Results

I. Implementation of a Marker-assisted Backcrossing Strategy for QPM Line Development

Two elite non-QPM parental inbred lines (CM137 and CM138) of a popular single cross hybrid PEHM-2 (Pusa Early Hybrid Makka-2) developed at the Division of Genetics, IARI, New Delhi, were selected as recurrent parents for QPM conversion in the present study. CML161, a medium-maturity QPM inbred line developed at CIMMYT, Mexico, and DMRQPM03-124, an early-maturing QPM inbred line developed at Directorate of Maize Research, New Delhi, were used as donor parents for conversion of CM137 and CM138, respectively, using the strategy illustrated in Fig. 1. Crosses among the recurrent and donor parents were made during Rabi 2005-06 at Maize Winter Nursery, Hyderabad, and BC₁F₁ progenies were generated through backcrossing with the respective recurrent parents at IARI Experimental Farm, Delhi during Kharif 2006.

Foreground and Background Selections on BC₁F₁ Progenies

The BC₁F₁ progenies for each of the two cross combinations were raised at the Maize Winter Nursery, Hyderabad, during Rabi 2006-07. Individual plants were tagged and leaf samples were collected at 3-4 leaf stage (Fig. 3). DNA isolations were undertaken using a modified CTAB protocol standardized at Maize Genetics Unit, IARI, New Delhi. MAS (foreground selection) on the BC₁F₁ progenies was undertaken for identifying the individuals with the target genotype (O2o2) using opaque2-specific SSR marker (umc1066).

According to Mendelian inheritance, the frequency of heterozygotes in a backcross population would be 0.50. Chi-square test was performed using the standard procedure for testing the goodness-of-fit of the observed segregation pattern with the expected in each of the backcross populations (CM137- and CM138-based). The populations showing normal segregation ratio (dominant homozygote: heterozygote = 1:1) were carried forward.
SSR polymorphism survey was undertaken on each of the two pairs of recipient-donor parents, leading to the detection of 32 polymorphic SSRs in each parent combination, covering all the 10 chromosomes of maize. The details of SSR markers used for background selection of CM137- and CM138-based populations are presented in Tables 1 and 2.

**CM37-based BC₁F₁ population:** A total of 176 individual plants from CM137 x DMRQPM03-124 based BC₁F₁ population were screened and 80 heterozygotes were identified (Fig. 3 & 5). Chi-square test indicated goodness-of-fit of the observed segregation pattern with the expected pattern (1 $O2o2$: 1 $O2O2$) (Table 3). All the heterozygotes were further analyzed using background SSR markers (32), for recovery of recurrent parent genome (Table 1). In the BC₁F₁ progenies, the usual expectation is that on an average 75% recovery of recurrent parent alleles are possible. However, using genetically co-dominant SSR markers which are polymorphic between specific recurrent and donor parents, it is possible to identify individuals with >75% recovery of recurrent parent genome in the BC₁F₁ progenies. Six individual plants with ≥85% of recurrent parent genome were selected and were also further backcrossed to CM137 to generate BC₂F₁ progenies (Table 4). Each of the selected six individual plants was also selfed to generate BC₁F₂ ears for comparing the kernel modification attributes (Fig. 8). Ears of selected individuals based on foreground and background selections were phenotypically compared with those of the respective recurrent parents, for further selections.

BC₂F₁ and BC₁F₂ ears harvested from the selected plants were compared for kernel modification and plant numbers MB-137-124, MB-137-113 and MB-137-144 (with recurrent parent genome recovery of 83.33%, 85.00% and 86.55%) were finally forwarded based on high degree of kernel modification attributes and ear phenotype resemblance with recurrent parent ears besides foreground and background selection. These three ears showed
high degree of endosperm modification (score 1.00-1.50), with the ear phenotype closely resembling that of the recurrent parent.

**CM138-based BC₁F₁ population:** In this case a, total of 229 individual plants (BC₁F₁ population) were screened (foreground selection) using umc1066 SSR marker, and 105 O2o2 heterozygotes were identified. Chi-square analysis revealed that the population conformed to the expected segregation pattern of 1:1 for O2O2: O2o2 (Table 3). A set of 32 polymorphic SSR markers between CM138 and CML161 were used for background selection on all the 105 plants (Table 2; Fig. 6 & 7). Eight individuals with >80% recovery of recurrent parent genome were identified. These plants were backcrossed with CM138 to generate BC₂F₁ and were also selfed to generate BC₁F₂ (Table 4; Fig. 4).

Phenotypic selection for endosperm modification was exercised on the resulting ears vis-à-vis the check (CM138) (Fig. 8 & 17). The study led to identification of two individual plants (MB-138-19 and MB-138-9) with heterozygous state at the opaque2 locus and a recovery of 84.22% (MB-138-19) and 86.55% (MB-138-9) of the recurrent parent genome, besides high endosperm modification (score 1.50) and high resemblance to the ear phenotype of recurrent parent.

**Foreground and Background Selections on BC₂F₁ Progenies**

Kernels from five selected ears each of the CM137- and CM138-based BC₂F₁ populations were planted at the IARI Experimental Farm, New Delhi, during Kharif-2007. Foreground selection was undertaken using the same procedure as done in BC₁F₁ generation for all the four populations and O2/o2 heterozygotes were identified. Background selection was also carried out using previously identified 32 SSR markers, specifically for those showing heterozygosity in the previous generation (BC₁F₁). χ² test was performed using the standard procedure for testing the goodness-of-fit of the observed segregation pattern in each of the families. Based on the foreground and
background selection, selected plants were selfed to raise BC2F2 generations. The procedure is described in detail below for each of the populations.

**CM137-based BC2F1 population:** MB-137-113 and MB-137-144 based BC2F1 population, consisting of 53 and 57 individual plants, respectively, were subjected to foreground selection. A set of 23 and 24 individuals, respectively, were found to be O2o2 heterozygotes (Fig. 9). All these selected 47 heterozygotes were analyzed using background SSR markers, specifically those which were found to be heterozygous in BC1F1 selected individuals (Table 1; Fig. 11). Of the 95 individuals in MB-137-124 based BC2F1 population, only five were identified to have heterozygosity at the opaque2 locus. χ² test indicated segregation distortion at opaque2 locus in one of the three families (MB-137-124 based population) was not forwarded (Table 5). Based on the background selection with 32 polymorphic SSR markers, 10 individual plants with >90% recovery of recurrent parent genome were selected for further phenotypic selection (Table 7).

Considering the extent of kernel modification (kernel vitreousness) and ear phenotype vis-a-vis CM137, specific ears, namely MB-137-113-13, MB-137-113-52, MB-137-113-144, MB-137-144-5, MB-137-144-8 and MB-137-144-56 with a high recovery of recurrent parent genome (93.33% to 96.66%) were selected for further advancement.

**CM138-based BC2F1 population:** Two specific families, namely MB-138-9 and MB-138-19, were raised in Kharif 2007 at IARI Experimental Farm, Delhi. In MB-138-19 based population, a total of 95 individuals were screened for foreground selection and 9 plants were found to have heterozygosity at opaque2 locus (Fig. 10). In case of MB-138-9 based population, a total of 90 progenies were screened for foreground selection and 40 heterozygotes were identified. χ² test revealed normal segregation of opaque2 locus in this particular population, and was therefore, further subjected to background selection, while segregation distortion was observed in one family (MB-138-19-based) which was not carried forward. Of the total 40 heterozygotes in MB-
138-9-based family, five plants were selected on the basis of >90% recovery of recurrent parent genome (Table 7; Fig. 12) and two plants MB-138-9-127 (with 95.55% recovery) and MB-138-9-165 (with 96.66% recovery) were finally identified, also taking into account kernel modification attributes and ear phenotypes of the recurrent parent CM138.

**Biochemical Analysis of BC2F2 Families**

Selections for endosperm modification were undertaken on the BC2F2 families from the selected plants using the 'backlit' kernel assay as described earlier. A set of 50 randomly taken kernels from each of the selected BC2F2 ears in both CM137- and CM138-based populations was subjected to biochemical analysis, that is, estimation of percent tryptophan in the endosperm flour. The lysine proportion in endosperm protein was not determined since a highly significant and positive correlation was well-established between lysine and tryptophan contents in the endosperm protein (Pixley and Bjarnason, 1993). The results of the biochemical analysis for each of the populations are discussed below.

**CM137-based BC2F2:** Kernels from all the six ears of CM137-based populations, namely MB-137-113-13, MB-137-113-52, MB-137-113-144, MB-137-144-5, MB-137-144-8 and MB-137-144-56, were analyzed biochemically for their endosperm tryptophan content (Table 8). MB-137-144-8 was found to be the best with 0.059% tryptophan in the endosperm flour. Among other ears, MB-137-144-56 exhibited 0.058% tryptophan followed by MB-137-113-144 (0.052%), MB-137-113-52 (0.051%), MB-137-144-5 (0.050%) and MB-137-113-13 (0.045%). Interestingly, recurrent parent, CM137 was found to contain 0.039% tryptophan whereas the donor parent DMRQPM03-124 recorded 0.091%. Since in the BC2F2 generation the opaque2 gene segregates in 1:2:1 ratio (O2O2 : O2o2 : o2o2), only the recessive homozygote (o2o2) would exhibit the benefit of the higher tryptophan, while O2O2 and O2o2 would be behaving like a normal maize with lesser content of the amino acid. This suggests that in the flour of the BC2F2 seeds, o2o2 genotypes would cause
25% increase in the tryptophan over the recurrent parent. Therefore, the seeds harvested from selected MAS derived ears should have >0.041% tryptophan in the endosperm flour. As expected all the selected ears were found to have tryptophan from 0.045-0.059%, indicating the nutritional worth of the MAS-derived lines.

**CM138-based BC₃F₂ ears:** Among the CM138-based populations, MB-138-9-127 was found to have 0.051% tryptophan in the endosperm flour, while, MB-138-9-165 showed 0.044% tryptophan (Table 8). In contrast, CM138 exhibited 0.0043% tryptophan while the donor parent CML161 revealed 0.090% tryptophan, indicating the nutritional superiority of MAS-derived inbred lines.

**Foreground and Background Selections in BC₃F₂ Generation**

The selected BC₃F₂ progenies were raised during Rabi 2007-08 at Maize Winter Nursery, Hyderabad. Foreground selections were undertaken on the individual plants (at the seedling stage) using opaque2-specific SSR marker to identify the recessive homozygotes (o2o2) (Fig. 13 & 14). Families conforming to the expected segregation pattern were identified and the recessive homozygotes from these families were selfed to generate BC₃F₃. The results were discussed in detail below.

**CM137-based BC₃F₂:** Six BC₃F₂ families of CM137-based population consisting of 235 individual plants were screened for opaque2 homozygotes (o2o2) (Table 9). Chi-square test was performed on the marker segregation data for each family (with expected segregation pattern as 1 O2/O2: 2 O2/o2: 1 o2/o2 (Table 10). On this basis, 34 individual plants belonging to three families were selected for generation advancement based on chi-square test.

A total of 40, 39, 46, 35, 35 and 40 seedlings of MB-137-113-13, MB-137-113-52, MB-137-113-144, MB-137-144-5, MB-137-144-8 and MB-137-144-56 respectively were screened for o2o2 homozygotes, leading to the identification of 12, 4, 19, 10, 5 and 12 o2o2 individuals. Of the six families,
three families (MB-137-113-13, MB-137-144-5 and MB-137-144-56) showed conformity with the expected segregation pattern (1:2:1), and were further analyzed using background SSR markers (Fig. 15). The identified homozygotes (34) from these three families were selfed to generate BC$_2$F$_3$ lines.

**CM138-based BC$_2$F$_2$:** Two BC$_2$F$_2$ families of CM138-based population, consisting of 100 and 98 individuals, respectively, were screened for opaque2 homozygotes (o2o2). Chi-square test was performed on the marker segregation data for each family (with expected segregation pattern as 1 O2O2: 2 O2o2: 1 o2o2); the analysis revealed goodness-of-fit of the observed segregation with the expected in both the families (Table 11). Based on foreground selection, a set of 26 and 27 homozygotes (o2o2), respectively, from each of the families were identified. These o2o2 individuals were subjected to background selection (Fig. 16), and selected homozygotes with high recovery of recurrent parent genome (mean ~95%) were selfed and BC$_2$F$_3$ lines were generated. Further on the basis of ear phenotype and kernel attributes, MB-138-9-165 was withdrawn from further advancement and only MB-138-9-127 was carried forward.

**Biochemical Analysis of BC$_2$F$_3$ Progenies**

In the BC$_2$F$_3$ progenies, the opaque2 allele is fixed in recessive homozygous state. This ensures the nutritional superiority of the MAS-derived families. However, to further verify this, the percent endosperm protein and percent tryptophan in endosperm protein were analyzed in each of the BC$_2$F$_3$ progenies. For this purpose, 50 randomly drawn kernels from each of the selected ears were subjected to biochemical analyses using the standard protocol as mentioned earlier.

**CM137-based BC$_2$F$_3$ progenies:** The seven selected CM137-based BC$_2$F$_3$ ears (from three selected BC$_2$F$_2$ families), namely MB-137-113-13-11, MB-137-144-5-19, MB-137-144-5-22, MB-137-144-5-39, MB-137-144-56-23, MB-
137-144-56-26 and MB-137-144-56-28, were analyzed for endosperm nutritional quality. While the recurrent parent (CM1137) recorded 11.93% endosperm protein, MB-137-144-5-39 revealed 10.53% endosperm protein, followed by MB-137-144-56-28 (10.37%). For percent tryptophan in the endosperm protein, MB-137-144-56-26 was found to be best (0.94%), followed by MB-137-144-5-22 (0.74%). The recurrent parent (CM1137) exhibited 0.39% tryptophan in the endosperm protein (Table 12).

**CM138-based BC2F3 progenies:** In this case, kernel samples from 17 individual ears belonging to a single BC2F3 family (MB-138-9-127) were analyzed for percent endosperm protein and percent tryptophan content. Among these, MB-138-9-127-6 was found to be the best for endosperm protein content (11.28%). Endosperm protein contents of other promising families were 11.24% (MB-138-9-127-26) and 10.42% (MB-138-9-127-91). For tryptophan content, MB-138-9-127-106 recorded the highest value (1.45%), followed by MB-138-9-127-25 (0.80%), MB-138-9-127-92 (0.76%), MB-138-9-127-43 (0.75%), and MB-138-9-127-51 (0.70%). The recurrent parent (CM138) revealed 10.1% and 0.42% endosperm protein and percent tryptophan, respectively (Table 12).

The biochemical analysis of the selected ears showed that tryptophan concentration in endosperm protein, which is the important indicator of protein quality in QPM genotypes, was enhanced almost two-fold in the selected MAS-derived lines of CM137 and CM138.

**Advancement of MAS-derived Inbred Lines to BC2F4**
The selected BC2F3 ears with high values of endosperm protein and percent tryptophan in protein content were considered for phenotypic selection based on kernel modification and conformity of ear phenotype with the respective recurrent parent. Based on these criteria, seven CM137-based BC2F3 ears, namely MB-137-113-13-11, MB-137-144-5-19, MB-137-144-5-22, MB-137-144-5-39, MB-137-144-56-23, MB-137-144-56-26 and MB-137-144-56-28
were selected for generation advancement. Similarly, 17 ears from CM138-based BC$_2$F$_3$ with average endosperm protein of 4.43% and average tryptophan percent in endosperm flour 0.090% were considered for generation advancement.

Each of the selected families was grown in three rows with 20 plants per row at the IARI Experimental Farm, New Delhi during Kharif-2008 (Fig. 18 and 19). Rigorous selection was undertaken on the basis of plant features, and the selected plants were control-pollinated (selfed) to generate BC$_2$F$_4$ lines. After harvest, ears from each of the families were tested once again for kernel modification (Fig. 17) and ears with a score of 1.00-1.50 (high kernel vitreousness comparable to the non-QPM maize) were selected for further advancement. Based on the flowering behavior, agronomic performance, vigour and ear characteristics coupled with kernel texture, three MAS-derived in CM137 genetic background, five MAS-derived lines in CM138 genetic background were selected for further utilization in QPM hybrid development.

II. Genetic analysis of Kernel Sugar Content and Yield Related Traits in Sweet Corn Inbred Lines

The present investigation was aimed to identify promising sweet corn inbred lines for utilization in sweet corn cultivar development in India. For this purpose, genetic analysis of a selected set of sweet corn inbred lines developed using Indian maize germplasm was undertaken for evaluation for their total kernel sugar content and yield performance.

Experimental crosses were generated using a Line x Tester (L x T) mating design. The lines included four sweet corn inbred lines developed at Directorate of Maize Research, New Delhi, and three sweet corn inbred lines isolated from a RIL (Recombinant Inbred Line) population developed earlier using CM139 and NAI116 as parental lines at Maize Genetics Unit, IARI, New Delhi. Three open-pollinated, popular sweet corn varieties in India were used as broad-based genetic testers.

The experimental crosses were generated at the IARI Experimental Farm during Kharif 2007 and the resulting crosses along with parental lines
were evaluated at two locations, (i) Maize Winter Nursery, Hyderabad during *Rabi* 2007-08 and (ii) IARI Experimental Farm, New Delhi during *Kharif* 2008. Eleven traits, namely plant height (cm), ear height (cm), days to 50% anthesis, days to 50% silking, total kernel sugar content, grain yield per plot (g), ear length (cm), ear diameter (cm), number of kernel rows per ear, number of kernels per ear row, and 100-kernel weight (g), were recorded and were further analyzed using WINDOSTAT 8.5 software package for various statistical parameters.

Variations due to lines were found to be significant for sugar concentration, yield and yield related traits at both the locations except for days to 50% anthesis at Delhi and days to 50% silking at Hyderabad (Table 13). Similarly, variance due to testers also showed significance for majority of the traits, except for grain yield, ear diameter and days to 50% silking at Hyderabad and ear diameter, plant height, ear height, days to 50% anthesis and silking at Delhi. Line x tester variance was observed to be significant for majority of the yield contributing traits, besides total sugar content (Table 13). The results also revealed that variation due to parents vs. hybrids was significant for all the traits except sugar content at Delhi. Significant variation among the hybrids was also noted for all the traits at both locations, except for days to 50% anthesis at Delhi and days to 50% silking at Hyderabad.

The variance due to lines was significant for grain yield and 100-kernel weight at Delhi, as well as 100-kernel weight and plant height at Hyderabad (Table 13). In contrast, variance due to testers was found to be significant for 100-kernel weight at Delhi and days to 50% silking and plant height at Hyderabad. Interestingly, line x tester effect was found to highly significant for total sugar content in kernels, grain yield and majority of the yield related traits at both the locations.

Pooled analysis revealed significant effect of environment on all the characters (Table 15). Variances due to lines, testers, and line x tester, were found to be significant for majority of the yield related traits, besides total sugar content. In case of line x tester effects, variance was found to be significant for
most of the traits, except days to 50% anthesis and silking. Environment x lines, environment x testers, environment x parents vs. hybrids, environment x hybrids interactions were also significant for majority of the yield related traits and total sugar content (Table 15). Although environment x lines effect and environment x testers effect were found to be important for some traits, environment x line x tester effects were significant for all the traits, except ear length and days to 50% silking (Table 15).

**Combining Ability of Sweet Corn Inbred Lines**

Combining ability analysis indicated significance in case of variance due to hybrids for all the traits (Table 14). Variance due to line and testers were found to be non-significant for majority of the traits under study. Interestingly, line x tester variance was found to be significant for all the traits, except ear height at Delhi, days to 50% anthesis at both the locations and days to 50% silking at Hyderabad. The results also revealed the predominance of dominance variance for all the characters, except plant height and days to 50% silking at Hyderabad which showed the predominance of additive gene action over non-additive type of gene action (Table 14).

Contribution of lines and line x testers to total variance was found to be of similar magnitude for kernel sugar content at both the locations. However, for majority of the yield related traits, contribution of line x tester variance was relatively higher than that of lines. Interestingly, the testers used in the present study contributed least to the total variance (Table 14).

Pooled analyses revealed the significant role of environment for ear length, ear diameter, 100-kernel weight, plant height, days to 50% anthesis and silking (Table 16). The analyses also showed the predominance of dominance variance for sugar concentration, yield related traits (except plant height, ear height and days to 50% silking). The contribution of lines was found to be higher than that of line x tester for sugar content, grain yield, 100-kernel weight, plant height and ear height. The reverse trend was observed in case of ear length, ear diameter, no. of kernel rows per ear, no. of kernels per
row and days to 50% anthesis (Table 16). Interestingly, in case of days to 50% silking, the lines, testers and line x tester contributed almost equally to the total variance. In general, except plant height, ear height and days to 50% silking, the contributions of testers were found to be minimal (Table 16).

**GCA and SCA Effects:** The estimates of General Combining Ability (GCA) and Specific Combining Ability (SCA) for sugar concentration and yield related traits based on Hyderabad and Delhi datasets, besides pooled dataset, are presented in Tables 17 and 18.

**Plant height:** The GCA effects were found to range from -34.55 to 11.62 at Hyderabad and from -6.35 to 6.23 at Delhi. Among the lines, L2 (11.62) was found to be the best general combiner at Hyderabad, followed by L3 (9.12), L4 (8.28), L1 (5.45) and L5 (4.62). While L3 (6.23) performed best at Delhi, L3 (7.67), L2 (4.44) and L4 (3.67) were observed to be the best general combiners in case of pooled analyses. Among the testers, T3 (8.97) was found to be best general combiner at Hyderabad, whereas pooled analysis revealed T3 (5.86) and T2 (1.85) as the best general combiners.

The SCA effects for hybrids ranged from -9.48 to 12.23 at Hyderabad and -13.14 to 7.92 at Delhi. Among these, L5 x T2 (12.23) recorded the highest SCA effect at Hyderabad, followed by L2 x T2 (8.74), L1 x T3 (7.19), L7 x T3 (4.69), L4 x T3 (3.86) and L3 x T1 (3.74). At Delhi, no hybrid combination was found to have desirable SCA effect for plant height. Pooled analysis identified L5 x T2 (9.79), L3 x T1 (5.16) and L7 x T3 (4.72) as the best specific combiner for this trait.

**Ear height:** The range of GCA effects for ear height was -15.93 to 10.40 at Hyderabad and -9.34 to 6.76 at Delhi, with L2 (10.40) being the best general combiner at Hyderabad. The other promising lines with high GCA effects included L5 (3.74), L7 (3.74) and L4 (2.08). In contrast, no line presented positive GCA effect for ear height at Delhi. Among the testers, T3 (7.62 at
Hyderabad, 5.89 at Delhi) performed as the best general combiner at both locations. Pooled analysis reported L2 (5.98) and L7 (5.25) among the lines and T3 (6.75) as the best general combiner.

The SCA effects for the trait ranged from -15.79 to 12.38 and -11.65 to 14.33 at Hyderabad and Delhi, respectively. Among the crosses, L2 x T3 (12.38) was found to be best specific combiner, followed by L6 x T1 (12.21), L1 x T3 (10.04), L5 x T2 (8.40), L3 x T1 (7.05), L7 x T3 (4.05), L6 x T2 (3.57) and L7 x T2 (3.40). On the contrary, at Delhi no hybrid combination was reported to have desirable SCA effect.

**Days to 50% anthesis:** GCA effects for days to 50% anthesis ranged from -2.33 and 1.33 at Hyderabad, while it was found to be -1.45 to 0.71 at Delhi. The lowest GCA effect, indicating early flowering behavior, was found in L3 (-2.33) at Hyderabad and in L5 (-1.45) at Delhi. No tester was found to be desirable for the early flowering trait. Pooled analysis revealed L2 (-0.94) as the best general combiner for earliness.

The SCA effects ranged from -2.67 and 4.19 at Hyderabad, while it was -1.29 to -1.31 at Delhi. At Delhi, L4 x T3 (-1.29) and L2 x T2 (-1.19) were reported to be as good specific combiners for earliness. However, no hybrid combination was found to be suitable for earliness at Hyderabad, while L6 x T1 (4.19) revealed late maturity. Pooled analysis also revealed no hybrid combination as a suitable specific combiner for earliness.

**Days to 50% female flowering:** For days to 50% silking, the range for GCA effects was found to be -1.93 to 1.79 and -1.12 to 0.71 at Hyderabad and Delhi, respectively. While no line at Hyderabad was found to have the desirable GCA, L5 (-1.12) was identified as the best general combiner for this trait at Delhi. Among the testers, T2 (-1.57) was found to be the best tester for early silking behavior at Hyderabad. Pooled analysis showed L3 (-0.86) and T2 (-0.76) as the best general combiner for earliness.
The SCA effect ranged from -3.28 to 3.21 at Hyderabad and from -1.21 to 1.14 at Delhi. Among the crosses, L2 x T2 (-1.21) was found to be best specific combiner at Delhi for earliness in female flowering, while no cross combination was found to be suitable for earliness at Hyderabad as well as in the pooled data.

**Kernel sugar content:** The GCA effects ranged from -2.85 to 3.43 at Hyderabad and from -4.02 to 5.06 at Delhi. Among the lines, L6 (3.43), L3 (0.59) and L7 (0.51) at Hyderabad, and L6 (5.06), L7 (1.64), L4 (1.05) and L5 (0.68) at Delhi showed significant positive GCA effects, and, in turn, were found to be good general combiner for sugar concentration. Importantly, L6 showed the highest and significant GCA value at both the locations. Pooled analysis also revealed the worth of L6 and L7 as promising general combiners. Among the testers, T3 was found to be the best general combiner for sugar concentration at both Hyderabad (0.29) and Delhi (1.96), besides pooled analyses (1.13).

Among the 21 crosses, eight experimental crosses were found to be showing positive and significant SCA effects at Hyderabad, while six cross combinations were identified as promising specific combiners for sugar concentration. Among the crosses at Hyderabad, L3 x T1 (3.95) was found to be best specific combiners, followed by L6 X T3 (3.94), L7 x T3 (2.08), L4 x T2 (1.89), L5 x T2 (1.56), L4 x T1 (1.18), L1 x T1 (1.16), and L2 x T2 (1.02). At Delhi location also, L6 x T3 (7.47) was identified as the best cross combination. Other promising cross combinations at Delhi included L3 x T2 (3.87), L5 X T1 (3.48), L7 x T3 (3.09), L5 x T2 (1.62) and L2 x T1 (1.14). Pooled analysis also revealed the potential of L6 x T3 (5.71) as a specific combiner, followed by L7 x T3 (2.59), L3 x T1 (1.79), L5 xT2 (1.59), L4 x T1 (0.94), L2 x T2 (0.90), L5 x T1 (0.88) L2 x T1 (0.84) and L4 x T2 (0.65).

**Grain yield:** GCA effects for grain yield per plot were found to vary between -0.30 and 0.28 at Hyderabad and -0.15 and 0.41 at Delhi. Among the lines, L4
(0.28) was found to be best general combiner at Hyderabad, followed by L1 (0.09), while at Delhi, L6 (0.41) was identified as the best general combiner followed by L4 (0.12). In case of testers, T2 (0.09) at Hyderabad and T1 (0.11) at Delhi were noted to be best general combiners for grain yield per plot. Analysis of pooled data set reveled the worth of L4 (0.20) and L6 (0.18) as the promising general combiners.

SCA effects for grain yield ranged from -0.40 to 0.39 and from -0.19 to 0.34 at Hyderabad and Delhi, respectively. Out of 21 cross combinations, seven showed positive SCA effects at Hyderabad, while one was found to be promising at Delhi. Among the crosses at Hyderabad, L7 x T2 (0.39) found to have the highest SCA, followed by L6 x T2 (0.22), L5 x T3 (0.22), L3 x T1 (0.21), L4 x T2 (0.18), L2 x T1 (0.16) and L5 x T1 (0.12). Among the crosses at Delhi, L3 x T2 (0.34) was found to be only promising specific combiner for grain yield. Pooled analysis did not identify any cross combination showing promising SCA effects at both the locations.

**Ear length:** Considering the data from Hyderabad, Delhi and pooled analyses, the overall range for GCA effect was found to vary from -0.16 to 1.01. L4 (0.63) showed maximum GCA effect at Hyderabad, followed by L3 (0.34). Among the testers, at Hyderabad, T2 (0.70) was identified as the promising testers for ear length. In case of Delhi, L3 (1.01) among the lines and T1 (0.51) among the testers were observed to be the promising good general combiners. Pooled analysis revealed the potential of L3 (0.67) and T2 (0.31) as the best general combiners for ear length.

Among the experimental crosses, L1 x T1 (2.18) was found to be best specific combiner for ear length at Hyderabad. Among other promising crosses, L3 x T3 (1.12), L6 x T3 (1.01), L5 x T2 (0.99), L4 x T1 (0.74), L6 x T2 (0.69), L7 x T2 (0.69), L2 x T1 (0.57) and L2 x T3 (0.32) showed significant positive SCA effects for ear length. In case of cross combinations evaluated at Delhi, L1 x T3 (1.85) and L5 x T2 (1.28) were identified as the best specific
combiners for ear length. Pooled analysis also demonstrated the potential of L5 x T2 (1.14) hybrid for ear length besides L6 x T3 (1.08) and L1 x T1 (0.99).

**Ear diameter:** The GCA effects ranged between -0.09 and 0.08 at Hyderabad and between -0.12 and 0.14 at Delhi. Among the lines, L6 (0.14) was found to be best general combiner for ear diameter at Delhi as well as in case of pooled analysis. Among the testers, T2 (0.07) was identified as the best general combiner at Hyderabad.

Among the crosses, L2 x T1 (0.24) at Hyderabad and L3 x T2 (0.19) and L6 x T2 (0.21) at Delhi were identified as the best specific combiners for ear diameter. Pooled data analysis revealed the importance of L2 x T1 (0.18) and L6 x T2 (0.18) as best specific combiners for ear diameter.

**Number of kernel rows per ear:** The overall range of GCA effects for this trait was -0.61 to 0.97 at Hyderabad and -0.59 to 0.41 at Delhi. At Hyderabad, L1 (0.97) among the lines and T2 (0.56) among the testers were identified as the best general combiners. Pooled data analysis also revealed the worth of L1 (0.61) and T2 (0.29) as best general combiners among lines and testers, respectively.

SCA effects varied between -0.09 and 1.75 and from -0.84 to 0.89 at Hyderabad and Delhi, respectively. Highest SCA effects were recorded for L6 x T2 (1.75) at Hyderabad, followed by L7 x T1 (1.33), while L1 x T2 (0.89) and L6 x T1 (0.83) at Delhi. Pooled analysis revealed L6 x T2 (0.88), L7 x T1 (0.54) and L1 x T2 (0.53) as the best specific combiners.

**Number of kernels per ear row:** The range of GCA effects was reported to be -3.19 to 1.80 at Hyderabad, and -2.85 to 3.09 at Delhi. Among the lines, L1 (1.80), L2 (1.70), L4 (1.56) and L3 (1.33) showed significant positive GCA effects at Hyderabad, whereas L1 (3.09) was found to be the only promising general combiner at Delhi. Pooled analysis revealed the importance of L1 (2.45) and L2 (1.27) as best the promising general combiners for the trait. In
case of testers, T2 (1.13) was found to be the best general combiner at Hyderabad.

SCA effects for the number of kernels per ear row ranged from -5.20 to 4.07 at Hyderabad, and from -3.99 to 6.16 at Delhi. Among the crosses, at Hyderabad, L1 x T1 (4.07) was identified as the best specific combiner, followed by L6 x T3 (3.69), L2 x T1 (3.27), L7 x T2 (2.06) and L4 x T2 (1.80). At Delhi, the promising combinations were L1 x T3 (6.16), L4 x T1 (2.78) and L5 x T2 (3.72). Pooled analysis also revealed the potential of L1 x T3 (2.55) as best specific combiner among all the hybrid combinations, besides L5 x T2 (2.63) and L6 x T3 (2.45).

**100-kernel weight:** The GCA effects for this trait ranged from -6.42 to 6.46 at Hyderabad, and from -4.33 to 7.07 at Delhi. L6 (6.46 at Hyderabad and 7.07 at Delhi) was found to be the best general combiner, followed by L4 (5.19 at Hyderabad and 4.13 at Delhi). Pooled analysis also revealed the worth of L6 (6.76), L4 (4.66) and T2 (0.54) as the best general combiners.

SCA effects at Hyderabad and Delhi were found to vary from -6.11 to 6.75 and from -5.95 to 7.27, respectively. At Hyderabad, L7 x T3 (6.75) was identified as the best specific combiner for this trait; other promising cross combinations include L4 x T2 (5.43), L5 x T1 (3.61), L3 x T3 (3.54), L2 x T1 (2.73), L6 x T2 (2.53), L1 x T3 (2.10) and L2 x T2 (1.48) recorded the high SCA effects. Among the crosses at Delhi, L6 x T2 (7.27) was found to be the best specific combiner, followed by L3 x T3 (3.70), L5 x T1 (3.36), L4 x T2 (2.18) and L7 x T3 (2.25). Pooled analysis also revealed L6 x T2 (4.90) as the best specific combiner, followed by L7 x T3 (4.50), L3 x T3 (3.62), L5 x T1 (3.48), L2 x T1 (2.23) and L4 x T2 (2.18).

**Heterosis for Total Sugar Content and Grain Yield**

Different heterosis parameters (better parent, mid parent and standard parent heterosis) were estimated for total sugar content and grain yield of the sweet corn cross combinations involved in the Line x Tester set evaluated at

For total sugar content in the kernels, the mid-parent heterosis ranged from -15.45 to 30.13% and from -26.30 to 79.16% at Hyderabad and Delhi, respectively. At Hyderabad, L3 x T1 showed highest mid-parent heterosis of 30.13%, whereas at Delhi, L6 x T3 was found to be the best combination with mid-parent heterosis value of 79.16%. Among other crosses, L6 x T1 (29.01%), L2 x T3 (24.77%), L4 x T3 (19.50%) at Hyderabad, and L7 x T3 (42.59%), L5 x T1 (24.87%) and L4 x T3 (19.92%) at Delhi, were identified to have high mid-parent heterosis values.

The best parent heterosis ranged from -45.70 to -28.78% at Hyderabad and from -27.04 to 54.00% at Delhi. L3 x T1 was found to be the best combination with 28.78% best parent heterosis, while at Delhi, L6 x T3 performed best with 54.00% heterosis, followed by L7 x T3 and L5 x T1 with 25.00% and 16.45% best parent heterosis, respectively.

Standard parent heterosis over ‘Golden Sweet Corn’ was found to vary from -9.80 and 56.16% at Hyderabad and from 0.99 to 136.80% at Delhi. The analysis revealed that L6 x T3 was the best hybrid combination at both the locations with highest heterosis value of 136.8% at Delhi and 56.16% at Hyderabad (Fig. 20). Among the other promising crosses, L3 x T1 (38.52%), L6 x T2 (33.19%) and L7 x T3 (29.41%) were found to be promising for this trait at Hyderabad. At Delhi, L7 x T3 (85.53%), L5 x T1 (58.24%), L4 x T3 (56.21%), L3 x T2 (51.40%), L5 x T2 (47.98%), L6 x T1 (45.42%), L4 x T1 (42.17%), L4 x T2 (35.34%), L6 x T2 (34.65%), L7 x T2 (31.75%) and L7 x T1 (31.58%) were identified as the best cross combinations.

Analysis of heterosis for sugar content over the popular sweet corn composites (Priya, WinOrange and Madhuri) revealed L6 x T3 (RIL62 x Madhuri) as the best cross combination among all the experimental hybrids with a heterosis value of 48.47%, 38.82% and 24.83% over Priya, WinOrange and Madhuri, respectively, at Hyderabad. At Delhi also, L6 xT3 (RIL62 x Madhuri) recorded the highest heterosis of 74.30%, 60.94% and 114.15% over
the three testers Priya, WinOrange and Madhuri, respectively. In case of heterosis over Priya, L3 x T1 (31.52%) and L6 x T2 (26.46%) were found to the promising cross combinations at Hyderabad, whereas at Delhi, L7 x T3 (35.06%) was found to be another promising combination. The results of heterosis over Winorange showed that L3 x T1 (23.13%), L6 x T2 (18.40%) and L7 x T3 (15.04%) at Hyderabad, and L2 x T3 (24.87%) and L7 x T3 (24.71%) were the best cross combinations. In case of heterosis over Madhuri, L7 x T3 (65.95%), L5 x T1 (43.08%), L4 x T3 (42.15%) and L3 x T2 (36.90%) were found to be the promising combinations at Delhi.

Grain yield heterosis for mid-parent ranged from -33.81 to 112.29% at Hyderabad and from -5.80 to 124.28% at Delhi. Among the experimental crosses, L4 x T2 (DMR-2322 x Winorange) found to be best cross with 112.29% heterosis at Hyderabad, followed by L7 x T2 (85.36%), L4 x T1 (49.25%) and L4 x T3 (47.92%) (Fig. 20). At Delhi, L6 x T1 emerged as the best cross combination as per mid-parent heterosis with a highest value of 124.28%; other promising crosses included L1 x T1 (68.74%), L7 x T1 (64.52%), L6 x T2 (59.87%), L3 x T2 (59.11%) and L6 x T3 (58.54%).

The result for best parent heterosis for grain yield demonstrated that the range varied from -38.88- 80.95% and -15.20-115.60% at Hyderabad and Delhi, respectively. L4 x T2 and L7 x T2 were found to be the best cross combinations at Hyderabad with highest heterosis of 80.95%, whereas at Delhi L6 x T1 (115.60%) was found to be best cross combination. Other promising cross combinations were L3 x T2 (75.54%), L1 x T1 (63.65%), L2 x T1 (46.22%), L6 x T2 (44.74%), and L6 x T3 (41.3%).

Standard parent heterosis (over Golden Sweet Corn) for grain yield was found to be in the range of -0.36-106.52% at Hyderabad and 3.72-106.38%. Analysis also revealed L4 x T2 and L7 x T2 as the best cross combinations for grain yield at Hyderabad with 106.52% grain yield heterosis, followed by L6 x T2 (74.81%), L5 x T1 (66.66%), L1 x T1 (64.13%), L5 x T3 (64.13%), L4 x T1 (63.04%), L1 x T2 (63.04%), L4 x T3 (61.59%), L2 x T1 (52.17%) and L1 x T3 (42.66%). On the other hand, L6 x T1 reported was identified as the best cross
combination for grain yield at Delhi with 106.38% heterosis followed by L6 x T3 (72.87%), L3 x T2 (71.80%), L4 x T1 (71.10%), L6 x T2 (70.92%), L4 x T3 (50.84%), L1 x T1 (44.50%) and L5 x T2 (43.62%).

Analysis of grain yield heterosis over three testers revealed that L4 x T2 (DMR-2322 x Winorange) and L7 x T2 (RIL91 x Winorange) were the best cross combinations over at Hyderabad with a percentage heterosis value of 50.79%, 80.95% and 50.79% over Priya, Win orange and Madhuri, respectively. At Delhi location, L6 x T1 reported to have percentage heterosis value of 133.73%, 74.77% and 68.40% over Priya, Win Orange and Madhuri, respectively, and was proved to be best cross combination for grain yield. The other promising crosses at Delhi depicting high heterosis value over Priya were L6 x T3 (95.78%), L3 x T2 (94.58%), L4 x T1 (93.78%), L6 x T2 (93.57%), L4 x T3 (70.83%), L1 x T1 (63.65%), L5 x T2 (62.65%), L1 x T3 (56.63%), L4 x T2 (55.62%), L5 x T1 (54.22%), L7 x T1 (53.61%) and L3 x T1 (50.60%). Heterosis over Win Orange revealed the superiority of L6 x T2 (53.17%), L5 x T1 (46.03%), L1 x T1 (43.81%), L5 x T3 (43.81%), L4 x T1 (42.86%), L1 x T2 (42.86%) and L4 x T3 (41.59%) at Hyderabad. Among the crosses at Delhi, the other promising crosses include L6 x T3 (46.40%), L3 x T2 (45.50%), L4 x T1 (44.89%) and L6 x T2 (44.74%). The result of grain yield heterosis revealed L6 x T3 (41.30%) and L3 x T2 (40.43%), L5 x T1 (39.86%) and L6 x T2 (39.71%) as other promising cross combination over Madhuri at Delhi.

**Correlations among Kernel Sugar Content and Grain Yield and Its Components**

The phenotypic and genotypic correlations among various traits under consideration, at both Hyderabad and Delhi locations, are presented in Tables 21 & 22.

*Phenotypic correlations*: Total sugar content in the kernels was found to have no significant correlation with grain yield or any of the yield related traits at Hyderabad. However, grain yield was found to be positively correlated with
number of kernel rows per ear (0.35) and number of kernels per ear row (0.47) at Hyderabad. In contrast, a negative correlation between grain yield and days to 50% anthesis (-0.36) and silking (-0.40) was recorded. Ear length at Hyderabad had positive correlation with ear diameter (0.46), number of kernel rows per ear (0.45) and number of kernels per row (0.70). Positive correlations of ear diameter with number of kernel rows per ear (0.41) and between number of kernel rows per ear and number of kernels per row (0.41) were also observed at Hyderabad.

Days to 50% anthesis and silking were found to be negatively correlated with some yield components such as number of kernel rows per ear, number of kernels per ear row and plant height. As expected, days to 50% anthesis and silking were observed to be positively correlated (0.87).

Correlations based on Delhi dataset also followed the similar trend as that found at Hyderabad. Total sugar content was found to be non-correlated with grain yield and other yield related traits. However, grain yield showed positive correlation with ear length (0.68), ear diameter (0.63), number of kernel rows per ear (0.40), number of kernels per ear row (0.43), 100-kernel weight (0.49) and plant height (0.50) at Delhi. Ear length was also found to be positively correlated with ear diameter (0.50), number of kernel rows per ear (0.53), number of kernels per ear row (0.65) and plant height (0.55). Ear diameter recorded positive correlations with number of kernel rows per ear (0.50), number of kernels per row (0.39), 100-kernel weight (0.50) and plant height (0.55). Plant height showed positive correlation with number of kernel rows per ear (0.50) and number of kernels per ear row (0.46). Interestingly, none of the grain yield and its component traits showed any association with flowering traits, although days to 50% anthesis had positive association with days to 50% silking.

**Genotypic correlations:** Akin to the phenotypic correlations, total sugar content showed no association with grain yield and any of the component traits at Hyderabad. Grain yield registered positive correlation with ear length
(0.50), ear diameter (0.43), number of kernel rows per ear (0.41), number of kernels per ear row (0.50) and 100-kernel weight (0.56). Ear length, on the other hand, was found to be positively correlated with ear diameter (0.58), number of kernel rows per ear (0.51) and number of kernels per ear row (0.73). While ear diameter was observed to be positively correlated with number of kernel rows per ear (0.44), number of kernels per ear row (0.38) and 100-kernel weight (0.37), ear height showed positive correlation with plant height (0.70). Interestingly, grain yield and many of the component traits were found to be negatively correlated with days to 50% anthesis and silking at Hyderabad, while the two flowering traits were found to be positive correlated.

Results based on Delhi location indicated positive correlations between grain yield and most of the component traits. Grain yield showed positive correlation with ear length (0.66), ear diameter (0.80), number of kernel rows per ear (0.45), number of kernels per ear row (0.38) and 100-kernel weight (0.59), plant height (0.59) and ear height (0.37). Similarly, both plant height and ear height were found to be positively correlated with grain yield, ear length, ear diameter, number of kernel rows per ear and number of kernels per ear row at Delhi. Ear length also registered positive correlation with ear diameter, number of kernel rows per ear and number of kernels per ear row. Similar trend was also observed in case of ear diameter. Plant height showed positive correlation with days to 50% anthesis (0.54) and days to 50% silking (0.37). The two flowering-related traits were found to be positively correlated at the Delhi location too, similar to Hyderabad.

In summary, considering both the phenotypic and genotypic correlations, total sugar content was not found to be significantly correlated to grain yield or any of the yield components traits at both Hyderabad and Delhi. However, grain yield, as expected, showed positive correlations with its many components traits, except with days to 50% anthesis and silking, which among them had strong positive correlation at both the locations.
Effect of Days after Pollination (DAP) on Total Kernel Sugar Content

Kernel sugar concentration in each of the genotypes was estimated at 20, 24 and 28 days after pollination (DAP) for understanding the effects, if any, on sugar concentration. In each of the genotypes, sugar concentration was measured from five random plants under both open- and controlled-pollination modes, and the same individual plants were tagged for estimation at different intervals. The results revealed the presence of significant variation for total sugar content among the genotypes (Table 23). Interestingly, the results also showed the significant effect of environment on the kernel sugar concentration (Table 24). Environment and genotype x environment interactions were found to be significant, indicating sensitivity of the trait to environmental fluctuations.

The mean data of sugar concentrations estimated at 20, 24 and 28 days after pollination (DAP) under both controlled- and open-pollination modes at both Hyderabad and Delhi are presented in Table 25. Among the lines evaluated at Hyderabad under controlled-pollination, L6 (RIL62) was found to be the best with 35.50% sugar at 20 DAP, followed by L1 (22.73%) and L5 (21.48%). At 24 DAP, L6 (RIL62) remained the best genotype with 35.53% sugar, followed by L4 (22.95%) and L7 (20.10%). At 28 DAP, L6 (RIL62) was once again identified as the best genotype with 34.15% sugar, followed by L7 (RIL91) (19.20%).

Among the lines evaluated at Delhi under controlled-pollination, L6 (RIL62) (22.50%) was judged as the best performing inbred lines at 20 DAP, closely followed by L4 and L2. However, at 24 DAP, L2 was identified as the best genotype with 27.08% sugar, with L1 (20.85%) being the second best genotype. Interestingly, L2 remained as best performing inbred line with 28.65% sugar at 28 DAP as well. Considering all the lines, L6 was found to be best inbred lines at Hyderabad with high and stable concentration of sugar, while at Delhi, L6 did not show stability in kernel sugar content at later stages of growth. At this location, L2 was identified as the best among the genotypes, with sugar content at its peak at 28 DAP.
Among the testers at Hyderabad, T3 (Madhuri) was identified as the best tester with 22.33% sugar at 20 DAP under controlled-pollination, while T2 (18.80%) and T3 (17.63%) were found to be the best testers at 24 and 28 DAP, respectively. At Delhi, T2 was found to the best genotype with 21.53% sugar, while T1 was the best genotype at both 24 and 28 DAP. Considering all the testers, T1 was found to be the most stable tester at Delhi, while no tester was found to be stable at Hyderabad.

Results on sugar concentration among the cross combinations at Hyderabad revealed L6 x T3 as the best genotype with 27.88% sugar at 20 DAP, followed by L3 x T1 (24.73%), L6 x T2 (23.78%), L7 x T3 (23.10%), L5 x T2 (21.38%), L4 x T2 (21.18%), L4 x T1 (20.63%), L1 x T1 (20.48%) and L3 x T3 (20.08%). At 24 DAP, L6 x T2 (32.68%) was found to be top ranking genotype, with L6 x T1 (27.98%), L1 x T2 (23.68%), L6 x T3 (22.60%) and L1 x T3 (20.78%). Results at 28 DAP revealed, L6 x T1 (21.48%) and L5 x T1 (20.18%) as the genotypes with over 20% sugar concentration. Among the crosses at Delhi, L6 x T3 remained to be the best genotype at 20 DAP with 34.65% sugar, followed by L7 x T3 (26.85%), L5 x T1 (23.15%), L4 x T3 (23.00%), L3 x T2 (22.15%), L5 x T2 (21.65%), L6 x T1 (21.18%) and L4 x T1 (20.80%). At 24 DAP, L6 x T3 (34.73%) was the best performing genotype with L6 x T1 (23.93%), followed by L7 x T2 (23.09%), L5 x T1 (22.03%), L3 x T1 (21.78%) and L3 x T2 (20.40%). Crosses that performed well at 28 DAP were L6 x T3 (35.50%), followed by L6 x T1 (26.78%), L5 x T1 (22.78%), L3 x T1 (22.78%) and L5 x T2 (21.54%). Considering the high and stable performance, L6 x T3 (RIL62 x Madhuri) was identified as the best cross combination for sugar concentration at Delhi, while at Hyderabad, no such promising cross combinations could be observed.

Considering the mean performance of all the genotypes (lines, testers, and crosses), the results revealed that, in general, the sugar concentration was its peak at 20 DAP, with gradual depletion at 24 and 28 DAP under controlled-pollination mode, The same trend was also observed under open-pollination mode, except that in case of testers sugar concentration started
depleting at 24 DAP as compared to 20 DAP; there was a little increase of sugar concentration at 28 DAP over 24 DAP at both Delhi and Hyderabad locations.

Paired t-test revealed significant differences in sugar concentrations between 20 and 24 DAP, 24 and 28 DAP, and 20 and 28 DAP under both the pollination modes at both locations (Hyderabad and Delhi). Interestingly, genotypes such as L6 x T2 had sugar concentration of 23.78%, while it rose up to 32.68% at 24 DAP and gradually depleted to 17.60% at 28 DAP under controlled-pollination at Hyderabad. Similar trend was also observed in case of L4, L7 (among inbred lines) and L6 x T1, L4 x T3, L4 x T1, L1 x T1, L1 x T2 at Hyderabad, and L7 x T2 at Delhi (among the crosses). Besides, genotypes such as L6 x T1 contained 21.28% and 23.93% sugar at 20 and 24 DAP, respectively, and finally reaching up to 26.78% at 28 DAP under controlled-pollination at Delhi. Other genotypes having highest percent sugar at 28 DAP included L1 and L2 under controlled-pollination at Delhi.

The rate of change of sugar concentration across genotypes was from -1.52 to 2.23% per day from 20 to 24 DAP under controlled-pollination at Hyderabad, while the same varied from -2.39 to 1.26% per day at Delhi. During the 24-28 DAP, the rate of change in sugar concentration among all the genotypes was between -3.77 to 0.06% per day and -1.08 to 0.71% per day at Hyderabad and Delhi, respectively, under controlled-pollination. Considering the 20-28 DAP period, the change in sugar concentration varied from 1.57 to 0.28% per day, while it was found to be -1.39 to 0.38% per day at Delhi.

During 20-24 DAP, L7 x T3 registered the highest depletion with 1.52% per day under controlled-pollination at Hyderabad (Table 26). Other genotypes such as L1 (-1.40% per day), L3 x T1 (-1.35% per day), L6 x T3 (-1.32 % per day), L3 (-1.26% per day), T3 (-1.14% per day) and L5 x T2 (-1.01% per day) also recorded depletion of more than 1% per day during 20-24 DAP. On the contrary, L6 x T2 had 2.23% increase of sugar concentration per day during 20-24 DAP, followed by L6 x T1 (2.18% per day) and L1 x T2 (1.29% per day) having gain of more than 1% per day.
During 24-28 DAP, L6 x T2 recorded a depletion of 3.77% sugar per day at Hyderabad under controlled-pollination, followed by L4 x T1 (-1.85% per day), L6 x T3 (-1.82% per day), L1 x T2 (-1.54% per day), L5 (-1.19% per day), L4 (-1.11% per day) and L1 x T3 (-1.03% per day).

Considering the 20-28 DAP period, L6 x T3 (-1.57% per day) showed the highest reduction of sugar concentration per day, followed by L7 x T3 (-1.12% per day), L1 (-1.17% per day), L5 (-1.03% per day) and L3 x T1 (-1.01% per day) having change of more than 1% per day. Similar trend was also observed in the Delhi dataset under controlled-pollination. L4 x T3 was found to have the highest depletion in sugar concentration with 2.39% per day during 20-24 DAP followed by L6 (-2.35% per day), L7 x T3 (-2.07% per day), T2 (-1.44% per day), L7 (-1.20% per day), L1 x T3 (-1.03% per day). Other genotypes such as L3 x T1 recorded a gain of 1.06% sugar per day, during 20-24 DAP. However, during 24-28 DAP period, the depletion per day was found to be much less as compared to the 20-24 DAP, with only L7 x T3 recording 1.08% depletion of sugar per day.

Considering 20-28 DAP period, L6 was found to have the highest depletion of sugar with 1.39% per day followed by L7 x T3 (-1.38% per day) and L4 x T3 (-1.35% per day). Similar trend was recorded in case of open-pollination mode too at both the locations.

Interestingly, L3 x T1 registered a depletion rate of 1.35% sugar per day at Hyderabad during 20-24 DAP under controlled-pollination, while the same cross combination showed a gain of 1.06% sugar per day at Delhi during the same period. In contrast, L1 x T2 had an increase of 1.29% sugar per day at Hyderabad during 20-24 DAP period (under controlled-pollination), while the same cross was found to have a depletion of 0.56% sugar per day at Delhi. Besides, in many cases, the direction of change of sugar concentration remained same at both Hyderabad and Delhi, however the extent of change varied. For example, in case of L5 x T2, the depletion of sugar per day was observed to be 1.01% per day at Hyderabad and it registered depletion rate of 0.34% sugar per day. In contrast, L7 x T3 had 1.52% depletion of sugar at
Hyderabad under controlled-pollination during 20-24 DAP, while it was -2.07% per day at Delhi during the same period. This complex nature of behavior of change in sugar concentration might be due to the difference in environments, genotypes and complex nature of genotype x environment interaction as already depicted in Table 24.

Despite the complexity described above, some genotypes such as L6 x T3 at Delhi and L6 at Hyderabad under controlled-pollination were identified to be the most promising and stable genotypes for kernel sugar concentration (Tables 25 & 26). Other genotypes, such as L1 at Delhi, L7 at Hyderabad, L3 x T2, and L5 x T2 at Delhi, were also found to be reasonably stable under controlled-pollination. In contrast, most of the genotypes were found to be unstable during the 20-28 DAP.

The present study also revealed that there is a general depletion of sugar concentration under open-pollination as compared to the controlled-pollination (Table 25). Genotypes such as L6 x T3 had 34.65% sugar under controlled-pollination at 20 DAP at Delhi, while the same genotype recorded 19.43% sugar under open-pollination. Similar trend of drastic change in sugar concentration under the two pollination modes was also observed in case of L7 x T3 (20 DAP, Delhi), L3 x T1 (20 DAP, Hyderabad), L6 x T3 (24 DAP, Delhi), L6 x T1 and L6 x T3 (28 DAP, Delhi), L6 (24 DAP, Hyderabad), L2 (24 DAP, Delhi), L1 and L2 (28 DAP, Delhi). However, reverse trend was also observed in case of few genotypes such as L2 x T3 (20 DAP, Hyderabad), L5 x T3 (28 DAP, Hyderabad) and L7 x T3 (24 DAP, Hyderabad), L3 and L4 (28 DAP, Hyderabad) and L4 and L5 (28 DAP, Delhi).

III. Analysis of Nucleotide Sequence Diversity in the sugary1 Alleles of Maize Genotypes and Relationship with Functional Diversity

The present investigation was aimed to identify promising sweet corn inbred lines with favourable alleles for high kernel sugar concentration and utilization of the same for sweet corn development in India. For this purpose, biochemical analysis of percentage sugar and starch content of a selected set
of sweet corn and normal maize genotypes developed in India was undertaken. Direct PCR sequencing methodology has been implemented to identify and analyze the nucleotide variation in the sugary1 (su1) alleles of selected genotypes. The results of various analyses are presented below.

**Phenotypic Analysis**

All the 14 genotypes were selected on the basis of their phenotypic contrast with respect to kernel appearance as well as pedigree-based diversity (Fig. 21). Analysis of total sugar and starch content in these lines indicated significant variation, with LSD values of 0.39 and 0.52 for sugar content and 1.63 and 2.21 for starch content at 0.05 and 0.01 levels of significance, respectively). ‘Madhuri’, a popular sweet corn cultivar, recorded the highest amount of total sugar content (12.72%), followed by DMR2319 (7.72%) (Table 29). Among the sweet corn inbreds developed at the Directorate of Maize Research (DMR), DMR2319 (7.72%) recorded the highest sugar content in mature kernels, followed by DMR2318 (7.72%) and DMR2323 (7.72%), while some genotypes like DMR2321, DMR2322 and DMR2324 were found to have relatively lower levels of total sugar content. The rest of the inbred lines showed varying levels of sugar content such as RIL10 (7.05), RIL62 (3.78) and RIL91 (5.06). As expected, the normal maize inbred ‘checks’, CM139 and CM140, recorded very low sugar content (3.38% and 3.91%, respectively).

Analysis of total starch content in the selected genotypes also showed significant variation. CM140 (70.16%) and CM139 (69.41%) revealed the highest starch content, as these are non-sugary lines. Among the DMR sugary inbred lines, DMR2324 (64.27%) and DMR2321 (63.81%) recorded the highest starch content. Among the sweet corn composites, ‘Madhuri’ revealed a high starch content (58.81%). Among the three sweet corn inbred lines identified at the Maize Genetics Unit, IARI, RIL62 showed the highest starch content (67.63%).
**Nucleotide Sequence Analysis**

Zea mays *su1* isoamylase sequence (Accession no. AF030882) was used to design the primer pairs for amplifying the 5' untranslated region (UTR) of the gene. Two primers pairs, namely *su1a* and *su1b*, covering together ~1150 bp region of 5'UTR of *su1* gene in two overlapping segments were designed in the study. The details about of primer pairs, nucleotide positions of amplicons, and amplicon sizes, are listed in Tables 27 & 28. After PCR amplification, the amplified products were purified and sequenced (in three replicates for each genotype) from both ends using forward and reverse primers. The nucleotide sequence variations uncovered in the targeted region, as revealed by analysis using CLUSTALW, are presented below.

**su1a primer pair:** This primer pair covered the first segment (762 bp) of the 5'UTR of *su1* gene (Fig. 22). Sequence analysis of this region in all the 14 genotypes revealed specific single nucleotide polymorphisms (SNPs), including eight transitions and one transversions; thus, a total of 11 SNPs are identified in this region (Fig. 23). The analysis also showed two insertion/deletions (InDels), all of which are single base type. The analysis using this primer pair revealed 0.014 percent sequence variation with a frequency of 1 variant per every 69 bp. Percent sequence variation was calculated by adding all the types of sequence variations (i.e. transition, transversions and InDels) and dividing the same by total number of bases in that region.

Among the eight transition events in the amplified region, the frequency of adenine (A) to guanine (G) transitions are more whereas there was only one guanine (G) to adenine (A), and one cytosine (C) to thymine (T) transition at this region in the selected genotypes. The A to G transition was reported at nucleotide positions 111, 115, 116, (Fig. 23) 190, 444 and 586. One cytosine to thymine (C-T) transition was reported at position 528 (Fig 23). At positions 111 and 116, A to G transition was exhibited by genotypes RIL10, RIL62 and RIL91, while at position 115, this transition was displayed only by RIL91.
Beside this, three A to G transitions are detected in CM140. Only one transversion event (T to A) was located in this region at position 480 in four genotypes, namely DMR2323, DMR2324, RIL91 and CM139.

The two InDels reported in this region were between nucleotide positions 728 and 730 (Fig. 23), and between 741 and 743. The first InDel was found in three genotypes, i.e., DMR2318, DMR2319 and DMR2320 where G was inserted between other nucleotides. The second InDel which was reported at position 742 was a single base (T) InDel in several genotypes (DMR2319, DMR2321, DMR2322, DMR2323, DMR2324, Madhuri, WinOrange, RIL60 and CM140).

**su1b primer pair:** The size of amplicon obtained using this primer pair was ~355 bp covering a region between nucleotides 1566 to 1921 of the 5’ UTR of *su1* gene. Sequence analysis revealed a total of 17 SNPs, including two transitions, seven transversions and eight InDels. The percent sequence variation in this region was 0.048, with a frequency of 1 variant per 21 bp. The extent of nucleotide variation shown by this primer pair was relatively higher than that of *su1a* primer pair.

The two transition events in this region were in genotype DMR2324 and RIL91 at nucleotide positions 937 and 963; in both cases, C was substituted by T. The seven transversion events were of C to G, G to C, and G to T type, at nucleotide positions 913, 923, 937, 981, 991, 995 and 1004. At position 913 and 923, C was replaced by G in RIL91, whereas a G to C event could be detected in DMR2324 at position 937. The four G to T transversion events were identified at positions 981, 991, 995 and 1004 in DMR2320, DMR2324 and RIL91.

The eight InDels reported in this region were a single base type, with occurrence at nucleotide positions 914, 938, 967, 980, 986, 990, 1006 and 1010. At position 914, T is present in CM140 whereas the rest of the genotypes displayed a single base deletion. Similarly, at nucleotide position 938, G is present in genotypes DMR2324 and RIL91, whereas the rest of the
genotypes showed a single base deletion. At positions 967 and 980, T is present in genotypes DMR2324 and RIL91 while the rest of the genotypes showed a deletion. At position 987, G is present in genotype CM140 whereas the rest of the genotypes exhibited a single base deletion at this position. At position 990, a single base InDel was detected in all the genotypes, except CM140. At position 1006, T insertion occurred in DMR2324, whereas at 1010, C insertion was found in DMR 2324 and A in RIL91.

A total of 28 sequence variations, including 10 transitions, 8 transversions and 10 InDels were identified in the 1100bp region of su1 in the selected genotypes, at a frequency of 1 nucleotide variation per 39bp (1 SNP per 40 bp) and 0.025 percent nucleotide variation. Out of 10 transition events 6 were A-G type, 3 were C-T type and 1 was G-T type. The frequency of purine to purine transition was found to be greater than pyrimidine to pyrimidine transition. Among the 8 transversion events, 4 were G-T type, 3 were G-C type and only 1 was T-A type; hence, six transversion events were purine to pyrimidine and two were pyrimidine to purine. There were a total of 11 single-base InDels identified in this study.

**Haplotype Analysis**

Sequence analysis of ~1.1kb region of 5’UTR of su1 gene revealed 11 putative haplotypes in the 14 genotypes (Table 30). Out of these haplotypes, three (Hap1, Hap2 and Hap3) were identified as ‘informative haplotypes’ with respect to the sugar content, while the rest did not show any specific relation with the trait of interest. These three haplotypes were reported in the genotypes DMR2318 (7.73%), DMR2319 (7.73%) and DMR2320 (7.14%). This preliminary observation needs further validation with a larger set of maize genotypes and statistical analysis.

Three haplotypes (Hap6, Hap10 and Hap11) showed a strong correlation with percentage starch content in the observed maize genotypes. Of these, Hap10 and Hap11 displayed a strong correlation with starch content in CM139 (70.16%) and CM140 (69.14%) which were used ‘checks’ (normal
non-sugary lines) in the present study. Hap6, which is present in DMR2324, showed a correlation with high starch content among the sweet corn inbred lines. This haplotype has 12 sequence variations including 5 deletions, 3 insertions, one transition and three transversions. However, two haplotypes (Hap5 and Hap 8) were not found to be non-informative as these were displayed in both high and low sugar lines.