounding, physiological deterioration, water stress, inland valley.

### Introduction

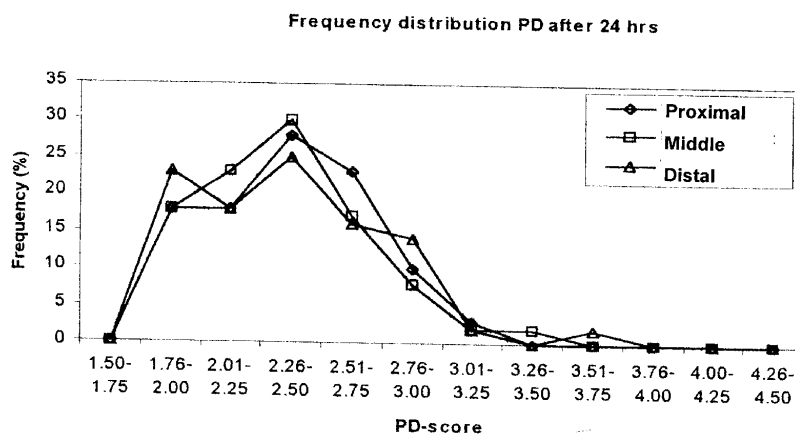
The storage roots of cassava (*Manihot esculenta* Crantz) provide a cheap source of food in tropical Africa. Fresh cassava roots consumed boiled or fresh are common in some parts of West Africa, Andean Latin America and Paraguay, and in 'Kerala' state of India<sup>1,2</sup>. Subsistence farmers and rural consumers rely on cassava roots for food substitution notably in marginal lands and under adverse environmental conditions such as prolonged drought. In West Africa, cassava is cultivated during the dry season, or after a rice crop, in hydro-morphic or seasonally dry paddy soils<sup>3,4</sup>. Cassava cultivated in hydro-morphic soils are exposed to lack of nutrients during the flooded period<sup>5</sup> causing in-ground deterioration of roots and a reduction of root quality<sup>4</sup> that further influence post harvest behavior of roots. Production and ecological aspects concerning changes in root quality traits such as cyanogenic potential in the cassava grown in hydro-morphic soils<sup>6</sup> or root rotting due to microbes<sup>7</sup> have also been described. Cassava storage root deterioration is the result of two causal and linked processes: physiological and microbiological<sup>8</sup>. The primary PD of cassava roots is physiological in nature and occurs when the storage roots are exposed to air and when detached from mother stem at harvest or after harvest, during washing to remove soil and prepare for marketing. PD is characterized by a bluish-brown or bluish-black discoloration of tissues due to leuco-anthocyanin pigment production<sup>9</sup>. This happens along the peripheral vascular bundles of roots, and spread over to adjacent root parenchyma, and is termed as "vascular streaking". It is a humidity-sensitive wound response that depends upon the degree of mechanical damage encountered by roots<sup>8</sup>. Reducing above described postharvest root quality losses associated with PD and increasing market value of the roots can aid to improve the status of poor involved with cassava cultivation in inland valley ecosystem of West African countries where cassava is a major income generating crop. Greater perishability of cassava roots in such stressful environments (water stress) and the need to use better adapted

genotypes and methods to reduce post harvest losses has been reported else where<sup>4,10,11</sup>. This study was therefore conducted to examine the usefulness of a quick, cheap approach based on a visual evaluation method to differentiate root PD at the early stage of preparation of the product ready for the market. To this end genotypic variation of 60 cassava clones of the African *Manihot* genotypes grown under hydro-morphic field conditions in an inland valley was evaluated for its root PD response from harvest to 48 h after harvest.

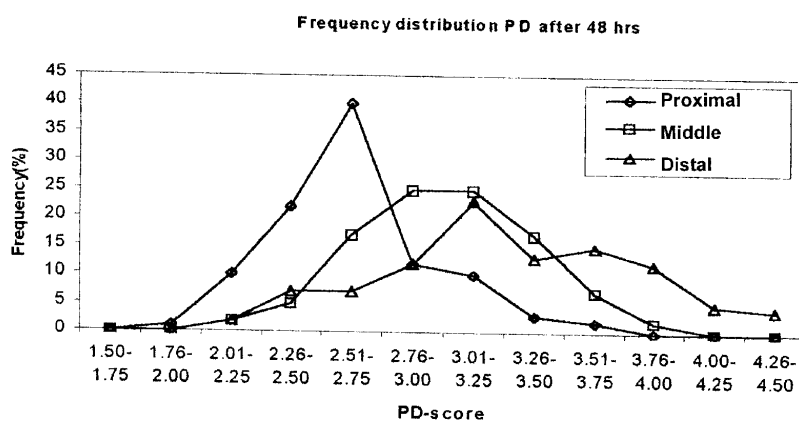
### Materials and Methods

**Field trials and design:** Field experiments were conducted during the dry season in a hydro-morphic soil at a research site located in the inland valley (IV) area at the International Institute of Tropical Agriculture (IITA) farm, Ibadan, Nigeria (latitude 7°30'N, longitude 30°54'E, altitude 210 m above sea level). Annual rainfall was 1253 mm, with a mean maximum temperature of 27°C and a mean minimum temperature of 21°C. Soils were sandy loam classified as Aeric Tropaquents. The experimental design was a factorial design with four replications, where the four water regimes were based on the changes in seasonal water table depth: a) dry at early (December – February) and late (March – June) seasons (dry/dry), b) dry at early season and wet at late season (dry/wet), c) wet at early season and dry at late season (wet/dry), and d) wet at both seasons (wet/wet). Water table depth was monitored during the crop season using piezometers installed at various depths in each replication and each moisture level treatment. Preparing high ridges, which thereafter dried due to natural drainage and evapo-transpiration, imposed moisture treatments. The rate of drying depended upon slope and proximity to water table. As the dry season progressed, with the gradual drying of ridged soil the following ranking of plots by moisture stress was created: dry/dry > dry/wet > wet/dry > wet/wet.

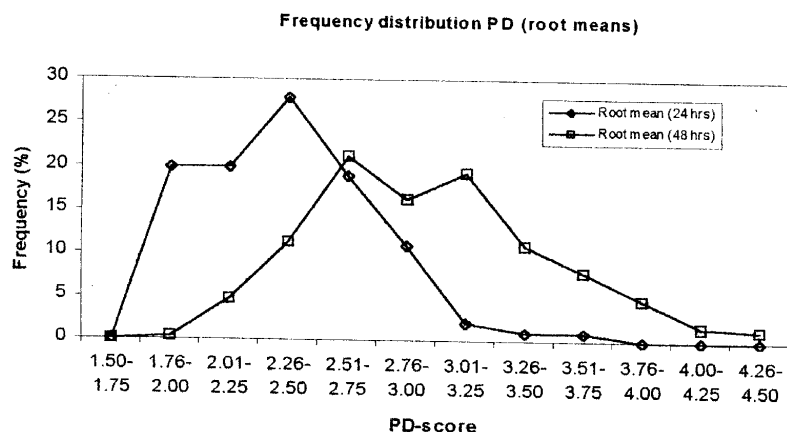
a)



b)



c)



**Figure 1.** Frequency distribution (%) of genotypes with varying physiological deterioration (PD) scores of the roots a) at proximal, middle and distal ends of roots evaluated at 24 h b) at proximal, middle and distal ends of roots evaluated at 48 h after harvesting, and c) mean root PD scores at 24h and 48 h after harvest.

**Table 1.** Scoring system for evaluating the physiological deterioration (PD) reaction of harvested cassava storage roots.

PD score	Qualitative evaluation term	Percentage of vascular streaking and storage root color change (%)
1	Very low	0-19
2	Low	20-39
3	Intermediate	40-59
4	High	60-79
5	Very high	80-100

**Plant culture:** Sixty cassava genotypes that represented the West African germplasm maintained at IITA composed of both farmers' landraces and some elite improved lines were used. Cassava stem cuttings were planted on 50 cm ridge of topsoil, at 1 m x 0.75-m spacing. Each treatment per replicated plot was a single row of 10 plants. Cuttings were planted at the end of the previous rainy season and mature plants were harvested 6 months after planting.

**Tuberous root sampling and quality analysis protocols.** Standard plant sampling procedure<sup>12</sup> was followed and care was taken to separate roots at the plant base. All roots were placed in paper bags and taken to laboratory for further analysis. Individual tuberous roots were cut to inflict wounding patterns similar to slic-

**Table 2.** Genotypes identified with relatively low tuberous root physiological deterioration (PD) responses 24 h and 48 h after harvest based on three positions (proximal, middle, and distal ends) of storage roots of cassava.

Proximal end of root	Middle of root	Distal end of root	Root section average
<b>24 h after harvest of roots</b>			
<i>Genotypes with very low PD scores (1.51 - 1.75)</i>			
90059	-	-	-
83672	-	-	-
82/00249	-	-	-
<i>Genotypes with low PD scores (1.76 - 2)</i>			
M86/00009	M86/00009	83672	TME 1
M85/00665	305555 P3-2	30555 P3-2	30001
M86/00106	M86/00106	M86/00106	M85/00665
M6298	M82/0249	M86/00009	81/00016
82/00249	M6298	M85/00665	M86/00106
087/00401	M85/00313	M85/00313	M85/00313
M41627	87/00691	82/00422	81/00942
81/00942	Bida Local	087/00401	087/00691
-	M6298	087/00691	M86/00009
-	M41627	M41627	M41627
-	81/00942	M6298	-
-	-	M85/00313	-
-	-	81/00942	-
-	-	087/00691	-
<b>48h after harvest of roots</b>			
<i>Genotypes with low PD scores (1.76 - 2)</i>			
82/00422	M85/00665	-	-
M86/00080	-	-	-
90029	-	-	-

ing and 2 cm root sections were selected from the proximal, middle, and distal ends of each root. These sections were used for evaluation. Physiological deterioration (PD) evaluations were made at 24 and 48 h after harvest. The root samples were stored under ambient conditions (26°C and 65% relative humidity and artificial light). PD of roots was evaluated using a visual score (Table 1) modified from the original scale<sup>13</sup>. Original evaluation procedures advocated for PD involves scoring 1.5-cm long root pieces after 3 days of storage, with a large number of replicates. In our tests, for PD evaluations during storage, coefficient of variation was relatively high at 25 to 31.5% but was acceptable for the objectives of this study. The use of a minimum of three tuberous roots per plant helped maintain CV at or below this level.

**Statistical analysis:** All data were analyzed using SAS package<sup>14</sup> and means and variances were computed. Significance was determined at  $P < 0.05$ . Frequency distribution of these genotypes by PD class was determined based on mean scores.

## Results and Discussion

**Genotypic variability for cassava root PD.** Differences in PD scores at 24 h after harvest were highly significant ( $P < 0.001$ ) for the 60 tested genotypes. Mean root deterioration score was 2.47 with 29.4% vascular streaking 24 h after harvest among all tested genotypes. At this time the differences were mainly expressed at the distal end of roots as compared to the proximal end of the roots. Forty-eight hours after harvest, mean PD reached 55% and the genotypic differences were more prominent at proximal root

**Table 3.** Mean physiological deterioration (PD) scores of cassava roots at 24 h and 48 h after harvest as influenced by water regime during the preharvest growing season.

Field location & water regime	Proximal end of root	Middle of root	Distal end of root	Root section mean
After 24 h				
Dry/dry	2.7a	2.7a	2.7a	2.7a
Dry/wet	2.8a	2.7a	2.6ab	2.6a
Wet/dry	2.1c	2.1c	2.2c	2.1b
Wet/wet	2.4b	2.4b	2.4b	2.1b
Significance of F-statistic	***	***	***	***
After 48 h				
Dry/dry	2.9	3.1b	3.2	2.9c
Dry/wet	2.8	3.3b	3.3	3.2b
Wet/dry	2.5	3.9a	3.4	3.9a
Wet/wet	2.8	3.2b	3.2	2.8c
Significance of F-statistic	ns	*	ns	*

Means in each column followed by the same letter were not significantly different at  $P < 0.05$  by DMRT ns, \* and \*\*\* denote non significance and significance at  $P < 0.05$  and  $P < 0.001$  levels, respectively.

ends rather than at distal ends; differences among genotypes were significant but at a lower probability level ( $P < 0.05$ ). Postharvest losses at the farm gates are generally concentrated at proximal and distal root extremes, which are often discarded in the preparation of food<sup>13</sup>. Genotypes evaluated 24 h after harvest was skewed to the right for PD scores (Fig 1a). The PD score distribution pattern at 24 h was not affected by the root section (Fig. 1c). No genotypes with PD scores at the lower end (scores less than 2) were observed after 48 h. Genotype frequency distribution was normal but with different distribution patterns at the second evaluation (Fig. 1b and c). More genotypes had PD scores between 2.5 and 3.75 irrespective of the root section. The median score for genotypic distribution at 24 hours which was 2.25 moved upwards to a PD score of 2.5, 48 h after. Interestingly, few of the tested genotypes recorded lower PD scores 48 h after harvest (Table 2) suggesting the availability of some genotypes with less deterioration that could be recommended for growing under inland valley conditions. Genotypes 90059, 8367, and 8/0049 had very low scores at 24 h after harvest. While genotypes 82/00422, 90029, M86/00080, and M85/00665 had lower scores 48 h after wounding. Genotype 90029 was the best in terms of maintaining low PD levels.

**Effect of preharvest water stress on PD.** Location of the field with respect to water regime significantly ( $P < 0.001$ ) affected the root PD response 24 h after harvest (Table 3). The location effect was highly significant at all 3 root sections but the sectional effect changed after 48 h. Roots harvested from very wet soils and wet to dry transition soils gave a delayed PD reaction 24 h after harvest than those harvested from dry soils and soils with dry to wet seasonal transition. Root PD response was less in those harvested from wet soils after 48 h. Therefore PD response was clearly affected by preharvest environmental factors, such as flooding or dry soils, and post harvest conditions such as length of storage period. The rapid screening test was useful in distinguishing genotypic differences for PD on the first day of harvest and wounding and clearly detected the changes occurring as a response to

wounding within the initial 24h period. After 48 h, the visual screening test was mainly able to distinguish the extremes. Sensitivity of the tests was therefore low at identifying genotypes with differential responses beyond 48h of wounding. Our study indicated the need to establish more robust and sensitive screening tools using biochemical assays. Cassava root PD is influenced by various environmental factors. Some of these factors are pre-harvest factors which reduce root dry matter contents and result in defoliation<sup>1</sup>, pruning<sup>15</sup>, and cut root pieces<sup>16</sup>. Dry matter content may be partly involved in PD performance; susceptibility is correlated with dry matter content<sup>1</sup>. Our data and the above reports confirm the need for location-independent mechanisms to prevent premature PD. The determination of enzymatic activities and formation of wound response-related chemical compounds in the selected genotypes in contrasting locations need to be investigated with reference to enzymes associated with wound-related processes. Genotypes with delayed PD response could prove useful as parents in breeding programs to develop genotypes with a reliable and robust wound healing response. If the genetics and biochemical pathways are understood, a genetic engineering approach could be more promising. Traditional breeding methods for tolerance to root PD has identified various and contrasting genotypes. Biochemical indices of root quality and postharvest response should be developed as an aid in cassava breeding<sup>8</sup>. Limited information is available on the genes involved in the biochemical pathways that are associated with cassava root PD. There is however, some knowledge on the biochemical phenomena involved. Several genes encoding key steps in this pathway have been cloned, sequenced and their regulatory mechanisms studied<sup>17</sup>.

### Conclusions

Improvements in the inherent storability of the crop through traditional and advanced breeding techniques and improved storage systems research can help ensure constant root quality and better storage life. Genotypic variability for this trait immediately after harvest and for 24 h to 48 h period was sufficiently promising to warrant their use as tests on dynamics of root PD over longer periods in order to select genotypes with longer shelf lives. The crude but rapid assay method described in this paper is expected to provide base-line information on root bio-deterioration under field conditions with minimum requirement of equipment (initial capital investment) vis-à-vis more sophisticated and sensitive analysis of root tissue assays. Data clearly indicated discrimination of genotypic responses to harvest related time-course changes, which in turn can be used to broadly categorize genotypes into groups for further analysis, genotypic selection and breeding purposes and for recommendation of desirable genotypes for inland valley ecosystems in West Africa.

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