Short Communication

Molecular and Biochemical Characterization of Short Duration Quality Protein Maize

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Fifty microsatellite or simple sequence repeat (SSR) markers, spread across the maize genome were used for analyzing a set of 19 elite Quality Protein Maize (QPM) lines, including seventeen lines developed in India and two at CIMMYT, Mexico. Polymorphic profiles for 47 SSR loci have aided in differentiating the QPM inbred lines. The polymorphism information content (PIC) values among the inbreds ranged from 0.06 (umc2229) to 0.70 (umc1071) with an average of 0.45 per primer-pair. The genetic relationships as indicated by the cluster analysis of SSR data were largely in congruence with the known pedigree of the QPM lines. The study resulted in identification of two SSR markers, umc1071 and umc1063 with higher PIC values of 0.70 and 0.64, respectively. The tryptophan content among the genotypes was found to vary considerably. Two genotypes viz., VQL 2 and VQL 8 were found to differ significantly for tryptophan content (0.51% and 0.94%, respectively). Both these QPM genotypes being derived from the same non-QPM parent CM 145, makes them ideal for mapping of modifiers for tryptophan content.

Key words: Quality Protein Maize, SSRs, tryptophan, Jaccard's similarity coefficient.

Maize (Zea mays L) plays a significant role in human and animal nutrition. With the discovery of opaque2 mutants (1) that produces enhanced levels of lysine and tryptophan, breeding for improved protein quality in maize began in mid-1960-s. Many research efforts at different research institutes, particularly CIMMYT, Mexico led to the development of Quality Protein Maize (QPM). India is one of the first countries who released QPM composites and hybrids. However, all those composites and hybrids developed were introductions from CIMMYT. Hence, there is a need to introgress QPM traits into Indian breeding materials for better adapted QPM genotypes. Over the years our institute has developed many short duration QPM inbreds. Those inbreds needs to be studied for their effective use in breeding programme.

The development of modern plant breeding techniques has greatly facilitated the wider use of a wealth of diversity from many sources including landraces and has allowed enhanced food production to keep pace with the population growth (2). The genetic diversity of plants has been assessed more efficiently after the introduction of methods that reveal polymorphism directly at the molecular level. Molecular markers like Simple Sequence Repeats (SSRs) have been applied in quantification of genetic diversity, genotype identification and marker assisted selection. The use of SSR markers in the assessment of maize germplasm has been well demonstrated in recent years (3, 4). The objectives of the present study were 1) assessment of genetic polymorphism in the selected QPM inbred lines developed by VPKAS (ICAR), India and CIMMYT, Mexico using microsatellite markers; 2) comparative analysis between molecular markers and protein and tryptophan content, and 3) identification of ideal parents for QTL mapping of modifiers for tryptophan content in QPM.

Our Institute (VPKAS) has developed 30 short duration elite QPM inbreds suitable to the hilly region of NW Himalayan states. Based on their agronomic performance and tryptophan content, a set of 19 genotypes was selected from them. Seeds of these 19 QPM inbreds along with two QPM inbreds from CIMMYT (CML 173 and CML 189) were used in the present study. These two QPM inbreds viz., CML 173 and CML 189 have been widely used as parents of promising hybrids and as donors for QPM traits. The parentage of the 19 maize genotypes under study is given in Table 1. The genomic DNA was isolated from fresh young leaves of 19 maize QPM inbred lines using CTAB method.

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Abbreviations: QPM - Quality Protein Maize; SSR - Simple Sequence Repeats; VQL – Vivek QPM Line; CIMMYT - International Maize and Wheat Improvement Center; PIC – Polymorphism Information Content.
The concentration of DNA was estimated on 0.8% agarose gel and using spectrophotometer. Fifty SSR markers, spread throughout the maize genome were used to detect polymorphism among the 19 maize genotypes. The sequences for the SSR loci under study were taken from the Maize Genetic Data Bank (http://www.maizegdb.org). The polymerase chain reactions and gel documentation were carried out using standard procedures (4) while the amplified products were resolved on a 3.5% agarose gel [Super Fine Resolution (SFR) Agarose; Amresco, USA]. The similarity matrix was constructed using NTSYS-pc 2.1 to produce an agglomerative hierarchical classification (6), by employing Unweighted Pair Group Method using Arithmetic Averages (UPGMA) with average linkage (7).

Polymorphism Information Content was determined as described by Smith et al (8), by using the formula PIC = 1 – Σfi^2, where fi is the frequency of the ith allele. For genotypes showing heterozygosity at a specific SSR locus, the PIC values were calculated after considering each allele as contributing one-half instead of one, as suggested by Narvel et al (9). The COPH module was used to compute a matrix of cophenetic (ultrametric) values for providing statistical support to the dendrogram created (10). This analysis provided an estimate of the goodness-of-fit of the original cluster analysis by generating a cophenetic correlation value, r. A cophenetic correlation value of r >0.9 is considered a very good fit, while r value between 0.8-0.9 considered good fit according to Mantel (11). The biochemical analyses for total protein content and tryptophan in endosperm samples of QPM genotypes were carried out following standard procedures as reported by Villegas and Mertz (12). All the biochemical analyses were performed in three replications.

The 50 SSRs used in the present study were spread over the maize genome. Among these SSR loci, 47 were found to be polymorphic while three markers viz., umc1514, bnlg1331 and bnlg149 were monomorphic. A total of 121 alleles were detected for 47 polymorphic SSRs with an average of 2.42 alleles per locus, which is also in close agreement with previous observations for maize (3). The banding pattern of 19 maize QPM genotypes with SSR loci umc1153 and umc1327 is shown in Fig. 1. Two out of 50 SSR loci named umc1071 and umc1555, showed 4 alleles each. The details of bin locations, repeat motif, number of alleles and PIC for each name are given in Table 2. PIC provides an estimate for the discriminating power of a locus by taking into account not only the number of alleles that are expressed but also their relative frequencies. The PIC values across the polymorphic loci ranged from 0.06 (umc2229) to 0.70 (umc1071), the mean being 0.45, which are in close agreement with earlier studies (3). Five SSR loci revealed PIC values more than 0.60, of which two markers viz., umc1071 and umc1063 have higher PIC values of 0.70 and 0.64, respectively. In the present study, SSR loci with tri-repeats represented...