Microsatellite marker-based genetic diversity among quality protein maize (QPM) inbreds differing for kernel iron and zinc

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Abstract Quality Protein Maize by virtue of opaque2 mutation is known to possess high lysine and tryptophan which help in improving biological value of maize protein. Improvement of these genotypes for kernel Fe and Zn holds immense promise. We report here wide variation for kernel Fe and Zn among 46 diverse Quality Protein Maize inbreds. Promising inbreds with high kernel Fe (>40 mg/kg) and Zn (>35.0 mg/kg) were identified. Profiling of inbreds using 75 microsatellite markers distributed throughout the genome produced a total of 256 alleles with a mean of 3.41 alleles per locus. Seven unique- and 26 rare- alleles were identified. The average polymorphism information content was 0.50, with a range of 0.11-0.79. Genetic dissimilarity coefficient varied from 0.38-0.86 with a mean of 0.72. Cluster analysis grouped the inbreds into three major clusters; and principal coordinate analysis depicted diverse genetic nature of inbred lines. Genetically diverse inbred lines with high kernel Fe and Zn can be used for development of Quality Protein Maize hybrids enriched with micronutrients. Phenotypically contrasting inbreds with high genetic divergence can serve as ideal parents in developing mapping population(s) for identifying loci underlying accumulation of Fe and Zn in maize kernel.

Keywords SSR; Genetic-diversity; Quality protein maize; Iron; Zinc

Background A major part of the world’s population depends on maize that provides 15% of global protein requirements to human (Shiferaw et al., 2011). Though maize kernel contains 8%-11% protein in endosperm, but is inherently deficient in two essential amino acids namely lysine and tryptophan (Vasal et al., 1980). The discovery of a recessive opaque2 mutant in maize resulted in enhanced concentration of lysine and tryptophan in endosperm protein (Mertz et al., 1964; Gupta et al., 2009). CIMMYT breeders later successfully combined the nutritional potential of opaque2 with the endosperm modifiers, and developed new maize genotypes, popularly referred to as ‘Quality Protein Maize’ (QPM) (Vasal et al., 1980; Gupta et al., 2013; Babu and Prasanna, 2014).

Micronutrient malnutrition is considered to be one of the major public health challenges to humankind (Black et al., 2008). Among the various micronutrients, iron (Fe) and zinc (Zn) have been identified as most important minerals that require urgent attention (Dalmiya and Schultink, 2003). It is estimated that over 60% of the world’s six billion people are Fe-deficient, and 30% of the population are affected due to Zn deficiency (White and Broadley, 2009; Gupta et al., 2015). Deficiency of Fe affects cognitive development, growth, reproductive performance and work productivity, while inadequate consumption of Zn leads to depression and psychosis, impaired growth and development besides affecting immune system (Solomons, 2003; Pixley et al., 2011).

Since human body cannot synthesize Fe and Zn, they must be made available through diet (Bouis et al., 2011). Development of micronutrient enriched maize holds immense promise as it provides sustainable and cost-effective solutions (Banziger and Long, 2000).
Further, Fe- and Zn-rich QPM genotypes would provide lysine and tryptophan in higher concentration (Gupta et al., 2015). Earlier reports suggested the possible role of opaque2 and its modifiers in enhancing Fe and Zn in maize (Arnold et al., 1977; Welch et al., 1993). Though various studies have analyzed variability for kernel -Fe and -Zn in maize, but it was performed predominantly among normal maize genotypes (Oikeh et al., 2003; Menkir, 2008; Pixley et al., 2011; Prasanna et al., 2011; Agrawal et al., 2012; Guleria et al., 2013). So far few research efforts have been directed towards analyzing QPM germplasm for their variability of kernel -Fe and -Zn. Further, understanding the genetic relationships of inbreds is of utmost importance for their effective utilization in the breeding programme (Choudhary et al., 2015). The present investigation, therefore, was aimed at to assess (i) the levels of variability for kernel Fe and Zn in diverse QPM inbreds and (ii) the genetic relationships among inbred lines, for their utilization in the biofortification programme.

1 Results

1.1 Genetic variability for kernel -Fe and- Zn

ANOVA revealed significant variation for kernel -Fe and -Zn among the diverse QPM inbreds (Table 1), where kernel-Fe ranged from 23.8-42.7 mg/kg and kernel-Zn varied from 12.6-39.4 mg/kg (Table 2). The mean kernel-Fe was 26.5 mg/kg, with five inbreds possessing ≥40 mg/kg of Fe viz. HKI170 (42.7 mg/kg), LQPM-20 (42.3 mg/kg), MGUQ-103 (41.6 mg/kg), DMRQPM-03-102 (41.2 mg/kg) and BQPML-10-1-1 (40.1 mg/kg). Further, 15 inbreds were found to possess 35-40 mg/kg of kernel-Fe (Table 2). The mean kernel-Zn among the inbreds was 33.4 mg/kg, with six inbreds possessing ≥35 mg/kg of Zn. Promising inbreds for kernel-Zn include BQPML-5204-1-5 (39.4 mg/kg), BQPML-199-2 (38.2 mg/kg), BQPML-5207-4-2 (37.9 mg/kg), MGUQ-102 (36.4 mg/kg), BQPML-10-1-1 (35.2 mg/kg) and LQPM-20 (35.0 mg/kg). The correlation between kernel -Fe and -Zn among the QPM genotypes was found to be 0.01.

1.2 SSR polymorphism

In total 256 alleles were detected across 75 SSR loci. The number of alleles per locus varied from two to seven, with an average of 3.41 alleles per locus (Table 3). The PIC value ranged from 0.11 (umc1066) to 0.79 (umc2165) with a mean of 0.50. Among 75 primers, 12 loci had di-repeat motif, 50 loci had tri-repeat, five loci had tetra-repeat motif, four loci were having penta-repeat motif and one locus belonged to hexa-repeat motif (Table 3). PIC (0.56) value of di-repeat based SSR loci were higher than other higher repeat motifs (0.46-0.55). The present study produced seven unique alleles by umc1857, phi125, umc2165, umc2360, umc1757, umc2021 and umc2373 among six genotypes (LQPM-2, LQPM-121, BQPML-5204-1-5, HKI161, BQPML-199-2 and MGUQ-103). Besides, 26 rare alleles (P<0.05) were detected; of which umc1757, umc1857, umc2360 and umc2143 were notable having lowest allele frequency. Allele present in maximum number of individuals among screened population is described as the major allele. In the present study the average major allele frequency was 0.54, with a range of 0.22 (umc2165) to 0.93 (umc1066). Gene diversity varied from 0.12 (umc1066) to 0.82 (umc2165) with an average of 0.56. The heterozygosity observed among the SSR markers varied from 0.00 to 0.17, with a mean of 0.03 (Table 3).

1.3 Genetic relationships

Genetic dissimilarity coefficient varied from 0.38 (VQL8 and VQL2) to 0.86 (MGU-107 and BQPML-5244; MGU-109 and LQPM-30), with an average dissimilarity value of 0.72. Inbred combinations having higher genetic dissimilarity include, VQL8 and CML161 (0.84), VQL5 and LQPM-40 (0.84), VQL5 and BQPML-5207-4-2 (0.83), MGUQ-109 and CML169 (0.83), MGUQ-103 and LQPM-19 (0.83). The phylogenetic analysis grouped 46 QPM inbred lines into three major clusters (Figure 1).

Table 1 Analysis of variance (ANOVA) for kernel-Fe and –Zn

| Sources of Variation | df | Kernel-Fe | Kernel-Zn |
|---------------------|----|-----------|-----------|
|                     |    | MSS       | t value   | Prob. | MSS       | t value   | Prob. |
| Replication         | 1  | 3.43      | 0.903     | 0.481 | 5.14      | 2.421     | 0.126 |
| Genotype            | 45 | 57.10     | 8.378     | 0.000 | 103.86    | 48.917    | 0.000 |
| Error               | 45 | 6.82      |           |       | 2.12      |           |       |

Note: df - degrees of freedom; MSS - Mean sum of squares; Prob. - Probability
Table 2 Details of QPM inbred lines used in the study

| S. No. | Inbred    | Source population   | Institution                                      | Kernel-Fe (mg/kg) | Kernel-Zn (mg/kg) |
|--------|-----------|---------------------|--------------------------------------------------|-------------------|-------------------|
| 1      | BQPML-5244| G33QC20             | ANGRAU, Hyderabad, India                         | 31.4              | 30.0              |
| 2      | BQPML-63-1-3 | P61C1                  | ANGRAU, Hyderabad, India                         | 24.7              | 31.4              |
| 3      | BQPML-5122 | 587(PC65Q)           | ANGRAU, Hyderabad, India                         | 27.0              | 33.7              |
| 4      | BQPML-5204-2-5 | P65C6                    | ANGRAU, Hyderabad, India                         | 32.2              | 34.9              |
| 5      | BQPML-5204-1-5 | P65C6                    | ANGRAU, Hyderabad, India                         | 33.8              | 39.4              |
| 6      | BQPML-5207-4-2 | P66CO                    | ANGRAU, Hyderabad, India                         | 32.5              | 37.9              |
| 7      | BQPML-10-1-1 | G17QC8                | ANGRAU, Hyderabad, India                         | 40.1              | 35.2              |
| 8      | BQPML-199-2 | G26QC23              | ANGRAU, Hyderabad, India                         | 31.1              | 38.2              |
| 9      | BQPML-62    | P61C1                | ANGRAU, Hyderabad, India                         | 26.2              | 34.9              |
| 10     | BQPML-412  | P66C0                | ANGRAU, Hyderabad, India                         | 29.8              | 31.8              |
| 11     | CML161     | G25QC18H520          | CIMMYT, Mexico                                   | 26.1              | 22.7              |
| 12     | CML162     | G25QC1F18            | CIMMYT, Mexico                                   | 28.6              | 30.1              |
| 13     | CML169     | G26QC22H7            | CIMMYT, Mexico                                   | 29.3              | 32.3              |
| 14     | CML170     | G26QC22H9            | CIMMYT, Mexico                                   | 33.6              | 20.9              |
| 15     | CML173     | P68C1F180            | CIMMYT, Mexico                                   | 27.0              | 20.8              |
| 16     | CML176     | P63-12-2-1/P67-5-1-1 | CIMMYT, Mexico                                   | 29.7              | 24.2              |
| 17     | CML180     | G32Q/18E944/SRBC4    | CIMMYT, Mexico                                   | 23.8              | 17.6              |
| 18     | LQPM-2     | S0/SN Comp(P)/SN6    | CSK-HPKV, Bajauria, India                        | 28.3              | 30.3              |
| 19     | LQPM-10    | 28FS(MS)/HEC         | CSK-HPKV, Bajauria, India                        | 39.8              | 25.7              |
| 20     | LQPM-19    | CIMMYT population-6482| CSK-HPKV, Bajauria, India                       | 35.7              | 22.2              |
| 21     | LQPM-20    | S0/SN Comp           | CSK-HPKV, Bajauria, India                        | 42.3              | 35.0              |
| 22     | LQPM-30    | 28FS(MS)/HEC         | CSK-HPKV, Bajauria, India                        | 39.4              | 31.8              |
| 23     | LQPM-34    | Shakti(S0)HE25       | CSK-HPKV, Bajauria, India                        | 37.7              | 21.3              |
| 24     | LQPM-40    | CIMMYT population-6482| CSK-HPKV, Bajauria, India                       | 26.4              | 12.6              |
| 25     | VQL1      | CM121/CML180         | VPAS, Almora, India                              | 36.0              | 28.9              |
| 26     | VQL2      | CM145/CML170         | VPAS, Almora, India                              | 34.5              | 22.0              |
| 27     | VQL5      | V25/CML184           | VPAS, Almora, India                              | 27.9              | 31.7              |
| 28     | VQL8      | CM145/CML170         | VPAS, Almora, India                              | 37.8              | 27.2              |
| 29     | VQL26     | V351/CML173          | VPAS, Almora, India                              | 37.1              | 24.5              |
| 30     | DMRQPM-60 | 28 FS (MS)6 HECC     | DMR, New Delhi, India                            | 39.3              | 19.7              |
| 31     | DMRQPM-03-102 | Derivative of ‘Shakti’ | DMR, New Delhi, India                         | 41.2              | 26.0              |
| 32     | DMRQPM-121 | Derivative of ‘Shakti’ | DMR, New Delhi, India                          | 36.1              | 21.7              |
| 33     | HKI161    | Selection from CML161 | CCS-HAU, Uchani, India                          | 26.2              | 18.4              |
| 34     | HKI163    | Selection from CML163 | CCS-HAU, Uchani, India                          | 27.9              | 31.6              |
| 35     | HKI170    | Selection from CML170 | CCS-HAU, Uchani, India                          | 42.7              | 23.8              |
| 36     | HKI193-1  | Selection from CML193 | CCS-HAU, Uchani, India                          | 37.0              | 28.9              |
| 37     | HKI193-2  | Selection from CML193 | CCS-HAU, Uchani, India                          | 39.7              | 32.9              |
| 38     | MGUQ-101  | HKI1105/CML161       | IARI, New Delhi, India                           | 35.9              | 27.2              |
| 39     | MGUQ-102  | HKI1128/HKI193-1     | IARI, New Delhi, India                           | 34.3              | 36.4              |
| 40     | MGUQ-103  | HKI323/HKI161       | IARI, New Delhi, India                           | 41.6              | 25.8              |
| 41     | MGUQ-104  | CM137/DMRQPM03-124   | IARI, New Delhi, India                           | 36.2              | 15.9              |
| 42     | MGUQ-105  | CM138/CML161         | IARI, New Delhi, India                           | 28.2              | 14.0              |
| 43     | MGUQ-106  | CM139/DMRQPM-58      | IARI, New Delhi, India                           | 38.4              | 13.9              |
| 44     | MGUQ-107  | CM140/CML161         | IARI, New Delhi, India                           | 36.9              | 15.8              |
| 45     | MGUQ-108  | CM150/CML161         | IARI, New Delhi, India                           | 30.8              | 19.7              |
| 46     | MGUQ-109  | CM151/DMRQPM-58      | IARI, New Delhi, India                           | 32.8              | 19.4              |
| Mean   |           |                     |                                                  | 33.4              | 26.5              |
| SE     |           |                     |                                                  | 2.61              | 1.45              |
| S.No. | Primers | Bin | Repeats | Major Allele Frequency | Alleles detected | Gene Diversity | Heterozygosity | PIC |
|-------|---------|-----|---------|------------------------|-----------------|---------------|---------------|-----|
| 1     | umc1353 | 1.00| (AAC)4  | 0.42                   | 3               | 0.64          | 0.02          | 0.57|
| 2     | bnlg1014| 1.01| (AG)14  | 0.50                   | 4               | 0.63          | 0.07          | 0.57|
| 3     | umc2226 | 1.02| (TGG)6  | 0.39                   | 3               | 0.65          | 0.00          | 0.58|
| 4     | umc1452 | 1.04| (GCC)4  | 0.67                   | 2               | 0.44          | 0.00          | 0.34|
| 5     | umc2083 | 1.06| (CGG)7  | 0.77                   | 3               | 0.37          | 0.09          | 0.33|
| 6     | umc1446 | 1.08| (TAA)17 | 0.46                   | 5               | 0.65          | 0.13          | 0.59|
| 7     | bnlg1331| 1.09| (AG)16  | 0.41                   | 4               | 0.68          | 0.07          | 0.62|
| 8     | umc1552 | 2.02| (GGA)7  | 0.36                   | 4               | 0.71          | 0.13          | 0.65|
| 9     | umc2193 | 2.02-2.03| (TCC)6 | 0.71 | 2 | 0.41 | 0.00 | 0.33 |
| 10    | phi083  | 2.04| AGCT    | 0.40                   | 4               | 0.68          | 0.07          | 0.61|
| 11    | umc2077 | 2.06| (AGC)4  | 0.85                   | 2               | 0.26          | 0.00          | 0.22|
| 12    | umc2129 | 2.07| (CGC)5  | 0.48                   | 4               | 0.68          | 0.00          | 0.63|
| 13    | bnlg1316| 2.08| (AG)13  | 0.48                   | 3               | 0.60          | 0.00          | 0.52|
| 14    | umc2101 | 3.01| (AG)7   | 0.34                   | 4               | 0.73          | 0.11          | 0.68|
| 15    | phi3744 | 3.02| ACC     | 0.43                   | 3               | 0.63          | 0.00          | 0.55|
| 16    | umc2369 | 3.02-3.03| (GCAC)4 | 0.60 | 4 | 0.56 | 0.07 | 0.49 |
| 17    | umc2259 | 3.03| (CGG)6  | 0.63                   | 3               | 0.53          | 0.00          | 0.47|
| 18    | umc2262 | 3.04| (CATCT)5| 0.48                   | 3               | 0.56          | 0.13          | 0.46|
| 19    | umc1102 | 3.05| GGAT    | 0.48                   | 4               | 0.62          | 0.04          | 0.55|
| 20    | umc1690 | 3.07| (GCA)4  | 0.35                   | 4               | 0.69          | 0.04          | 0.63|
| 21    | umc1915 | 3.08| (ACA)6  | 0.60                   | 2               | 0.48          | 0.00          | 0.36|
| 22    | bnlg1182| 3.09| (AG)19  | 0.46                   | 4               | 0.69          | 0.00          | 0.64|
| 23    | umc1641 | 3.1 | (TCGCC)4| 0.45                   | 3               | 0.65          | 0.02          | 0.57|
| 24    | umc1757 | 4.01| (TCC)7  | 0.32                   | 5               | 0.74          | 0.09          | 0.69|
| 25    | umc1758 | 4.02| (CTT)5  | 0.50                   | 3               | 0.62          | 0.00          | 0.55|
| 26    | umc1902 | 4.03| (CT)16  | 0.51                   | 4               | 0.61          | 0.17          | 0.54|
| 27    | umc2280 | 4.03-4.04| (CATT)A4 | 0.30 | 4 | 0.73 | 0.00 | 0.68 |
| 28    | bnlg490 | 4.04| -       | 0.44                   | 4               | 0.69          | 0.02          | 0.64|
| 29    | umc2061 | 4.05| (CTG)8  | 0.48                   | 4               | 0.65          | 0.09          | 0.59|
| 30    | umc2038 | 4.07| (GAC)4  | 0.48                   | 3               | 0.58          | 0.07          | 0.49|
| 31    | umc2384 | 4.08| (GCC)5  | 0.80                   | 2               | 0.31          | 0.04          | 0.27|
| 32    | umc2360 | 4.09| (GCC)4  | 0.87                   | 3               | 0.23          | 0.02          | 0.21|
| 33    | umc1532 | 4.10| (AAAT)4 | 0.34                   | 4               | 0.71          | 0.07          | 0.65|
| 34    | bnlg1890| 4.11| (AG)26  | 0.51                   | 5               | 0.67          | 0.15          | 0.64|
| 35    | umc1761 | 5.02| (GCA)5  | 0.63                   | 3               | 0.51          | 0.00          | 0.43|
| 36    | umc2296 | 5.03| (AGT)4  | 0.61                   | 2               | 0.48          | 0.04          | 0.36|
| 37    | umc2298 | 5.03-5.04| (GCG)4 | 0.63 | 2 | 0.47 | 0.00 | 0.36 |
| 38    | umc2373 | 5.04| (GCT)4  | 0.30                   | 6               | 0.76          | 0.04          | 0.72|
| 39    | bnlg118 | 5.07| -       | 0.56                   | 2               | 0.49          | 0.00          | 0.37|
| 40    | umc2143 | 5.08| (TTC)4  | 0.47                   | 4               | 0.64          | 0.04          | 0.57|
| 41    | umc1153 | 5.09| (TCA)4  | 0.54                   | 4               | 0.63          | 0.00          | 0.58|
| 42    | umc1186 | 6.01| (GCT)5  | 0.50                   | 3               | 0.61          | 0.00          | 0.53|
| 43    | umc1723 | 6.02| (CTT)6  | 0.83                   | 3               | 0.30          | 0.00          | 0.28|
| 44    | umc1257 | 6.02-6.03| (CAC)4 | 0.52 | 3 | 0.59 | 0.00 | 0.51 |
| 45    | phi389203| 6.03| AGC     | 0.52                   | 3               | 0.54          | 0.00          | 0.43|
| 46    | umc1837 | 6.04| (TAA)6  | 0.61                   | 3               | 0.48          | 0.02          | 0.38|
| 47    | umc2141 | 6.05| (CT)8   | 0.48                   | 3               | 0.63          | 0.00          | 0.55|
| 48    | umc2375 | 6.06-6.07| (GCG)4 | 0.74 | 3 | 0.42 | 0.00 | 0.38 |
| 49    | umc2165 | 6.07| (CTT)6  | 0.22                   | 7               | 0.82          | 0.09          | 0.79|
| 50    | umc2324 | 6.08| (CAC)4  | 0.53                   | 2               | 0.50          | 0.00          | 0.37|
| 51    | phi057  | 7.01| GCC     | 0.76                   | 3               | 0.38          | 0.00          | 0.33|
Continued Table 1

| S.No. | Primers | Bin  | Repeats     | Major Allele Frequency | Alleles detected | Gene Diversity | Heterozygosity | PIC  |
|-------|---------|------|-------------|------------------------|------------------|---------------|----------------|------|
| 52    | umc1066 | 7.01 | (GCCAGA)5   | 0.93                   | 2                | 0.12          | 0.00           | 0.11 |
| 53    | umc1831 | 7.02 | (AG)8       | 0.38                   | 5                | 0.72          | 0.00           | 0.67 |
| 54    | umc1456 | 7.03 | (AACC)5     | 0.65                   | 2                | 0.45          | 0.00           | 0.35 |
| 55    | umc2332 | 7.04 | (CTC)5      | 0.46                   | 4                | 0.68          | 0.00           | 0.62 |
| 56    | phi079  | 7.05 | AGATG       | 0.58                   | 5                | 0.57          | 0.09           | 0.50 |
| 57    | umc2334 | 7.06 | (GGA)4      | 0.51                   | 2                | 0.50          | 0.00           | 0.37 |
| 58    | umc1327 | 8.01 | (GCC)4      | 0.44                   | 4                | 0.64          | 0.00           | 0.58 |
| 59    | phi125  | 8.03 | AG          | 0.67                   | 3                | 0.45          | 0.00           | 0.37 |
| 60    | bnlg240 | 8.06 | -1          | 0.39                   | 6                | 0.74          | 0.00           | 0.71 |
| 61    | bnlg1065| 8.07 | (AG)21      | 0.46                   | 4                | 0.63          | 0.00           | 0.56 |
| 62    | umc2393 | 9.00 | (ACG)7      | 0.46                   | 4                | 0.67          | 0.00           | 0.61 |
| 63    | bnlg1724| 9.01 | (AG)31      | 0.69                   | 3                | 0.45          | 0.00           | 0.38 |
| 64    | phi028  | 9.01-9.02| GAA         | 0.46                   | 4                | 0.69          | 0.00           | 0.65 |
| 65    | umc2130 | 9.02 | (CGG)6      | 0.65                   | 3                | 0.49          | 0.00           | 0.42 |
| 66    | umc2336 | 9.02-9.03| (TGT)4     | 0.63                   | 2                | 0.47          | 0.00           | 0.36 |
| 67    | umc1743 | 9.03 | (GGC)4      | 0.65                   | 3                | 0.48          | 0.09           | 0.40 |
| 68    | umc2134 | 9.05 | (TTC)6      | 0.46                   | 3                | 0.60          | 0.00           | 0.52 |
| 69    | umc2358 | 9.06-7.07| (CGC)5    | 0.60                   | 2                | 0.48          | 0.00           | 0.36 |
| 70    | umc2089 | 9.07 | (CGC)4      | 0.90                   | 3                | 0.18          | 0.00           | 0.17 |
| 71    | umc2018 | 10.01 | (CCT)7      | 0.80                   | 2                | 0.31          | 0.00           | 0.27 |
| 72    | umc1318 | 10.02 | (GTC)5      | 0.43                   | 4                | 0.63          | 0.12           | 0.55 |
| 73    | phi084  | 10.04 | GAA         | 0.50                   | 3                | 0.62          | 0.00           | 0.55 |
| 74    | umc2043 | 10.05 | (TCC)4      | 0.48                   | 3                | 0.64          | 0.00           | 0.56 |
| 75    | umc2021 | 10.07 | (TGG)4      | 0.37                   | 6                | 0.72          | 0.13           | 0.67 |
| Mean  |         |      |             |                        |                  | 0.54          | 3.41           | 0.03 |
|       |         |      |             |                        |                  | 0.56          | 0.31           | 0.50 |

Figure 1 Cluster analysis depicting genetic relationship among 46 QPM inbreds. Bootstrap value of ≥30 is presented.
Cluster A comprised of 13 inbred lines, all of which were indigenous in nature. Subcluster A1 had nine inbreds, while subcluster A2 contained four inbreds. Cluster B could be sub-divided into two subgroups, in which subcluster B1 had six Indian lines and one CIMMYT line, while subcluster B2 had four inbreds of Indian origin. Cluster C having four sub-clusters emerged as the largest cluster, with subcluster C1 having seven indigenous inbreds, while five inbreds of both exotic and indigenous origin were in each of subcluster C2, C3 and C4 (Figure 1). PCoA revealed diverse nature of the inbreds with both indigenous and exotic lines were found to be distributed in all quadrangles (Figure 2).

**Figure 2** Principal Coordinate Analysis (PCoA) among 46 QPM inbreds using 75 SSR markers

### 2 Discussion

Wide genetic variations observed for kernel -Fe and -Zn among the QPM genotypes suggested the potential for genetic improvement of target traits. Guimaraes et al. (2004) while analyzing 189 QPM inbreds reported a range of 14-54 mg/kg of Zn in kernel. Chakraborti et al. (2009) analyzed 10 QPM inbreds in India and reported variation of kernel -Fe (16.7-31.1 mg/kg) and -Zn (25.5-32.9 mg/kg). Besides, presence of wide variation predominantly among the normal inbreds have been reported by various researchers (Oikeh et al., 2003; Menkir, 2008; Prasanna et al., 2011; Agrawal et al., 2012; Guleria et al., 2013).

The study did not show any correlation between kernel -Fe and -Zn. Similar observations were also observed by Arnold and Bauman (1976), Agrawal et al. (2012) and Prasanna et al. (2011). This could be possibly due to the fact that majority of QTLs identified for kernel -Fe and -Zn in maize were unique (Simic et al., 2011). This is also evident from the present results that some of the lines showed high value for one micronutrient (HKI170, Fe: 42.7 mg/kg), but were low in other (HKI170, Zn: 23.8 mg/kg). Similar observations were also recorded in MGUQ-106, MGUQ-107, MGUQ-108, MGUQ-104, DQPM-60, DQPM-121, VQL2, LQPM-34 and
VQL26. However, due to random segregation followed by accumulation of favourable genes for both the traits, it was possible to identify inbreds promising for both kernel micronutrients. Considering this, BQPML-10-1-1 (Fe: 40.1 mg/kg; Zn: 35.1 mg/kg), HKI193-2 (Fe: 39.7 mg/kg; Zn: 32.9 mg/kg), LQPM-30 (Fe: 39.4 mg/kg; Zn: 31.8 mg/kg), MGUQ-102 (Fe: 34.3 mg/kg; Zn: 36.4 mg/kg) and BQPML-5204-1-5 (Fe: 33.8 mg/kg; Zn: 39.4 mg/kg) were identified as promising lines that can be readily utilized in the breeding programme. The study thus suggested that both kernel -Fe and -Zn can be improved independent of each other. However, several studies have reported positive correlation between kernel -Fe and -Zn (Brkic et al., 2003; Oikeh et al., 2003; Guimaraes et al., 2004; Menkir, 2008; Chakraborti et al., 2009). Interestingly, Pixley et al. (2011) could find positive correlation only in testcross hybrid trial, while the same was not observed in other two hybrid trials. This contrast could be attributed to the inherent nature of the specific type of germplasm used in these studies.

Some of the inbreds used in the study have been derived from the same source population. In majority of such cases, the kernel -Fe and -Zn were comparable among the sister lines; possibly due to inheritance of same set of genes from the source population. For example, (a) HKI193-1 and HKI193-2; (b) CML161, HKI161 and CML162; and (c) BQPML-5204-2-5 and BQPML-5204-1-5 are noteworthy to mention (Table 2). In contrast, CML169 possessed 32.3 mg/kg of kernel-Zn, while CML170 contained low Zn (20.9 mg/kg). This could be possible due to segregation and fixation of different gene sets during derivation of the inbreds.

A number of SSR markers were found to have selective efficiency in separating one inbred from rest by amplification of unique alleles that can be useful in fingerprinting studies. The major allele frequency with low value observed in the study is indicative of highly diverse locus among the selected panel of genotypes (Nepolean et al., 2013). Low mean heterozygosity as revealed in the study was indicative of attainment of high level of homozygosity among the inbred lines (Choudhary et al., 2015). However, few primers showed high heterozygosity like umc1902 (0.17), bnlg1890 (0.15), umc1446 (0.13), umc1552 (0.13) and umc2262 (0.13). This could be attributed to residual heterozygosity, due to which inbreds tend to segregate for few loci regardless of repeated cycles of selfing over many generations (Kaur et al., 2011). As compared to doubled haploid based inbred derivation, conventionally bred inbred lines often show some degree of residual heterozygosity (Sivarajanji et al., 2014). Other possible reasons that contribute to heterozygosity include mutation at specific SSR locus or amplification of similar sequences from different genomic regions due to duplication (Semagn et al., 2006).

Broadly, clustering pattern depicted robust congruence with pedigree data (Table 2). Majority of BQPML-inbreds developed at ANGRAU, Hyderabad were grouped in Cluster B, suggesting that inbreds were related to each other by descent. Besides, HKI193-1 and HKI193-2 developed from CML193 were grouped together in sub-cluster C3. Similar observations were also noted in case of HKI161 and CML161, HKI170 and CML170 in Cluster C; VQL2 and VQL8 in Cluster A; BQPML-5204-2-5 and BQPML-5204-1-5 in Cluster B. Earlier, Chakraborti et al. (2011) grouped 24 normal maize inbreds differing for kernel -Fe and -Zn into four clusters using 50 SSR markers. The genetic relationships as depicted by cluster analysis were further reconfirmed by PCoA, where both indigenous and exotic lines were found to be distributed in all quadrangles, thereby suggesting wide genetic variability among these inbreds (Fig. 2). HKI193-1 and HKI193-2; and VQL2 and VQL8 of Indian origin could be placed in the same quadrangle due to their similar pedigree. Exotic inbreds, CML169 and CML170 also showed similar trend.

Based on kernel micronutrients and genetic diversity, systematic crosses among selected lines could be generated in the biofortification programme (Sivarajanji et al., 2014; Choudhary et al., 2015). Genetically diverse parents with contrasting micronutrients can be crossed to generate F2:3 and/or RIL mapping population(s) to identify quantitative trait loci (QTL) governing the accumulation of kernel Fe and Zn. LQPM-30, MGUQ-102, BQPML-5204-1-5 and BQPML-10-1-1 having a higher concentration of kernel -Fe and -Zn can be crossed with inbreds having low kernel micronutrients.
such as CML180 and LQPM-40, to generate such mapping populations (Fig. 1, Table 2). Though QTL for kernel -Fe and -Zn in maize have been mapped by several researchers (Simic et al., 2011; Lungaho et al., 2011; Qin et al., 2012; Baxter et al., 2013), in all such studies normal maize inbreds were used in generating mapping populations. So far no QTL mapping experiment(s) for kernel -Fe and -Zn in maize has been reported using QPM inbreds. Since, modifiers in opaque2 genetic background were found to have possible pleotropic effects on accumulation of micronutrients in maize (Arnold et al., 1977; Welch et al., 1993), mapping experiments using QPM inbreds would effectively help to map such unique loci present in QPM genetic background.

Further, enhancing the genetic base of elite germplasm through introgression of exotic germplasm is important to exploit heterotic potential (Ron-Parra and Hallauer, 1997). Genetic divergence among the parents is one of the key factors that determines the extent of heterosis (Moll et al., 1965; Prasad and Singh, 1986). Based on the genetic relationships and high kernel -Fe and -Zn concentration, the following potential heterotic combinations were identified in the present study: MGUQ-102 × HKI193-2, MGUQ-102 × BQPML-5204-1-5, MGUQ-102 × BQPML-10-1-1, HKI193-2 × BQMPL-5204-1-5 and HKI193-2 × BQPML-10-1-2. These combinations could be attempted to develop hybrids with a desirable concentration of micronutrients.

The present investigation thus led to the identification of promising QPM inbreds for kernel -Fe and -Zn. Microsatellite marker-based genetic diversity analysis among the selected set of QPM inbred lines from indigenous and exotic origin revealed high degree of genetic variability. The study also identified potential cross combinations that could be planned and generated for further use in genetic and breeding strategies pertaining to improvement of kernel micronutrients in maize.

3 Materials and methods
3.1 Plant materials
A set of 46 QPM inbreds comprised of exotic and indigenous origin were selected for assessing the genetic diversity (Table 2). Of these, 39 inbreds were developed by different maize breeding centres of India, while seven inbreds were selected from CIMMYT, Mexico. All these inbreds were grown in the field during 2012 rainy season at IARI Experimental Farm, New Delhi.

3.2 Estimation of kernel -Fe and -Zn
Five selfed ears per genotype were hand-harvested with husk and dried under clean shade to reduce post harvest grain moisture concentration to ~14%. Grains collected from all the ears were bulked and representatives were drawn by quartering method. Individual samples in duplicates were ground into fine powder using iron free Cyclotech Sample Mill. Biochemical analysis for kernel -Fe and -Zn concentration was carried out by digestion with 9:4 diacid mixture (HNO$_3$: HClO$_4$) followed by observation in atomic absorption spectrometer (AAS-Analytikjena, Zeenit 700), as per the protocols described by Zarcinas et al. (1987) and Singh et al. (2005). Analysis of variance (ANOVA) following complete randomized design (CRD) was calculated using Windostat 8.0. Pearson’s simple correlation coefficient was calculated using Office-Excel.

3.3 SSR markers and PCR amplification
Total genomic DNA was extracted from leaves of three-week old seedlings using modified CTAB procedure (Saghai-Marof et al., 1984). 75 microsatellite/SSR markers based on their broad coverage of the maize genome were used for the analysis (Table 3). The primer information for the selected SSR markers is available in public domain (http://www.maizegdb.org). The PCR amplification of SSR primers was carried out as per Bantte and Prasanna (2003). The amplified products were resolved using 3.5% agarose (Amresco, USA) gel.

3.4 SSR data analysis
The allele size was determined by comparing the 100bp DNA ladder. Gene diversity, major allele frequency, unique and rare alleles, and polymorphism information content (PIC) values were computed using Power Marker 3.25 (Liu and Moose, 2005). Genetic dissimilarity was calculated using Jaccard’s coefficient. Cluster analysis following unweighted neighbour-joining method and principal coordinate analysis (PCoA) were undertaken using DARwin5.0 (Perrier et al., 2003).

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