

# Development of Cassava Germplasm Resources for the Improvement of High Value Root Quality Traits through Induced Mutation and Marker Aided Breeding in Nigeria



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## BACKGROUND

Improvement of cassava for nutritional and industrial traits is strategic in enhancing the market potential of this crop in addition to addressing the food requirements of the impoverished and malnourished poor that largely rely on this crop for food. Value addition in cassava is important for increased income and improved livelihood for poor resource farmers who mainly grow this crop. The National Root Crops Research institute (NRCRI), Nigeria in collaboration with the International Center for Tropical Agriculture (CIAT) and International Atomic Energy Agency (IAEA), Austria, is developing its cassava germplasm resources for the improvement root quality traits through induced mutation and marker-aided breeding for increased beta carotene and protein content, delayed post-harvest physiological deterioration, and high dry matter content. Because of the limited genetic variation for value-added traits in cassava in Nigeria, irradiation of elite cassava lines and introduction of exotic germplasm from Latin America, the origin of *Manihot* species were explored as strategies for improving cassava germplasm resources at NRCRI for high value quality trait for development of value-added cassava. For mutation breeding activities, in vitro plants of elite varieties and F<sub>1</sub> botanical seeds were irradiated with gamma rays and evaluated for key traits of economic importance while exotic germplasm for value added traits were introduced into Nigeria through seeds and in vitro plant materials. We report here the results and progress obtained in the improvement of germplasm resources for these traits in Nigeria.

## MUTATION BREEDING

### Latin American germplasm

Irradiated Latin America (LA) varieties (SM 909-25 and Col 2215) were introduced via in vitro plantlets into Nigeria. A set of 108 plants was evaluated for cassava mosaic disease and quality traits. The germplasm which was susceptible to cassava mosaic disease (CMD) was harvested at 12 MAP and evaluated for beta carotene and dry matter content.

### Beta carotene

Carotenoid analysis of the irradiated LA materials was done using 100g samples. Screening was done by extraction and spectrophotometric method as described by Rodriguez-Amaya and Kimura (2004). Results from quality traits analyzed showed interesting data for carotenoid analysis where five genotypes (SM 909-25-15Gy/080; SM 909-25-15Gy/84; SM 909-25-15Gy/086; SM 909-25-15Gy/090 and Col 2215-10Gy/001) showed high carotenoid content above 100ug/100g fresh weight of roots (Fig. 1). The highest carotene content observed was 237.27 ug/100g.

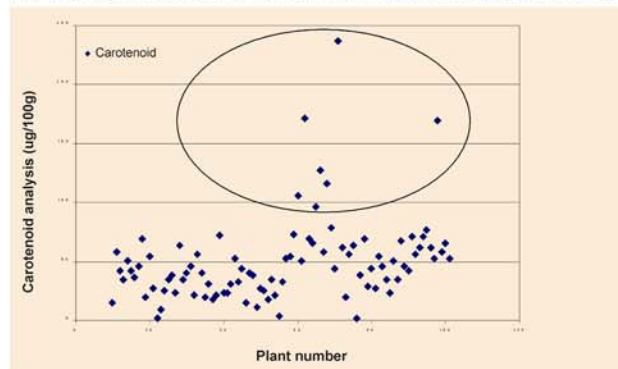


Fig. 2 Carotenoid content distribution in Latin American germplasm with six clones (in the circle) showing good carotenoid content

### Dry matter

Fresh root samples of 10g were used for assessing dry matter content. The samples were oven dried, and the final weight on drying was expressed as a percentage of the original fresh weight. Some 12 genotypes of the LA materials showed high dry matter percentage 40% (Fig. 2).

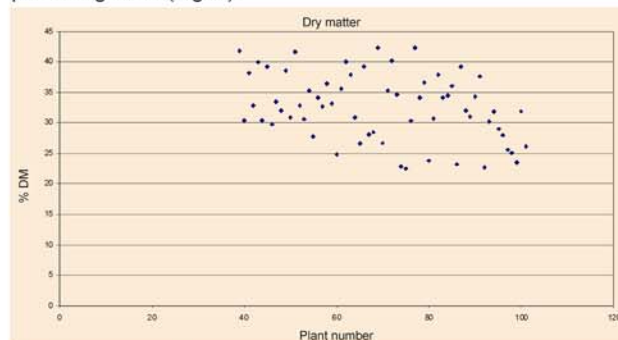


Fig. 2 Dry matter distribution in Latin American germplasm

## African germplasm

LA are generally very susceptible to CMD, and in line with efforts to develop well adapted disease resistant useful mutants, CMD resistant clones in NRCRI breeding programme were used as plant materials for irradiation. The clones used were TME3, TMS98/002, NR87184, TMS95/0379. Over 2500 plantlets of these clones were irradiated at IAEA. They were micropropagated to enhance uniformity and reduce chimeras and established on the field (Fig 3). The materials will be evaluated for root quality traits at harvest. Another 2000 seeds from open pollinated sources from five CMD resistant varieties (TMS 30572, AR-9-62, TMS 30555, TMS 97/4762 and AR 15-5) were also irradiated and have been planted on the field. The seeds are from open pollinated source from five varieties. Due to their good CMD resistance, a number of the genotypes from plant materials generated from in vitro plants and seeds have already started flowering and crosses to generate M<sub>2</sub> populations have begun.

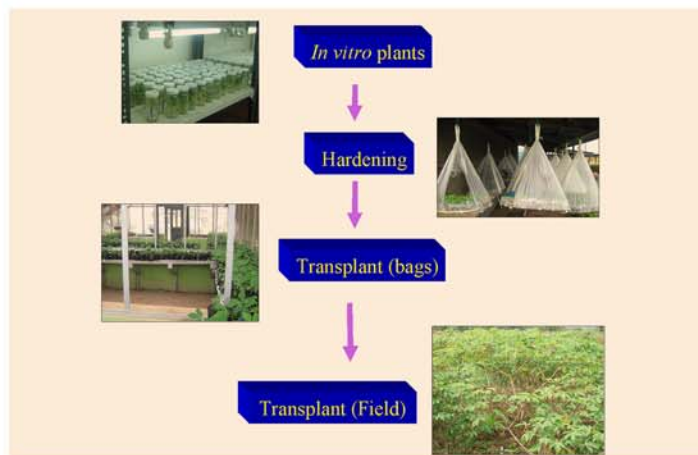


Fig 3. Process of deployment of irradiated plant materials to the field

## MARKER AIDED BREEDING

### Beta Carotene/Protein

Beta carotene germplasm of 600 genotypes introduced as seeds from CIAT were evaluated for yellow pulp color which is associated with beta carotene. Results indicate that 87% of the genotypes evaluated had cream pulp color and 10% were yellow and 3% were white. The results indicate that the germplasm had fair to high carotene content. Ten of the materials with good CMD resistance have been integrated into the breeding scheme at NRCRI for yield trials. Additional crosses were made at CIAT to generate populations with combined enhanced beta carotene and high protein contents in cassava roots. The parents have cream or deep yellow colored-roots and crude protein contents of 3-8%. A total of 1555 seeds were derived from 17 parents in different combinations. The number of plants successfully established in vitro from embryo axes was 981. DNA was extracted from these genotypes and used for marker-assisted selection (MAS) for resistance to CMD using SSR marker, NS158 and a SCAR marker, RME 1. A total 138 plants were selected for CMD resistance from 16 families and further micro-propagated. The selected CMD-resistant genotypes with enhanced nutritional attributes are to be evaluated for protein and beta carotene.

### Delayed Postharvest physiological deterioration

Cassava roots generally start to deteriorate 24 – 48 hours after harvest. Previous studies revealed limited genetic variability for post-harvest physiological deterioration (PPD) (Sanchez et al. 2005). A new source of genes for dramatically delayed PPD was identified in an inter-specific hybrid between cassava and a wild relative *Manihot walkerae* at CIAT. This inter-specific hybrid (CW429-1) was used as parent to develop segregating populations for genetic mapping and to introgress delayed PPD into cassava gene pools. In this study CW429-1 and 8 other elite genotypes (MCOL 1505, MPER183, MTA18, CM523-7, HMC-1, MBRA337, MCOL2279 and CM2772-3) having different degrees of PPD response were evaluated. PPD quantification was as described by Wheatley et al. (1985) with a slight modification. Evaluations were done at 5, 10 and 14 days after harvest (DAH) with 5 roots each. A half-sib backcross population (BC<sub>1</sub>) was developed using CW429-1 as the donor parent to two elite but PPD susceptible genotypes, (MTA1 8 and SM 909-25) for QTL mapping for delayed PPD. The BC<sub>1</sub> population, numbering 122 individuals (66 from cross CW429-1 x MTA18 denoted as family B1PD284, and 56 from cross CW429-1x SM 909-25 denoted as family B1PD289), was utilized for genetic mapping using the MAPMAKER version 2.0 (Lander et al. 1987). QTL map was done using the QGENE software (Nelson 1997). Genes for delayed PPD were successfully transferred into cassava from the wild relative (Fig 4). Mean PPD values at 5 DAH was 0% in CW 429-1. At 10 DAH, mean values ranged from 0% in CW429-1 to 58% in CM 523-7 (Figure 1). Up to 33% of the individuals in the population studied showed low PPD values (<35%) with about 6% of them exhibiting low deterioration (<2%). Significant variation was also observed in the magnitude of PPD in the roots of the same genotype (Fig. 5). Seven genotypes which showed high resistance to PPD at 14 days after harvest are good candidates for use

in breeding for delayed PPD. The parental genotypes CW 429-1, MTA1 8 and SM 909-25 revealed 45% polymorphism for a total of 423 SSR markers evaluated (Fig 6). Eight markers on three linkage groups were significantly associated with a putative QTL for delayed PPD with a range of 6.2 to 12.8% of explained phenotypic variance (Fig. 7). The BC<sub>1</sub> derivatives showing delayed PPD were crossed to a LA variety resistant to CMD and cassava green mite. A total of 665 BC<sub>2</sub> progenies were derived from 18 families. Molecular markers associated with CMD were used to screen the BC<sub>2</sub> progenies for resistance to CMD. Selected plants will be evaluated for PPD in Nigeria.

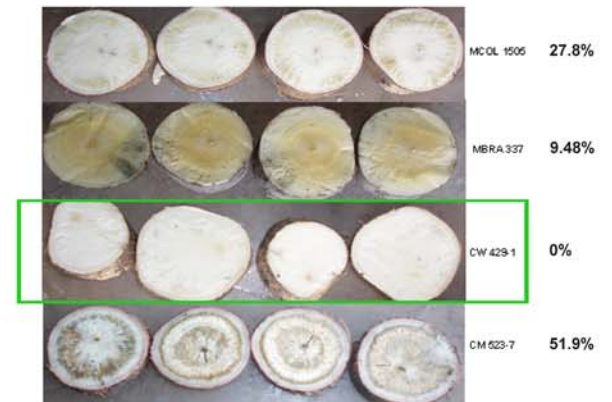


Fig. 5. Delay in post-harvest physiological deterioration (PPD) of the inter-specific CW 429-1 fifteen days after harvest.

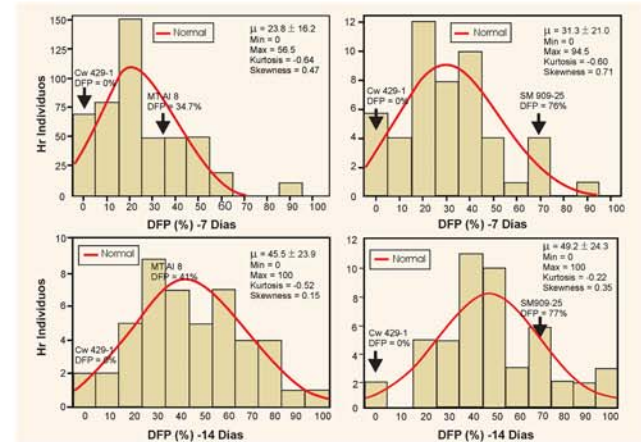


Figure 5. Frequency distribution of genotypes evaluated for post-harvest physiological deterioration at the 7th and 14th days after harvest in (a) family B1PD284 (n=47) and (b) family B1PD289 (n=49). Means for parentals are shown by arrows.

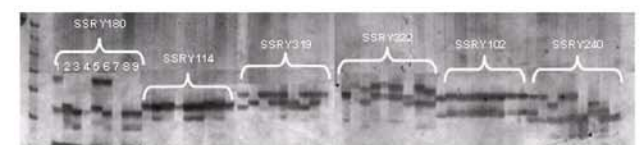


Fig. 6. Tests for amplification and polymorphisms in 4% polyacrylamide gels using different SSR markers from parents and selected progenies: 1 – CW 429-1; 2 – SM 909-25; 3 – MTA1 8; lanes 4 to 9 represent segregating progenies.

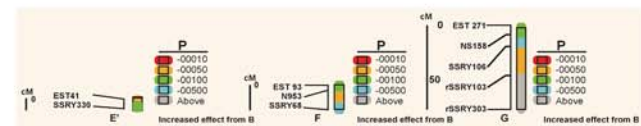


Fig 7. Map of putative QTLs for PPD in linkage groups E, F and G of family B1PD 284. Colours denote the probability of association of marker with a QTL

## CONCLUSIONS AND PERSPECTIVES

- Identification of candidate mutants for high carotenoid levels and dry matter attests to the usefulness of mutagenesis in breeding for rare novel traits
- Enhanced flowering of irradiated CMD resistant African cassava germplasm aided M<sub>2</sub> population development
- Developed germplasm resources represents a major step towards enhancement of commercialization cassava
- Developed germplasm to be explored in the development of improved high value –added cassava varieties

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