Discussion

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

India is one of the first few countries to focus on opaque2 (o2) maize (Dhillon and Prasanna, 2001) and released three o2 composites (Shakti, Rattan and Protina) in 1970 for commercial cultivation. In 1997, one hard-endosperm o2 composite ‘Shakti 1’ was released in 1997. Later on, a series of eight QPM hybrids were developed and released, mainly using CIMMYT QPM lines (adapted to Indian conditions) as parental lines. Four of these QPM hybrids were developed at the Dholi centre (in Bihar), three from the Uchani centre (in Haryana) and one from the Almora centre (in Uttarakhand). Interestingly, all the seven hybrids developed at Dholi and Uchani Centre is of full season maturity, while the Almora hybrid is of early maturity. The present study pertains to conversion of parental lines of Pusa Early Hybrid Makka 2 (PEHM-2), developed at Division of Genetics, IARI, New Delhi, into QPM versions.

Development of a QPM genotype requires not only the desired opaque2 allele in homozygous condition, but also the endosperm modifiers (for kernel vitreousness) which are polygenic in nature. This makes conversion of a non-QPM line into a QPM version a complicated strategy, warranting progeny testing (through selfing) at each backcross generation to differentiate the heterozygotes from the dominant homozygotes, besides selection for endosperm modifiers and rigorous biochemical tests to ensure enhanced lysine and tryptophan levels in the selected materials in each breeding generation. Also, a minimum of 6-7 backcross generations are required to recover satisfactory levels of recurrent parent genome. All this would require enormous labor, time and land resources. However, recent advances in genome research and molecular technology led to identification of microsatellite/SSR markers within the opaque2 locus that can effectively differentiate O2O2, O2o2 and o2o2, and background selection for recurrent parent genome (Young and Tanksley 1989; Hospital et al., 1992; Visscher et
al., 1996; Frisch et al., 1999a, b), thereby enhancing selection efficiency and expediting the development of QPM cultivars (Dreher et al., 2003; Gupta et al., 2009).

Using o2-specific markers (*phi057, umc1066 and phi112*), the individual plants in any segregating population could be scored directly for the gene (at the vegetative stage itself). However, in the present study, among these three markers, *phi112* is genetically dominant and was, therefore, not considered for MAS. Among the two codominant SSR markers, *umc1066* was used for foreground selection, while 30 polymorphic SSR markers, evenly distributed in the genome, were used for background selection.

The success of a marker-based breeding system depends on three main factors: (i) a genetic map with an adequate number of uniformly-spaced polymorphic markers to accurately locate desired QTLs or major gene(s); (ii) close linkage between the major gene/QTL of interest and adjacent markers; (iii) adequate recombination between the markers and rest of the genome; and (iv) an ability to analyze a larger number of plants in a time- and cost- effective manner (Babu et al., 2004). A major advantage of MAS lies in the possibility of performing selection prior to flowering. In addition, the PCR-based co dominant markers, such as microsatellites/Simple Sequence Repeat (SSR) markers, aid in discriminating between the homozygotes and heterozygotes, which is a great advantage in marker-assisted backcrossing schemes, such as that implemented in the present study.

**Choice of Recurrent and Donor Parents**

PEHM-2 is an early maturing single cross hybrid (CM137 x CM138) developed at the Division of Genetics, IARI, New Delhi, in the year 2004. An orange flint grain type having yields of approximately 50 q/ha and has wider adaption in different states of India, besides showing resistance to some important diseases such as turcicum leaf blight (TLB), maydis leaf blight, and ear and stalk rot. However, the level of two essential amino acids (lysine ad tryptophan) in PEHM-2 were low as was normally observed in the normal
(non-QPM) maize genotypes, thereby making it a nutritionally poor hybrid although it has an excellent agronomic performance. Therefore, to ameliorate this deficiency, the parental lines of PEHM-2 (CM137 and CM138) were selected for the QPM conversion programme. Since the desired recessive opaque2 (o2) allele that confers nutritional advantage in QPM has to be in the homozygous state, both parental lines of the single-cross hybrid need to be converted to QPM versions.

In the present study, the QPM donors chosen were DMRQPM-03-124 and CML161, for conversion of CM137 and CM138, respectively (Fig. 1). CML161 is a yellow kernel type, medium-maturity QPM inbred line developed at CIMMYT, Mexico, while DMRQPM03-24, an early-maturing, yellow kernel type QPM inbred line was developed at Directorate of Maize Research (DMR), New Delhi. CML161 has been widely used as one of the parental lines in QPM hybrid development in India as well as many Asian and Latin American countries (Prasanna et al., 2001). For instance, CML161 is the female parent of a hybrid (CML161 x CML165) named ‘INIA’ in Peru and ‘HQ2000’ in Vietnam. In India, the QPM hybrid ‘Shaktiman-3’ (CML161 x CML163) and Shaktiman-4 (CML161 x CML169) were released in 2006. Also, HKI-161, one of the parental lines of the hybrid ‘HQPM-5’ (HKI-163 x HKI-161) and ‘HQPM-7’ (HKI-193-1 x HKI-161) was basically derived through selection from CML161 at the Uchani Centre in India. These two hybrids were released for commercial cultivation in India in the year 2007 and 2008, respectively. Thus, CML161 has been nationally and internationally recognized as a good combiner in the QPM hybrid breeding programmes, besides its nutritional superiority and kernel vitreousness.

DMRQPM-03-124, another QPM donor used in the present study, was developed by Directorate of Maize Research (DMR) under the All-India Coordinated Maize Improvement Project. This yellow kernel colored inbred line is another excellent QPM genotype with high per cent protein in the endosperm (11.0) and percent tryptophan in endosperm protein (0.85). In addition, use of one donor parent from India (DMRQPM-03-124) and another
from CIMMYT (CML161) was expected to aid in exploitation of heterosis in terms of grain yield and agronomic performance in the F₁ hybrid, besides better complementation among the genes responsible for kernel modification.

In a similar effort on conversion of non-QPM lines developed at VPKAS, Almora, Babu et al. (2005) used CML176, a white kernel type inbred of CIMMYT origin, as the donor parent for the conversion of V25, a non-QPM orange kernel type inbred. However, the use of a white kernel genotype as a QPM donor for the conversion of an orange kernel genotype complicates the QPM conversion programme as the selection for opaque2 locus had to be undertaken simultaneously with phenotypic selection for the yellow kernel phenotype conferred by Y₁Y₁Y₁ endosperm genotype. The genotype having opaque2 allele in the Y₁Y₁y₁ background in case of BC₁F₁ generation had to be rejected. This factor increases the population size during the conversion programme. However, in the present study, since the recurrent and donor parents were of orange/yellow kernel colour, it facilitated relatively less cumbersome conversion of non-QPM inbred lines.

**Marker-assisted Foreground and Background Selections**

Marker-assisted foreground selection helps in identifying the gene of interest without extensive phenotypic assays ( Tanksley, 1983; Melchinger, 1990); marker-assisted background selection expedites significantly the rate of genetic gain/recovery of recurrent parent genome in a backcross breeding program (Young and Tanksley, 1989; Hospital et al., 1992; Visscher et al., 1996; Frisch et al., 1999a,b). With respect to the gene-specific markers, such as phi057, umc1066 and phi112, which are located within the opaque2 gene itself, the individual plants in any segregating population could be scored directly for the gene, eliminating the probability of occurrence of false positives and false negatives. In the present study, phi112 was not considered as it behaves in a dominant manner and would not facilitate to identify the heterozygote. The codominant nature of the polymorphism exhibited by phi057
and umc1066 enables their potential utility in MAS programs to successfully discriminate between homozygotes and heterozygotes.

Identification of heterozygotes in the seedling stage prior to pollination aided in the rejection of non-target BC progenies resulting in substantial saving of labor and material resources. Among the two codominant SSR marker, umc1066 was used for foreground selection, while 32 polymorphic SSR markers (available in the public domain: www.maizegdb.org) evenly distributed and covering whole genome were used for background selection.

The objective of the background selection is to recover rapidly maximum proportion of recurrent parent genome at non-target loci through markers that are distributed evenly throughout the genome (Young and Tanksley, 1989; Hospital et al., 1992; Visscher et al., 1996; Frisch et al., 1999a,b). Availability of robust anchored marker maps in maize renders application of marker-aided background selection an easy and attractive proposition. The earlier simulation studies of Frisch et al. (1999a, b) and Ribaut et al. (2002) have indicated that application of background selection in one later generation along with foreground selection in each BC generations could be efficient and cost-effective. In the present investigation, we followed a two-generation marker-based breeding program.

In the present study, the marker-assisted backcrossing scheme was carried up to two backcross generations. Very stringent selection for recurrent parent genetic background was not performed deliberately as the population size was limited in each generation (since the target was to improve nutritional quality in as many as two different recipient-donor combinations) and also to avoid losing the endosperm modifiers conferring hard vitreous kernel. Thus, in BC$_2$ generation, theoretically 87.5% of the whole genome recovered was the recipient allele type. However, through background selection, the recurrent parent genome was recovered up to an extent of >90% in different lines.

Based on the marker-aided background selection, at least one BC$_2$ individual each with highest proportion of recurrent parent genome needs to be chosen in CM137- and CM138-based populations for generation
advancement. In the present study, we opted for slightly higher number of individuals in each of the populations (six in CM137-based BC₂ generation and two in CM138-based BC₂ generation) However, it must be noted here that high recovery of recurrent parent genome (>95%) could be obtained in only one each in these selected families, while the rest showed 90-95% recovery. In a similar study, Babu et al. (2005) selected three MAS-derived BC₂F₁ individuals in QPM conversion programme showing a range of 92.75–95.75% recovery of the recurrent parent (V25) genome. Thus, the background selection in the present study was as effective as that reported by Babu et al. (2005).

Although some portion (approx. 8% to 10%) of the selected BC₂F₃ lines could still have the donor parent genome, by applying background selection, it is still possible to further recover BC₂F₄ lines with not more than 5% donor parent genome, as has been demonstrated in a study on MAS in maize (Bouchez et al., 2002). Also, it must be noted that the donor parents used in this study are also agronomically elite, and are not unadapted germplasm or landraces or wild relatives. Therefore, retention of some donor segments could also provide favourable effects on agronomic performance of the MAS-derived lines. Whether the residual, “linkage drag” particularly on the carrier chromosomes has specific effect on the agronomic performance of the selected BC₂F₄ genotypes need to be ascertained through further analysis.

By retaining considerable proportion (>5%) of the donor parent genome in some of the BC₂F₄ lines, it is also possible to obviate the possibility that the MAS-derived lines fall under the category of “Essentially Derived Varieties” (Heckenberger et al., 2005). Thus, some of the MAS-derived lines obtained in the present study could be considered as newly derived inbred lines, rather than being the EDVs of the existing inbred lines.

**Importance of Phenotypic Selection in QPM Conversion Programme**

Molecular markers are often assumed to have 100% heritability. However, even with markers, mistakes while scoring the marker data or incorrect marker
data sometimes could make them less than perfect. Thus, selection based
only on markers occasionally will result in selecting the wrong plant, though
this frequency could be very low (Hospital et al., 1997).

The present study to convert non-QPM elite maize inbred lines into
QPM version was based on integrated strategy of marker assisted genotypic
selection coupled with the phenotypic selection and biochemical analyses.
While, marker data were used for precise estimation of foreground and
background selection, phenotypic selection was carried out for the estimation
of endosperm modification, plant and ear phenotype. On the other hand,
biochemical analyses helped in selecting the best family having superior
nutritional quality.

The biochemical analysis of the selected ears showed that tryptophan
concentration in protein, the chief indicator of protein quality was enhanced
more than twice in all the selected ears of CM137 and CM138-based
backcross progenies. Babu et al. (2005) converted some early maturing
inbreds (e.g., V25, CM145, CM212) developed at VPKAS (Almora) into QPM
versions with higher percent tryptophan in the endosperm protein as
compared with the respective recurrent parents, while the protein content was
found to comparable.

Lysine proportion in endosperm protein was not determined since a
highly significant and positive correlation was recorded between lysine and
tryptophan contents in the endosperm protein (Pixley and Bjarnason, 1993)
indicating that kernels having higher tryptophan would invariably have higher
amounts of lysine. Also, lysine estimation is procedurally more demanding and
more cost-intensive as compared to colorimetric estimation of tryptophan
content.

Babu et al. (2005) found that the QPM version of V25 had
approximately two- fold increase in percent tryptophan in the endosperm
protein as compared to the recurrent parent, but the endosperm protein
content was comparable to the recurrent parent. Recurrent parent V25
showed 9.6% protein and 0.41% tryptophan in protein while three MAS-
derived families showed a range of 8.3-9.1% mean total protein content and a range of 0.78-0.94% mean tryptophan in protein.

In the present study, the significant range of tryptophan content in the endosperm protein (0.43-1.45%) of the MAS-derived lines indicated the nutritional worth of the materials selected in this backcross scheme. Also, the observed variation in the tryptophan content among the individual families indicated the segregation of other regulatory genes or modifiers influencing the levels of the limiting amino acids in the maize endosperm. Earlier it was believed that only opaque2 locus and endosperm modifier genes are completely responsible for amino acid balance in maize kernel. Recently multiple genes have been identified in controlling amino acid content in maize kernel. At least three gene loci have been implicated in controlling the levels of a protein synthesis factor correlated with lysine levels and these have been mapped to locations on chromosomes 2,4 and 7 (Wang et al., 2001; Wu et al., 2002). This distinct set of genes is now referred to as “amino acid modifiers” (Krivanek et al., 2007).

Endosperm modification is complex polygenic trait governed by a set of modifier genes for which no reliable molecular markers have been identified so far. Phenotypic screening of the individual kernels under transmitted light and selection of kernels that have less than 25% opaqueness is by far the most convenient and efficient strategy employed in all the QPM breeding programs (Vasal et al., 1993a,b). We preferred kernels with less than 25% opaqueness over 25–50% and more than 50% opaqueness due to the semi-soft/soft nature of the endosperm and susceptibility to ear rot of latter categories (Vasal et al. 1993a, b).

Besides the endosperm modification, phenotypic selection based on the recurrent plant features, ear phenotypes, grain colour were also employed for selection of individual plants for further advancement. Thus phenotypic selection employed in the present study not only helped to recover recurrent parent genome indirectly, but were also helpful to keep the key phenotypic features of the original parent intact. On the other hand, selection of families
based on the percent protein and tryptophan content ensuring the indirect selection for regulatory genes responsible for endosperm protein and tryptophan content.

Thus, rapid line conversion strategy outlined in this investigation brings together the salient features of both marker aided and phenotypic selection approaches such as fixing the large segregating generation for the target locus, recovery of maximum amount of recurrent parent genome within two BC generations and provides ample scope for exercising phenotypic selection for as many desirable agronomical and biochemical traits as possible.

**Future Possibilities**

The strategy adopted in the present study is less time-consuming and less expensive, although the background selection strategy giving priority to carrier chromosomes was recommended when at least 3-4 backcross generations are performed (Hospital and Charcosset, 1997). Similar approach was followed by Bouchez et al. (2002) for selection of three QTL involved in maize flowering time and productivity. Thus, MAS based on SSR markers for conversion of normal maize lines into QPM is simple, rapid, accurate, efficient, cost-effective and complementary to existing breeding protocols.

The MAS-derived inbred lines would be further crossed between CM137- and CM138-based families in all possible combinations. Each of the experimental hybrid combinations would be analyzed at multi locations and their performance in terms of yield, kernel modification, protein and tryptophan content over PEHM-2 and check varieties. Besides, responses the MAS experimental hybrid combinations to the key biotic and abiotic stresses would also be tested over the locations. For the registration of varieties under the PPV&FR Act, 2001, DUS testing is one of the key components for testing of distinctiveness, uniformity and stability. All the phenotypic features as per the maize guidelines would be tested vis-à-vis PEHM-2. Test of EDV status is also an important aspect for registration either as independent variety (IV) or EDV. For all the MAS derived putative QPM version of PEHM-2 hybrids, EDV status
would be tested based on the protocol standardized at the Maize Genetics Unit, IARI.

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

Significant increase in production and productivity of maize in both the developed and developing countries, including India, could be attributed mainly to the success of hybrid technology. Single-cross hybrids have particularly revolutionized maize cultivation in the developed countries, and in India, this technology is gaining momentum day by day. One of the key factors responsible for this technology is the development of an array of inbred lines with excellent *per se* performance. However, the hybrid technology has not been popularized in case of sweet corn in India due to relatively low level of adoption of sweet corn. Composites like Madhuri, Priya and WinOrange still have dominated the sweet corn market.

Considering the constantly increasing demand of sweet corn in the Indian as well as in international market, hybrid technology would be the key to the success of sweet corn cultivars in India due to its increased grain yield potential over composites. One of the major aims of sweet corn breeding programmes worldwide, including India, has been the development of new generation sweet corn inbred lines that show not only superior productivity but also improved sugar concentration and other kernel attributes. The present investigation was particularly aimed at identifying promising sweet corn inbred lines with desirable agronomic performance, combining ability and sugar content for their potential utilization in sweet corn cultivar development in India.

‘Combining ability’ is defined as the measure of gene action, which refers to the capacity or ability of a genotype to transmit superior performance to its crosses. The value of an inbred line depends on its ability to produce superior hybrids in combination with the other inbreds (Sprague and Tatum, 1942). Combining ability is one of the most important areas hybrid research
and it has a significant impact on inbred line evaluation and population improvement in maize breeding (Hallauer and Miranda, 1988; Crossa et al., 1990). The estimate of combining ability not only provides information about the component of genetic variance involved in the expression of various polygenic characters and but also aid in selection of desirable parents for utilization in the hybrid breeding programme. In the present study, a line x tester set, along with their parental lines/populations, have been evaluated for kernel sugar concentration, various yield and kernel related traits for identification of promising genotypes (parental lines and crosses) for sweet corn breeding. A set of 21 experimental crosses generated using 7 line x 3 tester mating design were evaluated at two locations [Hyderabad (Rabi, 2007-08) and Delhi (Kharif, 2009)].

Genetic analyses revealed that both additive and non-additive gene action are important, which was in agreement with another recent study on sweet corn lines in India (Kumari et al., 2008). When proportion of dominance variance and additive variances were compared, the dominance variance were found to be relatively higher for most of the characters, signifying the utility of heterosis breeding in sweet corn genotypes. Fabricio et al. (2009) and Kumari et al. (2008) in their experiments on combining ability of sweet corn inbred lines also indicated the predominance of non-additive gene action with respect to total sugar, reducing sugar, non-reducing sugar, grain yield per plant and other agronomic traits. In contrast, Rosenbrook and Andrew (1971) reported highly additive type of gene action for carbohydrate fractions in the US sweet corn genotypes.

ANOVA for combining ability in the line x tester set revealed that both the line and tester variances were significant for kernel sugar concentration and most of the yield contributing traits analyzed in the study. This indicates that the 7 sweet corn lines (inbred lines) as well as the 3 testers (sweet corn composites) used in this analysis were significantly different in terms of sugar concentration and all other relevant yield components. Variances for hybrids in case of most of the characters were observed to be significant, suggesting the
presence of wide array of variation of different traits among the cross combinations. The analysis also indicated prominence of contribution of line x tester variance to the overall variance of the hybrids over lines and tester variances. The significant contribution by the line x tester variance is also in congruence with the results of Kumari et al. (2008).

Pooled analysis revealed significant effects of the environment on sugar concentration and almost all the yield related traits indicating the prominent role of environment in determining the extent of expression of these traits. Besides this environment x lines, environment x tester and environment x crosses were also found to be significant, suggesting the presence of genotypes x environment interactions. Non-additive gene action was also observed to be playing a major role in the pooled data set, once again indicating the possibility of success of heterosis breeding in sweet corn cultivars.

In the present study, three lines (L6, L3 & L7) were found to be the promising general combiners for sugar concentration at Hyderabad, while at Delhi, L4, L5, L6 and L7 among the lines were identified as the suitable genotypes for sweet corn trait. Considering all the traits, L6 (RIL62) was found to be the best general combiner at Delhi for total sugar content, besides having significant positive GCA effects for grain yield, ear diameter and 100-kernel weight. At Hyderabad, the same line showed 34.93% sugar concentration and was also identified as the best general combiner for sugar concentration and 100-kernel weight. Pooled analysis also proved L6 (RIL62) as the best general combiner for sugar concentration, grain yield and related traits, such 100-kernel weight, days to 50% anthesis and silking. Among the testers, T3 (Madhuri) was observed to be the best genotype for sweet corn and other agronomic traits. However, L1 (DMR-2317), L2 (DMR-2318), L4 (DMR-2320) and T2 (Winorange) showed promise as good general combiners for grain yield and majority of component traits, but were identified as poor combiners for sweet corn traits. Therefore, there is a need for further breeding efforts in combining the sweet corn trait with the yield component traits in
otherwise agronomically superior genetic backgrounds. Such efforts are presently being undertaken at the Maize Genetics Unit, IARI. Kumari et al. (2008), Revilla et al. (2000), Malvar et al. (1997) and Cartea et al. (1996) analyzed field corn x sweet corn cross combination and identified best field corn donors for favorable alleles for grain yield and yield contributing traits.

The present study also led to the identification of L6 x T3 (RIL62 x Madhuri) as the best specific combiner for sweet corn trait with total kernel sugar content of 27.50% and 34.65% at Hyderabad and Delhi, respectively. Among other crosses, L7 x T3 (RIL91 x Madhuri) and L5 x T2 (RIL10 x Winorange) were also found to be the promising cross combinations for the same trait at both the locations. Considering the mean performance and combining ability for sugar content, grain yield and its component traits, L6 x T3 (RIL62 x Madhuri) and L7 x T3 (RIL91 x Madhuri) were identified as the best genotypes. The analysis of SCA effect revealed that in most of the cases the parents involved in experimental crosses having higher SCA possess higher GCA effect as was also reported by Betran et al. (2003). In the present study, both L6 and T3 were found to be the best general combiners for sugar concentration and L6 x T3 also showed high SCA effects, suggesting that having one or both parents with high GCA effects would lead to desirable SCA effects in the cross combinations.

Several studies (e.g., Fabricio et al., 2009; Kumari et al., 2008; Rivella et al., 2003; Malver et al., 1997; Michaels and Andrew, 1986) analyzed the combining ability of sweet corn x sweet corn or field corn x sweet corn crosses for diverse traits such as sugar content, grain yield and its components, prolificacy, seed processing potential and resistance to biotic and abiotic stresses. These studies led to the identification of promising sweet corn inbred lines and cross combinations for various target traits based on the GCA and SCA estimates.

The sweet corn experimental crosses in the present study showed different levels of heterosis over the best check (Golden Sweet Corn) at both Hyderabad and Delhi, confirming that the sweet corn hybrids not only have
desirable sweetness trait, but also possess high yielding potential. In the present study, L6 x T3 (RIL62 x Madhuri) has revealed highest standard heterosis for kernel sugar concentration at both the locations signifying its utility across the environments. Interestingly, this particular cross combination was also found to be the best combination when compared against Priya, WinOrange and Madhuri. Among the other promising crosses, L3 x T1 and L6 x T2 at Hyderabad and L7 x T3, L5 x T1, L4 x T3, L3 x T2, L5 x T2, L6 x T1, and L4 x T1 at Delhi performed well over the standard check. Interestingly, most of the cross combinations exhibited negative, better-parent heterosis for sugar concentration indicating that although non-additive gene action is playing an important role (as evident from the proportion of additive and dominance variance), there is a need to accumulate favorable alleles in the inbred lines so that the heterosis for sugar concentration can be achieved in the positive direction. For examples, L3 x T1, L6 x T3, L7x T3 and L5 x T1 at Hyderabad exhibited positive better parent heterosis indicating the positive complementation of alleles responsible for sweet corn trait. Results on best parent heterosis for grain yield also showed that many of the cross combinations exhibited positive heterobeltiosis suggesting the presence of complementing favourable alleles among the parents.

Considering both kernel sugar concentration and grain yield, L6 x T3 (RIL62 x Madhuri) was identified as the best cross combinations at both the locations followed by L3 x T1 (DMR-2320 x Priya) and L6 x T2 (RIL62 x Winorange) at Hyderabad and L5 x T1 (RIL10 x Priya), L4 x T3 (DMR-2322 x Madhuri), L3 x T2 (DMR-2320 x Winorange), L5 x T2 (RIL10 x Winorange), L6 x T1 (RIL62 x Priya), and L4 x T1 (DMR-2322 x Priya) at Delhi. However the potential of these promising cross combinations needs to be further validated by evaluating them at multiple locations/seasons. Although, the cross combinations refers to the top cross hybrids (since the female parent being a inbred line and male parent being a composite), the promising combinations can be considered for release as they are better than the existing composites
(Priya, WinOrange and Madhuri) in terms of both sweet corn trait as well as for yield performance.

Phenotypic and genotypic correlation analyses revealed that kernel sugar concentration was not correlated with any of the grain yield and its component traits. This suggests that improvement of kernel sugar concentration in sweet corn genotypes can be undertaken independently of that of grain yield and its component traits. Saleh et al. (2002) also reported non-association between ear yield and brix value while experimenting with nine advanced sweet corn population at the University Putra Malaysia during 2000 and 2001. The study also showed that grain yield was positively correlated with most the yield component traits (Oktem et al., 2008; Saleh et al., 2002), indicating that selection for yield component traits could help in improving grain yield. Interestingly, flowering traits (days to 50% anthesis and silking) was found to be negatively correlated with grain yield at Hyderabad during Rabi 2007-08, while the same did not show any association at Delhi during Kharif 2009.

The analyses for kernel sugar concentration at 20, 24 and 28 days after pollination revealed significant variation at different time intervals suggesting the presence of wide variation amenable for genetic improvement. The study also indicated the significant effect of environment as well as genotype x environment interactions in this regard. This indicates that kernel sugar concentration is highly influenced by specific environment; however selection of promising and stable genotypes in different environments would help in release of sweet corn cultivars across locations.

The results also revealed that, in general, the sugar concentration attains its peak at 20 DAP and gradually depletes at 24 and 28 DAP under controlled-pollination mode. Creech et al. (1968) also demonstrated the attaining of highest sugar concentration at 20 DAP while experimenting with su1/su1, sh2/sh2 and su1su1/sh2sh2 genotypes. The significant differences in sugar concentrations between 20 and 24 DAP, 24 and 28 DAP, and 20 and 28 DAP at both locations (Hyderabad and Delhi) further establishes the significant
decrease in sugar concentration over days. However, some cross combinations such as L6 x T2 (RIL62 x Winorange) had sugar concentration of 23.78% at 20 DAP, while it rose up to 32.68% at 24 DAP and gradually depleted to 17.60% at 28 DAP under controlled-pollination at Hyderabad. On the other hand, genotypes such as L6 x T1 (RIL62 x Priya) revealed 21.28% and 23.93% kernel sugar content at 20 and 24 DAP, respectively, and finally reached up to 26.78% at 28 DAP under controlled-pollination at Delhi. This suggests that although kernel sugar concentration reaches its peak at 20 DAP, there could be specific genotypes which attain the peak at later stages, indicating that not necessarily all the sweet corn hybrids are amenable for harvesting at 20-22 DAP (used as a standard practice), but this should be empirically analyzed and suitable practice should be followed.

One of the characteristics of a successful sweet corn variety is the stability of high kernel sugar concentration over days. In this regard, the rate of change of kernel sugar concentration over days is an important aspect. The present study indicated the decrease of sugar concentration could be as high as 3.77% per day in case of some genotypes like L6 x T2 (RIL62 x Winorange) at Hyderabad (24-28 DAP). On the contrary, the same genotype showed 2.23% increase of sugar concentration per day during 20-24 DAPS at Hyderabad. Therefore, selection of promising genotypes with negligible or little decrease in kernel sugar concentration would aid in successful sweet corn breeding programme. The stable genotypes would provide farmers a period of 7-8 days within which harvesting can be undertaken at any time with no depletion of sugar level.

The study also showed that the rate of change of kernel sugar concentration over days could be influenced by the environment, as observed in case of L3 x T1 (DMR-2320 x Priya) which showed a gain of 1.06% sugar per day at Delhi during the 20-24 DAP under controlled pollination, while it registered a depletion rate of 1.35% sugar per day at Hyderabad. In contrast, L1 x T2 (DMR2317 x Winorange) was found to have a depletion of 0.56% sugar per day at Delhi during 20-24 DAP period (under controlled-pollination),
while it had an increase of 1.29% sugar per day at Hyderabad. Besides, in many cases, the direction of change of sugar concentration remained same at both Hyderabad and Delhi, but the extent of change varied. This trend further establishes the presence of strong genotype x environment interaction for the sweet corn trait. Michaels and Andrew (1986), George et al. (2003), Gupta et al. (2005), William (2008), Garcia et al. (2009) also found significant influence of planting and harvesting dates on sweet corn traits, establishing strong G x E effects.

Despite the complexity described above, the study led to the identification of some promising genotypes. Among the experimental hybrids, L6 x T3 (RIL62 x Madhuri) is the best cross combination for high and stable sugar concentration (34.65% at 20 DAP, 34.73% at 24 DAP and 35.60% at 28 DAP) at Delhi, while at Hyderabad, no such promising and stable cross combinations could be observed. Among the lines evaluated at Hyderabad under controlled-pollination, L6 (RIL62) was found to be best inbred line with high and stable concentration of sugar (35.50% at 20 DAP, 35.53% at 24 DAP and 34.15% at 28 DAP), while it showed instability for kernel sugar concentration at Delhi. Other genotypes, such as L1 (DMR-2317), T1 (Priya), L3 x T2 (DMR-2320 x Winorange), and L5 x T2 (RIL10 x Winorange) at Delhi, L7 (RIL91) at Hyderabad, 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. Michaels and Andrew (1986) while experimenting with shrunken-2 sweet corn genotypes, reported that warmer season resulted in lower reducing sugar and higher sucrose, while the cooler season had opposite effects, indicating the role of different environmental factors in determining the extent as well as quality of sweetness in the sweet corn genotypes.

Under the Indian scenario, once the farmer harvests the sweet corn ears, it normally takes 2-3 days to take the same to the nearest market/mandi as the transportation facilities are not so strong. Further, it takes another 4-5 days before it reaches the consumers/processing units. Therefore, altogether
it takes 7-10 days from the farmers’ field to the consumers; thus, stability of sugar concentration even after harvest over at least 7-10 days is an important factor for success of sweet corn cultivation. Therefore, the studies on change of sugar concentration after harvest would be required to be undertaken for better marketability and profitability of sweet corn genotypes.

The present analyses also revealed that there is a general depletion of sugar concentration under open-pollination as compared to the controlled-pollination. Genotypes such as L6 x T3 (RIL62 x Madhuri) had 34.65% sugar under controlled-pollination at 20 DAP at Delhi, while the same genotype recorded 19.43% sugar under open-pollination. 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). The situation where kernel sugar concentration was found to be more under controlled-pollination and less under open-pollination is due to the ‘dilution effects’ caused by the foreign pollen coming from other sweet corn genotypes grown in the trial. The foreign pollen had led to the poor complementation of alleles coming from the female and male plant for sweet corn trait. In the reverse case where kernel sugar concentration under controlled-pollination was lower than that of open-pollination indicates that in these specific genotypes, complementation of alleles was not favourable when controlled-pollination was done.

Interestingly, in the farmer’s field where a single sweet corn hybrid is usually grown, the farmer harvests the sweet corn ears under open-pollination. In this regard, it is important to note that open-pollination mode in the farmer’s field is akin to the controlled-pollination mode in the experimental trial. Since the pollen coming from the same sweet corn genotype being grown in the farmer’s field would pollinate the ears, the concentration of kernel sugar would be determined by the alleles of the same sweet corn hybrid except the few border rows where the chances of pollinating by foreign pollen could be high. Therefore, selection for kernel sugar concentration under open-pollination
during the breeding programmes may lead to the faulty selection of genotypes. On the other hand, selection for grain yield and its component traits should always be undertaken from the open-pollinated trial, as under controlled-pollination there is possibility of lesser ear size and in turn the harvest. Therefore, the experimental trials can be designed such that, in each row, 5 plants can be control-pollinated, while the rest can be allowed for open-pollination. While the selection of genotypes for grain yield and its component traits should be taken based on observations recorded on open-pollinated plants, the control-pollinated ears could be used for the purpose of selection of genotypes for sweet corn trait.

The present investigation, thus, led to the detailed analyses of sweet corn genotypes in terms of identification of promising and stable sweet corn inbred lines as well as hybrid combinations based on estimates of combining ability and heterosis. The study also highlights the possible relationships among the kernel sugar concentration, grain yield and its component traits. The effects of environment and genotype x environment interaction are important in determining kernel sugar concentration in the sweet corn genotypes. The study also demonstrated the role of open- vs. controlled-pollination for identifying promising genotypes in breeding programmes for kernel sugar concentration as well as for yield and its contributing traits.

III. Allele Mining in the sugary1 (su1) Gene of the Maize Genotypes
Among the crop plants, maize has enormous diversity both at the phenotypic and genetic levels which was as well confirmed through gene sequence analyses (Whitt et al., 2002). Modern genomic technologies and development of robust statistical analysis methods especially in the field of bioinformatics now enable researchers to analyze the diversity at the DNA level. When considering nucleotide polymorphism in genes, it has been well said that two maize lines are on average are as diverged from one another as humans are from chimpanzees (Tenaillon, 2001). This increase in diversity in maize provides tremendous opportunity to modern breeders for crop improvement.
A single nucleotide polymorphism (SNP) is an individual nucleotide base difference between two DNA sequences. SNPs can be categorized according to nucleotide substitution as either transitions (C/T or G/A) or transversions (C/G, A/T, C/A, or T/G) and InDels. As a nucleotide base is the smallest unit of inheritance, SNPs provide the ultimate form of molecular genetic marker. They also represent the most frequent type of genetic polymorphism, and the potential number of such markers are present throughout the species (Rafalski, 2002 a,b). Sequence variation can have a major impact on how the organism develops and responds to the environment. Furthermore, they are evolutionarily stable, not changing significantly from generation to generation (Lopez et al., 2005).

SNPs provide an important source of molecular markers that are useful in genetic mapping, map-based positional cloning, and detection of marker-trait relationships. The low mutation rate of SNPs makes them excellent markers for studying complex genetic traits and as a tool for the understanding of genome evolution (Syvanen, 2001). SNPs are suitable for automated discovery and detection, and can be applied to wide range of purposes, including rapid identification of crop cultivars, construction of high resolution genetic maps, mapping traits, genetic diagnostics, analysis of the genetic structure of populations, phylogenetic analysis, marker-assisted selection, etc.

SNPs, at any particular site, could in principle involve four different nucleotide variants, but in practice they are generally biallelic. However, this disadvantage, when compared to multiallelic markers such as SSRs, is compensated by the relative abundance of SNPs. A major advantage of SNPs is their abundance in the genome. For example, in maize, even conservative estimates would predict over 20 million polymorphisms to be available for analysis. In maize, it has been determined by direct PCR product sequencing that the frequency of polymorphisms in the US elite germplasm is very high; on average 1 SNP per 48 bp in non-coding regions, and 1 SNP per 131 bp in the coding regions (Bhattaramakki et al., 2001, 2002). InDel polymorphisms are also very frequent, on average 1 in 126 bp, but they occur almost exclusively
in non-coding regions (Bhattaramakki et al., 2001). These data are based primarily on the examination of coding sequences and 3' untranslated segments of genes in the US elite maize germplasm. In nongenic regions of the genome or in different populations the frequency of polymorphisms may be different.

The most direct approach to the discovery of DNA polymorphisms is direct sequencing of PCR products from a number of diverse individual which has been used in the present study. PCR primers are either designed on the basis of known DNA sequences of genes available from GenBank, or from EST sequences. In an example of SNP allelic diversity in maize at DuPont and Pioneer Hi-Bred, the available sequences of maize genome were used for designing the primers to amplify sequences (300 bp long) preceding the poly-A sites at the 3' untranslated segments of several maize genes. The alignment and analyses of products from 20 loci in 30 genotypes revealed the occurrence of one SNP per 70 bp, and one InDel per 160 bp (Gupta et al., 2001).

In the present study, we had approached the strategy of direct sequencing of the PCR product. SNPs reported in this study are also of three types i.e., transition, transversion and InDels. The frequency of InDels in the present study (1 InDel per 110bp) is found be very close to what has been reported earlier in maize (Bhattaramakki et al., 2001, 2002). On an average 1 SNP is reported per 40bp in the non-coding region of su1 gene; Whit et al. 2002, analyzed the both coding and non-coding region and reported the same low level of nucleotide diversity.

In a comprehensive study of variation within a maize chromosome, the diversity at 21 loci varied by 16-fold (Tenaillon, 2001). The variation between loci partly reflects sampling effects, but selection and other factors also play an important role. One major factor which plays a key role in determining the nucleotide diversity is background selection. In background selection, reduced diversity at neutral sites can result from selection against linked deleterious alleles that have arisen by mutation. In inbreds, the high incidence of selfing
reduces the effective recombination rate, and should reduce diversity in selfing species. Background selection pointed that the diversity should be shaped by recombination at the intragenomic scale and by outcrossing rate at the species level. At the gene level, Tenaillon et al. (2001) found a strong correlation between locus recombination rates and overall levels of diversity.

In the first candidate-gene association mapping study in plants, DNA sequence polymorphisms within the Dwarf8 (D8) locus in maize were associated with flowering time (Thornsberry et al., 2001). Later studies of the same population associated the candidate gene su1 with sweetness taste (Whitt et al., 2002), bt2, sh1 and sh2 with kernel composition, and ae1 and sh2 with starch pasting properties (Wilson et al., 2004). Association has been successfully established for traits with only moderate heritability, such as starch concentration in maize.

Nucleotide diversity in a diverse set of pedigree-based germplasm can lead to identification of superior alleles that were not captured by breeding practices and support introgression of these alleles into elite breeding germplasm (Zhu et al., 2008). Promoter, intron, exon, 5’ and 3’-untranslated regions (UTR) are all reasonable targets for identifying candidate gene SNPs (Single Nucleotide Polymorphisms) as the non-coding regions (which do play an important role in regulation of gene expression) are expected to have higher levels of nucleotide diversity than the coding regions (Thorup et al., 2000; Whitt and Buckler, 2003).

The results obtained in the present study signifies the importance of the 5’ UTR region of the su1 gene in the phenotypic variation for the trait, coupled with variations in other important genes influencing the starch biosynthetic pathway. The study demonstrated the significant three genotypes, DMR2318, DMR2319 and DMR2320 which showed high percentage sugar content. Analysis of sequence variations led to the identification of 11 haplotypes, of which three haplotypes Hap1, Hap2 and Hap3 which comprised of DMR2318, DMR2319 and DMR2320 could be considered as ‘informative’ with respect to variation for total percentage sugar content. These informative haplotypes
need to be further validated by analyzing a larger set of high sugar valued sweet corn inbred lines, for conclusively relating the nucleotide variation with the phenotypic diversity for the sugar concentration in the maize endosperm. This would lead to identification of favorable allelic variants for important allelic variants influencing the starch biosynthetic pathway through the strategy of ‘allele mining’ (Prasanna, 2007), and potential utilization of such variants in improving the sweet corn breeding which has a great economic importance. The nucleotide diversity analyzed in this study can be potentially used in future genetic and breeding studies to manipulate starch biosynthetic pathway in maize, besides selection of superior sweet corn genotypes for use in breeding strategies.

The future of starch biosynthesis research in maize is promising as advances in molecular techniques and the increased availability of germplasm resources move us ever closer to dissecting the molecular and functional diversity of starch biosynthesis in maize. Finding new favourable alleles for high sugar and starch concentration will lead to new insights about these biosynthetic pathways besides enabling development of improved sweet corn cultivars.

The high throughput DNA sequencing techniques allows the easy analysis of nucleotide diversity with large number of sample size which can be further analyzed for association studies. Important and unanswered questions related to starch biosynthesis in maize can be addressed using this wealth of knowledge. Association tests in natural populations are providing an exciting opportunity to simultaneously use diversity to understand the function of genes and to find useful alleles for plant breeding and crop improvement. Association approaches are amendable to high-throughput genomics and could be used to characterize all of the genes in a genome.

In the future, we may find ultra-high resolution analysis of intra-specific diversity by re-sequencing every gene from a comprehensive collection of genotypes. Such efforts will allow in silico association studies of any trait for which reliable phenotypes are available. Still, accurate determination of
phenotypes remains a significant challenge, especially for highly environmentally influenced traits. It is the access to accurate phenotyping information, and not genotyping that will limit in silico genetic mapping. Taken together, comprehensive phenotyping and genotyping of large germplasm collections with modern tools will open new vistas in molecular genetics and breeding of crop plants like maize.