NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 49 Number 2, October 2018. Pp. 258-262
Available online at: http://www.ajol.info/index.php/naj

# SINGLE NUCLEOTIDE POLYMORPHISMS - ESTIMATED HETEROZYGOUSITY ON PARTIAL CASSAVA INBRED LINE DEVELOPMENT

**Ezenwaka, L.C.**
National Root Crops Research Institute, NRCRI, P.M.B, 7006 Umudike, Nigeria
Corresponding Author's email: lydiaezenwaka@yahoo.com

**ABSTRACT**
The effect of selfing and heterozygousity were analyzed in five $S_1$ families. Results are discussed with a view to selecting the best performing and least heterozygous plants during inbreeding to isolate useful genes and superior partially inbred parental lines. A reduction of heterozygousity was found in the $S_1$ progenies for all other families except for family TMS 98/0002 which recorded the highest missing data. The result further indicated that over 55% of the progenies had heterozygousity values less than 25%. This implied that there were good materials to carry to the second generation of inbreeding which probably will reduce heterozygousity in the next breeding generation.

**Keywords: Inbreeding, Inbreeding depression, SNPs, Partial inbred line, $S_1$ and Cassava**

## Introduction

Cassava is highly heterozygous and for much of the last four decades, inbreeding was not been fully exploited. Inbred lines are better parents for breeding and genetic studies as they do not have confounding effect of dominance and carry lower levels of genetic load (undesirable alleles). The speed of inbreeding depends upon the average heterozygousity of the original parental lines, the homozygousity level of the selected genotypes at the end of the self-pollinating phase and the process of selection of progenies to be self-pollinated (Scotti *et al*., 2000). In the inbreeding process, phenotypically, there is a decrease in vigour, which is correlated with increased levels of homozygousity. The aim is to select vigorous plants (tolerant to inbreeding depression); in the process, plants may be selected that are less homozygous than the expected average for their generation. Selection in inbreeding is biased by the differences in homozygousity levels of segregating partially inbred genotypes (Blair *et al*., 2007). Single nucleotide polymorphisms (SNPs) have rapidly become the molecular marker of choice in assessing heterozygousity in cassava, enabling the selection of plants with true tolerance to inbreeding. The objective of the study was to assess the suitability of SNPs for estimating the heterozygousity level in five $S_1$ families in Nigeria toward development of partially inbred lines. At each selfing generation, genotypes with the highest degree of homozygousity showing vigour will be selected and used for further selfing, and this will continue until high levels of homozygousity (i.e. least heterozygous) are attained with individuals showing tolerance to inbreeding depression leading to the identification of the best performing superior partially inbred parental lines.

## Materials and Methods

Five of the elite cassava genotypes (TMS 30572, TME 419, TMS 98/0505, TMS 01/1371 and TMS 98/0002) were obtained from the germplasm of International Institute of Tropical Agriculture, (IITA) Ibadan. These five elite cassava genotypes were used as progenitors ($S_o$) to generate $S_1$ progenies. For each elite genotype, 20 stem cuttings were planted at a spacing of 1m x 1m. The plots were isolated by a distance of 100m from neighbouring cassava plots to ensure that only self-pollination occurs. Harvested $S_1$ botanical seeds were allowed a two - month dormancy period before sowing in the nursery under screen house conditions. The seeds were sowed in trays filled with sterilized soil with a mixture of loamy and sandy soil in a ratio of 2:1, respectively in the screen house. Harvesting was done at 12 months after planting, after which they were cloned to generate at least 10 stem cuttings per seedling for clonal evaluation. All progeny belonging to the same family, together with the parents, were established in the same block. Each row represented a clone and spacing within rows was 1 m, with between row spacing of 1.5 m to minimise inter-plot

interference. The parental clones served as the check. Evaluation was conducted in a single location, using augmented experimental design (AED) Federer (1956). NPK 15:15:15 fertilizer was applied at the rate of 600kg/ha. Fertilizer was applied at eight weeks after planting. The field was weeded regularly following appropriate agronomic practices.

**DNA extraction and SNP genotyping**

The DNA extraction was carried out at the biotechnology laboratory of the KBiosciences, Hoddesdon, London, United Kingdom. A total of 200 SNPs markers were analyzed in 260 DNA samples. 5µl DNA samples were arrayed into PCR plate. The DNA was genotyped as liquid samples. KASP genotyping mix was dispensed robotically into the PCR plates containing 5µl of DNA samples and briefly vortexed. The plate was sealed with optically-clear seal and thermal cycled using Peltier block-based thermocycler at 94°C for 15 mins. The plate was read on a suitable fluorescent plate reader under an ambient temperature. The genotyping data was analysed using most FRET- capable plate readers.

**Data Analysis**

Variables analyzed were plant height (cm), measured from the soil level to the highest apical point of the plant at harvest time; fresh root yield (kg/plant); harvest index (root biomass as proportion of total biomass); dry matter content (measured as %) was determined by the specific gravity methodology as suggested by Kawano *et al*., (1987).

Inbreeding depression (ID) was estimated for each variable as a percentage of the $S_0$ average:

The ID was obtained following the equation suggested by Rojas *et al*., (2009):

$$\text{ID} = \frac{S_0 \text{ mean} - S_1 \text{ mean}}{S_0 \text{ mean}} \times 100$$

Inbreeding depression was also measured at the family level. Therefore, the lower the ID value, the lower the depression, which implies that the performance of the $S_1$ progenies is close to that of the $S_0$ progenitor.

The heterozygousity index was obtained following the equation suggested by Scotti *et al*., (2000):

$$100 \times {}^{(S_nH - S_{n-x}H)}/_{S_{n-x}H},$$

where $S_n$ is the inbreeding generation in which the effect was calculated and $S_{n-x}$ is the generation taken as reference (the parental $S_0$) and $H$ is the mean of heterozygousity loci.

Summary statistics (average, range, variance and skewness) were performed on the traits for each family using excel programme.

**Results and Discussion**

**Ranges of inbreeding depression for the yield traits of five $S_1$ cassava *(Manihot esculenta Crantz)* families**

The ranges of ID for each family for yield traits are provided in Table 1. For plant height, all the families produced $S_1$ genotypes with plant heights superior to those of their $S_0$ progenitor genotypes. The average maximum ID observed for plant height was 10.69 and the minimum ID averaged -68.36. The highest variability in plant height as reflected by the variance was observed in family TMS 98/90505 with negative skewness. Negative values indicate asymmetrical distribution when there are a small proportion of unusually low values which results in the mean being less than the median. This also indicated that not much improvement would be expected in the next breeding generation. For fresh root yield and fresh foliage yield, ID averaged 86.55% and 86.69% respectively (Table 1). This suggested an almost negligible production. Fresh root yield, fresh foliage yield and vigour were positively skewed. Positive values indicate asymmetrical distributions, when there are small proportion of unusually high values which result in the mean being higher than the median. This indicates that there would be an improvement for these traits in the next breeding generation. Only family TMS 98/0505 showed $S_1$ genotypes with harvest index values below its progenitor clones. In the case of dry matter content, however, families TMS 30572, TMS 98/0002 and TMS 98/0505 failed to produce $S_1$ genotypes with a performance superior to their respective progenitor clones while TME 419 and TMS 01/1371 produced $S_1$ genotypes with a performance superior to their respective progenitor clones. For vigour, families TMS 01/1371, TMS 98/0002 and TMS 98/0505 produced $S_1$ genotypes with a performance superior to their respective progenitor clones while TME 419 and TMS 30572 failed to produce $S_1$ genotypes with a performance superior to their respective progenitor clones.

**Estimation of heterozygousity in the five $S_1$ cassava families using single nucleotide polymorphisms ($SNP_s$) molecular marker**

$SNP_S$ were used to assess heterozygousity in the five $S_1$ cassava families, enabling the selection of plants with true tolerance to inbreeding. A total of 200 SNPs markers were analyzed in 260 individuals. Two hundred and fifty- five $S_1$ lines were analyzed (33 genotypes from family TME 419, 63 genotypes from family TMS 01/1371, 90 genotypes from family TMS 30572, 44 genotypes from family TMS 98/0002 and 25 genotypes from family TMS 98/0505) and 1 from each of their parents ($S_0$ progenitors). The average heterozygousity reported for $S_1$ lines was 27.02% and the range was from 23.27% in TMS 98/0002 to

31.04% in TME 419 (Table 2). A reduction of heterozygousity was found in the $S_1$ progenies for all other families except for family TMS 98/0002 which recorded the highest missing data with SNPs markers. It is ascertained that the missing data led to the biased estimate of the heterozygousity observed in the family. Figure 1 showed the distribution of $S_1$ progenies based on their percentage heterozygousity using SNPs marker. The result further indicated that over 55% of the progenies had heterozygousity values less than 25%. This implied that there were good materials to carry to the second generation of inbreeding which probably will reduce heterozygousity in the next breeding generation. Castro *et al*., (2006) estimated heterozygousity in cassava. The authors observed that average heterozygousity in the $S_1$ families was 22.3%. The current study recorded a slightly higher average value of 27.02%. The SNPs adequately showed good estimation of heterozygousity compared to SSRs as found based on earlier studies (Castro *et al*., 2006). Five $S_1$ individuals, GCO 274-2 (11.17%), (a progeny derived of TMS 98/0505), GCO 219-15 (11.46%), (a progeny derived of TME 419), GCO 223-53 (13.76%) and GCO 223-13 (14.09%), (progeny derived of TMS 01/1371) and GCO 228-23 (13.14%), (a progeny derived of TMS 30572) showed relatively low heterozygousity based on SNP data. These genotypes have therefore, been identified as candidate genotypes for the generation of $S_2$ population. Comparison among the four $S_1$ families (TME 419, TMS 01/1371, TMS 30572 and TMS 98/0505) having very good SNP data points indicated that the $S_1$ family TMS 01/1371 showed the least reduction in heterozygousity (24.37%) and least inbreeding depression in vigour (-66.67) in Table 2. This is an excellent family for the production of $S_2$ progeny. Moreover, the family also had good number of survival materials and had less inbreeding depression in the different agronomic characteristics that were evaluated. It is the most tolerant to selfing and could reduce the heterozyousity more than expected in the next generation of selfing. For these reasons, it is commendable to choose this family to continue with $S_2$.

**Conclusion**

This study presented the first report on cassava inbreeding in Nigeria, information of which will be important for the general cassava breeding community; the effects of inbreeding on agronomic traits and estimation of heterozygousity using molecular markers (SNPs) have for the first time been quantified on African germplasm. Of particular interest was the generation of vigorous $S_1$ progenies (with high phenotypic values), which appeared to have benefitted from either additive and non-additive genetic effects or a combination of the two. These improved individuals should be targeted by breeders.

**References**


Blair, M.W., Fregene, M.A., Beebe, S.E. and Ceballos, H. (2007). Marker assisted selection in common beans and cassava. *In*: E.P. Guimaraes, J. Ruane, B.D. Scherf, A. Sonnino and J.D. Dargie (Eds.). Marker-Assisted Selection (MAS) in Crops, Livestock, Forestry and Fish: Current Status and the Way Forward. FAO, Rome, Italy. pp. 81-115.

Castro, A.M., Perez, J.P., Fregene, M. and Ceballos, H. (2006). Use of Molecular markers to estimate level of heterozygosity in selfed lines of Cassava (Manihot esculenta Crantz). Project IP3, Improved cassava for the developing world. CIAT Annual report 2006. Centro internacional de Agricultura Tropical (CIAT), Cali, Colombia.

Federer, W. T. (1956). Augmented (or hoonuiaku) designs. Hawaiian Planters' Record LV(2): 191-208.

Kawano, K, Gonçalves, F.W., Fukuda, M and Cenpukdee, U. (1987). Genetics and environmental effects on dry matter content of cassava roots. Crop Sci. 27: 69-74.

Rojas, M.C., Pérez, J.C., Ceballos, H., Baena, D., Morante, N. and Calle, F. (2009). Analysis of inbreeding depression in eight S1 cassava families. *Crop Science* 49:543-548.

Scotti, C., Pupilli, F., Salvi, S. and Arcioni, S. (2000). Variation in vigor and in RFLP-estimated heterozygosity by selfing tetraploid alfalfa: new perspectives for the use of selfing in alfalfa breeding. Theor. Appl. Genetics 101: 120-125.

Table1: Ranges of Inbreeding depression (ID, as a percentage of the performance from the $S_0$ generation) measured in five $S_1$ cassava (*Manihot esculenta* Crantz) families based on the yield traits

| Family | Variables | Max | Min | Variance | Skewness |
|---|---|---|---|---|---|
| TME 419 | Plant height | -47.06 | -129.41 | 1686.57 | 1.27 |
| TMS 01/1371 | | 2.38 | -14.29 | 92.98 | 0.38 |
| TMS 30572 | | 65.00 | -95.00 | 7066.00 | 0.66 |
| TMS 98/0002 | | -22.87 | -91.13 | 370.00 | -0.33 |
| TMS 98/0505 | | 56.00 | -12.00 | 7900.00 | -1.35 |
| **Average** | | **10.69** | **-68.36** | **3423.11** | **0.13** |
| TME 419 | Fresh Root Yield | 91.84 | 89.22 | 0.89 | -0.56 |
| TMS 01/1371 | | 74.38 | 19.66 | 5.69 | 0.99 |
| TMS 30572 | | 89.31 | 75.77 | 5.53 | 1.59 |
| TMS 98/0002 | | 88.31 | 80.51 | 2.35 | 0.47 |
| TMS 98/0505 | | 88.89 | 88.85 | 5.92 | -1.68 |
| **Average** | | **86.55** | **70.80** | **4.08** | **0.16** |
| TME 419 | Fresh Foliage Yield | 84.75 | 22.03 | 4.86 | 1.80 |
| TMS 01/1371 | | 76.67 | -26.67 | 4.55 | 1.03 |
| TMS 30572 | | 90.48 | 19.58 | 17.69 | 2.07 |
| TMS 98/0002 | | 86.75 | 28.48 | 2.69 | 0.82 |
| TMS 98/0505 | | 94.79 | 90.63 | 0.21 | -1.73 |
| **Average** | | **86.69** | **26.81** | **6.00** | **0.80** |
| TME 419 | Harvest Index | 40.70 | 9.75 | 0.02 | 0.11 |
| TMS 01/1371 | | 26.46 | 1.06 | 0.03 | -0.64 |
| TMS 30572 | | 28.86 | 9.59 | 0.01 | -0.27 |
| TMS 98/0002 | | 35.32 | 11.57 | 0.02 | 0.68 |
| TMS 98/0505 | | -4.63 | -6.02 | 0.14 | -1.73 |
| **Average** | | **25.34** | **5.19** | **0.04** | **-0.37** |
| TME 419 | Dry Matter Content | 26.81 | -14.21 | 23.12 | 0.79 |
| TMS 01/1371 | | 33.43 | -35.03 | 23.13 | 0.79 |
| TMS 30572 | | 34.29 | 8.38 | 8.44 | -0.52 |
| TMS 98/0002 | | 26.23 | 18.24 | 6.26 | 1.64 |
| TMS 98/0505 | | 23.24 | 16.85 | 1.08 | -1.73 |
| **Average** | | **28.80** | **-1.15** | **12.41** | **0.19** |
| TME 419 | Vigour | 60.00 | 0.00 | 0.79 | 1.03 |
| TMS 01/1371 | | 33.33 | -66.67 | 0.99 | 0.43 |
| TMS 30572 | | 60.00 | 20.00 | 0.44 | 0.26 |
| TMS 98/0002 | | 50.00 | -25.00 | 1.80 | -0.17 |
| TMS 98/0505 | | 50.00 | -25.00 | 3.00 | -1.73 |
| **Average** | | **50.67** | **-19.33** | **1.40** | **-0.04** |

Table 2: Heterozygousity Index (as a percentage of the performance from the $S_0$ generation) measured in five $S_1$ cassava (*Manihot esculenta* Crantz) families

| Family | $S_0$ | $S_1$ | Heterozygousity Index |
|---|---|---|---|
| TME 419 | 39.45 | 31.04 | -21.32 |
| TMS 01/1371 | 26.98 | 24.37 | -9.69 |
| TMS 30572 | 39.23 | 30.26 | -22.87 |
| TMS 98/0002 | 7.32 | 23.27 | 218.02 |
| TMS 98/0505 | 46.03 | 26.18 | -43.13 |
| Average | **31.80** | **27.02** | **24.21** |

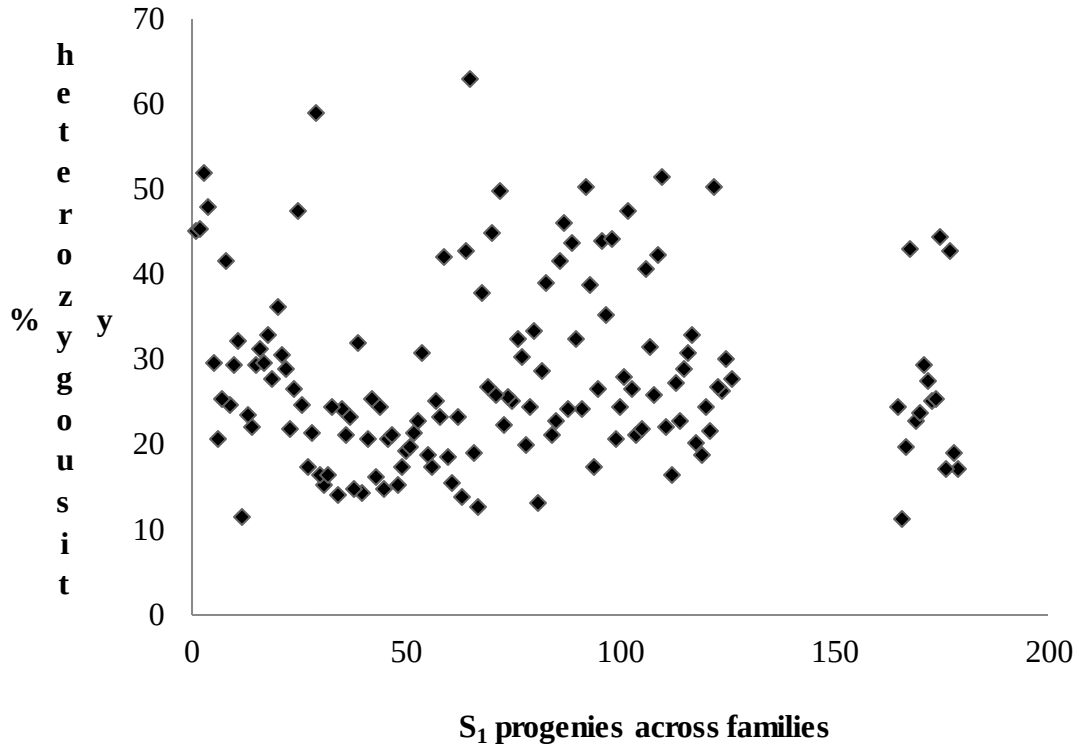


Figure 1: Distribution of $S_1$ progenies based on their percentage heterozygousity using SNPs markers