

**DNA MARKER-BASED DIVERSITY ASSESSMENT AND  
CHARACTERISATION OF ALLELES OF CANDIDATE  
GENES FOR CAROTENOID BIOSYNTHESIS IN YELLOW  
MAIZE INBRED LINES**

**BY**

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## **DEDICATION**

To my husband Adeniyi and to my children, Ayomide, Adeibukun and Oluwadetan.

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## ABSTRACT

The extent of genetic diversity among tropical-adapted maize inbred lines with varying carotenoid concentrations (two sets, 38 and 122) and to assess the relationship between markers based grouping and carotenoid concentration based grouping were assessed. Two  $\beta$ -carotene candidate genes in the maize carotenoid biosynthetic pathway were evaluated using allelic specific PCR-based markers, by validating these markers and evaluating them among these diverse sets of maize inbred lines for the level of allelic diversity present. Diversity assessment of 38 tropical-adapted yellow endosperm maize inbred lines was made using 17 AFLP primer combinations and 87 SSR markers. Also, SNP analysis of *PSY1* gene was studied to determine the genetic diversity in the 38 lines. Genetic distance (GD) values among all pairs of inbred lines varied from 0.08 to 0.64 with an average of 0.48 for AFLPs, and from 0.007 to 0.59 with an average of 0.45 for SSRs. Cluster and principal coordinate analyses based on AFLP and SSR-based GD estimates separated these lines into grouping consistent with their pedigrees. No nucleotide variations were observed in the *PSY1* regions that were sequenced. The extent of genetic diversity and relationship among 122 tropical-adapted maize inbred lines were elucidated using 62 SSR markers. Genetic distance (GD) values among all pairs of inbred lines varied from 0.02 to 0.61 with an average of 0.41 for the SSR markers. Cluster and principal coordinate analyses revealed clear separation of tropical-adapted maize inbred lines into SSR-based and carotenoid-based groupings. Cluster based on SSR markers showed consistency with the pedigree information of the 122 inbred lines. The 38 and 122 inbred lines exhibited a substantial level of genetic diversity. The Euclidean genetic distances calculated from carotenoid composition of the 38 lines varied from 0.30 to 9.00 with an average of 3.27. The Euclidean genetic distances for the 122 maize inbred lines varied from 1.00 to 9.97 with

an average of 3.81 for the carotenoid data. Correlation using Mantel's test between carotenoid-based GD estimates and matrix from SSR marker-based GD estimates for the 122 maize inbreds also gave a low  $r$  value (-0.06). SSR and AFLP markers were more efficient for studying genetic variability in the 38 and SSR markers for 122 maize inbred lines than carotenoid-based data. The correlations of molecular marker-based clustering with carotenoid-based clustering for the 38 and 122 inbred lines were not strong, suggesting that the molecular analysis could be used as the basis to identify diverse lines with high pro-vitamin A that can be used as parents for making bi-parental crosses or hybrids to increase the level of pro-vitamin A in tropical maize. The GD estimates can be used as the basis for effective utilization of the yellow inbred lines with diverse genetic backgrounds to enhance the rate of genetic gain from selection for high pro-vitamin A in the national maize breeding programs. The *LCYE* and *crtRB* genes in the carotenoid biosynthesis and allelic variants such as single nucleotide polymorphisms (SNPs), insertion and deletions (InDels) have been revealed to affect beta carotene accumulation in the endosperm of yellow maize grains. One hundred and twenty-two and thirty-eight (total, 160) tropical adapted maize inbred lines were screened for allelic characterization at *LCYE* and *crtRBI* using allele-specific PCR primers designed against allelic variation. Different alleles (SNPs/Ins/Del) representing favorable and unfavorable alleles were detected in the two sets of inbred lines in different frequencies. Furthermore, the donor parents having maximal favorable alleles at the *LCYE* and *crtRBI* loci were identified in breeding program for development of new maize inbred lines. This study has shown that some tropical adapted maize inbred lines harbor most favorable  $\beta$ -carotene content alleles at the two loci. Therefore, potential parental genotypes can be used to breed for higher  $\beta$ -carotene content for *LCYE* and *crtRBI* favorable alleles using the allelic-specific marker assisted selection. This

study suggests that these gene specific functional markers are reliable and efficient. They will be useful for recombination and introgression of alleles in maize breeding programs for increased level of pro-vitamin A concentration in maize. The groupings of 122 inbred lines generated from SSR-based data demonstrated that the lines belonging to the same groupings varied for the gene variants. Information from the current study will be useful in the breeding program for increasing higher  $\beta$ -carotene in maize grain.