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Genetic analysis of kernel modification in Quality Protein Maize (QPM) genotypes

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Abstract

Kernel vitreousness, besides agronomic performance and endosperm protein quality, is important for the successful adoption of the Quality Protein Maize (QPM) genotypes. The present study was undertaken to analyze in detail different attributes of kernel modification (endosperm modification, crown opaqueness and ear appearance) in QPM inbred lines and a set of experimental crosses (7 x 7 full diallel). Significant differences among the QPM genotypes for kernel modification were observed in the diallel set, indicating segregation of several kernel modifier genes. Correlation analysis revealed significant and positive associations among endosperm modification, crown modification as well as ear appearance under open-pollination. Analysis of ears obtained from different pollination modes (open *vs*. controlled-pollination) indicated significant interaction of the genotypes with the pollination mode, suggesting the importance of the source of pollen and its genetic constitution in conferring the kernel texture. The diallel analysis also indicated almost equal contribution of additive and non-additive effects for endosperm modification; however, there was predominance of non-additive gene effects on crown modification and ear appearance. Reciprocal cross differences for kernel modification in the diallel set were also observed, suggesting the possible dosage effects of the endosperm modifiers. Overall, for analysis of combining ability and for estimation of genetic variance components in relation to kernel modification in the QPM genotypes, experiments employing controlled-pollination mode could be more reliable than those using the open-pollination mode.

Key words: Endosperm modification, QPM, pollination mode, gene effects

Introduction

The average protein content of common maize (*Zea mays* L.) is about 9-10%, which is intermediate between rice and wheat [1]. However, cereal storage proteins, including those of maize, are deficient in two essential amino acids, lysine and tryptophan [2]. Therefore, healthy diets for monogastric animals, including humans, must include alternate source of these amino acids [3, 4].

The discovery of the nutritional value of the *opaque2* mutation in maize [5] was a significant breakthrough. The recessive *opaque2* mutant alters the amino acid composition of the endosperm protein, resulting in enhanced concentration of lysine and tryptophan [5]. This finding provided an immediate opportunity for breeding new cultivars with high lysine protein [1] around the world. In India, under the All-India Coordinated Maize Improvement Project (AICMIP), three *opaque2* composites, namely Shakti, Rattan and Protina were released for commercial cultivation in 1970. However, the euphoria related to *opaque2* mutation and its direct utilization in breeding programmes was soon tempered by the pleiotropic effects of this mutation, especially soft endosperm along with effects on other agronomic traits also. The soft kernel texture not only made the *opaque2* maize cultivars more vulnerable to maize weevil infestation and breakage of kernels during mechanical threshing and polishing, but also led to the non-preference of these cultivars by the farming community due to its pale and chalky appearance of the kernels.

Intensive breeding efforts at CIMMYT [International Maize and Wheat Improvement Center], Mexico led to the successful combination of the high lysine potential of *opaque2* with the genetic endosperm modifiers that led to hard kernel texture. The new maize genotypes were collectively referred to as “Quality Protein Maize” (QPM) [1, 6]. Several countries in Asia,

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Africa and Latin America, are actively pursuing QPM breeding programmes. In India, QPM cultivars occupy nearly 50,000 hectares and there is a growing demand for the QPM cultivars both as a source of human food and animal feed.

Endosperm modification is quantitatively inherited [1, 7] and the degree of kernel vitreousness and increased synthesis of g-zein in modified endosperms were both dosage-dependent and directly correlated [8]. Vasal *et al*. (1993a) found that the genotype x environment interactions were significant for endosperm hardness. Several reports [6, 9-13] indicated preponderance of additive gene action in kernel modification. Favourable general combining ability for kernel vitreousness and kernel hardness was positively correlated with an accumulation of dominant kernel modifiers. Due to the complex genetic control of kernel modification and lack of reliable molecular markers linked to endosperm modifier genes, the only effective approach at present is to physically screen the kernels using a 'light box' for identification of promising QPM genotypes with desirable kernel modification attributes. Further intensive studies are required to understand the genetic and molecular bases of endosperm modification. Also, since the QPM germplasm has to now compete with the normal-endosperm maize, information regarding combining ability of the QPM inbred lines coupled with important characters such as kernel modification is required for breeders to utilize this germplasm more effectively in the breeding programmes.

The objectives of the present study were (i) to analyze the kernel modification attributes in a selected set of elite QPM lines developed in India, besides a set of QPM hybrids derived using these lines; (ii) to explore the possible effects of the pollination mode (controlled versus open-pollination) on the kernel modification in the QPM genotypes as well as combining ability for kernel modification; and (iii) to identify the mode(s) of gene effects influencing kernel modification.

Materials and methods

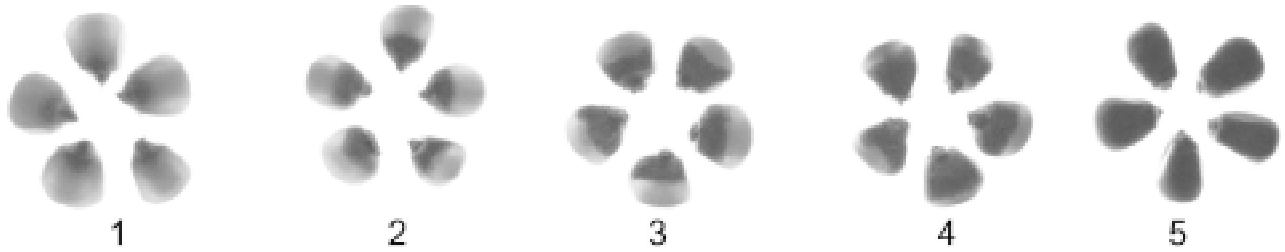
The genetic materials selected for the study consisted of (i) a set of seven QPM inbred lines, which were mainly developed from high-lysine *opaque2* composites, such as Shakti-1, under the All India Coordinated Maize Improvement Project (AICMIP); these lines were designated as 'DMRQPM' lines.

To analyze the complementation effects of kernel modifier genes from different parents, experimental crosses were derived using 7 x 7 diallel mating design (including reciprocals), comprising DMRQPM-56, DMRQPM-60, DMRQPM-401, DMRQPM-28-3, DMRQPM-403, DMRQPM-17-4 and DMRQPM-45 as parents.

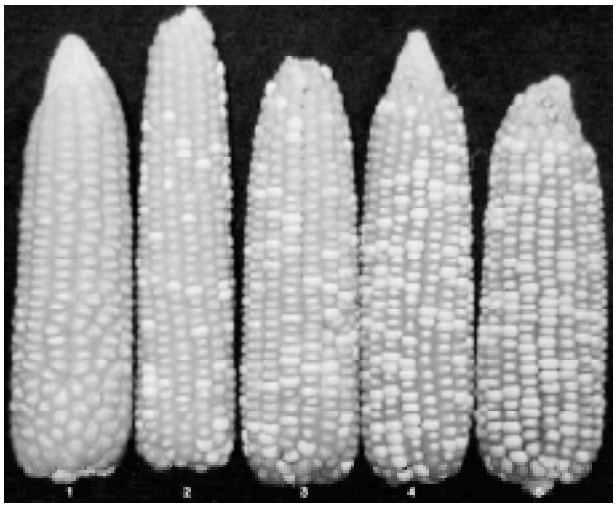
The QPM experimental hybrids along with the parental lines were evaluated in a trial at the IARI Experimental Farm, New Delhi, during *kharif* 2003, with three replications in a randomized complete block design. Shakti-1, a hard-endosperm *opaque2* composite was used as a check. The trial was maintained in two sets: one in open-pollination mode and another through controlled pollinations (bulk sibs). The trial was isolated from the normal-endosperm maize by difference in time of planting and by QPM border rows. Plots were of 5-m row length, spaced 75 cm apart. Standard agronomic practices were followed, and the material was hand-harvested.

Kernel modification of the QPM hybrids and their parental lines was rated using a procedure reported by Bjarnason and Vasal [1]. Three attributes of kernel modification, namely (a) endosperm modification (extent of opaqueness in the endosperm irrespective of the position in the kernel), (b) crown opaqueness (presence of opaqueness only on the crown of the kernels), and (c) ear appearance (in terms of opaqueness/ vitreousness) were evaluated in each genotype. For analysis of endosperm modification, the backlit kernels were rated on a scale of 1-5, with 1 indicating 100% normal (vitreous), 2 indicating 25% opaque, 3 indicating 50% opaque, 4 indicating 75% opaque, and 5 indicating 100% opaque (Fig. 1A). Endosperm modification scores were derived based on analysis of 100 randomly chosen kernels from the ears of QPM genotypes. Crown opaqueness was evaluated in terms of percent kernels showing crown opaqueness when screened using the back-lit procedure. The ears were also rated on a scale of 1-5, with 1 indicating 0% opaque kernels in an ear, 2 indicating 25% opaque kernels, 3 indicating 50% opaque kernels, 4 indicating 75% opaque kernels and 5 indicating 90-100% opaque kernels (Fig. 1B).

The data thus generated were analyzed for ANOVA, and DMRT (Duncan's Multiple Range Test) and was further carried out to rank the genotypes based on the kernel modification, using MSTAT-C. Cumulative index of kernel modification, based on ranks for the three different kernel modifications attributes, was computed, using the procedure suggested by Arunachalam and Bandopadhyay [14].



(A)



(B)

Fig. 1. (A) Kernel modification rating scale (1: 100% modified; 2: 25% opaque; 3: 50% opaque; 4: 75% opaque; 5: 100% opaque); (B) Ear appearance rating scale (1: 100% modified; 2: 25% opaque; 3: 50% opaque; 4: 75% opaque; 5: 90-100% opaque)

For analysis of gene effects related to kernel modification, the data recorded for the three kernel modification attributes were analyzed using appropriate models for the diallel set [15]. Combining ability for the target traits were analyzed using SPAR1 software (developed by the Indian Agricultural Statistical Research Institute, New Delhi) for combining ability analysis. Data recorded on both open- and controlled-pollinated ears were analyzed for combining ability analysis as well as for the comparison of mean performances for the three attributes.

Pearson's correlation coefficients among three kernel modification attributes (endosperm modification, crown modification and ear appearance), were computed and tested for their significance following standard statistical procedures [16], to ascertain the associations, if any, among these important attributes of the QPM genotypes.

Results and discussion

Accumulation of endosperm modifier genes in the *opaque2* genotypes led to the amelioration of the soft and chalky texture of *opaque2* mutant, leading to the development of QPM genotypes. However, during the QPM breeding programmes, it is not uncommon to find genotypes with varying degree of opaqueness or kernel modification, which could possibly affect the preference of these genotypes by the farmers as well as consumers. Therefore, successful commercial utilization of the QPM genotypes warrants combination of high-yield potential and superior endosperm protein quality with proper kernel modification.

The study revealed significant differences among the QPM genotypes for the three kernel attributes, namely endosperm modification, crown modification and ear appearance (Table 1) indicating the presence of enough variation for these kernel attributes among the experimental QPM genotypes. ANOVA also showed significant interaction of the pollination mode with the genotypes, suggesting the significance of the pollen source and its genetic constitution in affecting kernel modification in QPM genotypes. However, the effect of pollination mode (open vs. controlled) on kernel modification was found to be non-significant for all the three kernel traits.

Table 1. ANOVA of kernel modification attributes of the QPM genotypes in the diallel analysis

Sources of variation	d.f.	Mean Sum of Squares		
		EM	CM	EA
Pollination mode	1	0.0044	0.0157	0.1224
Replication	2	0.0228	0.0011	0.2278
Genotypes	48	1.9025**	0.1026**	2.215**
Pollination mode x genotype	48	0.7376**	0.0415**	0.8655**
Error	194	23.8689	0.0084	0.2519

d.f.: degrees of freedom; EM: endosperm modification; CM: crown modification; EA: ear appearance; *Significant at P = 0.05; ** Significant at P = 0.01

It was analyzed in detail for the first time the effects of modes of pollinations (open- vs. controlled-pollination) on kernel modification attributes. The results from controlled-pollination reflect the situation in which a farmer or a group of farmers in a contiguous area grow a particular QPM cultivar, whereas open-pollination reflects the situation where the foreign pollen from the nearby fields might contaminate a QPM genotypes, and thereby affecting kernel modification. The present study clearly revealed interaction between the pollination modes (pollen source) with the genotype of the seed plant. Wessel-Beaver and Lambert [10] indicated xenia effect in an experiment involving S2 lines derived from a modified-*o2* synthetic. The possible reason for significant xenia effect on QPM ear appearance could be the 'quality' of QPM genotypes available in the 1970s with respect to endosperm modification, compared to the present-day, improved QPM lines available in the international QPM breeding programmes. In another study, Pixley and Bjarnason [17] indicated no significant xenia effect on QPM genotypes, although kernel modification in open-pollination mode may be slightly higher than that observed in controlled-pollination.

A comparison of the mean values for various attributes revealed better kernel texture of majority of the QPM inbred lines and their experimental crosses over the QPM check, Shakti-1 (Table 2). Cumulative indices for kernel modification computed for the genotypes, based on DMRT ranks of genotypes for the individual attributes, indicated DMRQPM-403 (2.65) was the most promising among the Indian QPM lines followed by DMRQPM-56 (2.49) under controlled pollination. However, several of the DMRQPM lines displayed higher degree of kernel opaqueness. Significant differences among the QPM genotypes for endosperm modification, crown opaqueness and ear appearance in the diallel set indicated that several endosperm modifier genes were segregating in the QPM genotypes under study. Most of the QPM inbred lines and their experimental crosses outperformed Shakti-1 in terms of kernel modification. Interestingly, most of the DMRQPM lines were isolated from Shakti-1, suggesting that the selection procedures resulted in accumulation of some of the favourable combinations of the modifier genes. However, none of the QPM genotypes exhibited complete vitreousness, reaffirming the need for further accumulation of favourable endosperm modifier genes in the QPM inbred lines. Similar observation was made by Kassahun and Prasanna [18].

Among the QPM hybrids, DMRQPM-17-4 x DMRQPM-28-3 revealed the highest cumulative index (2.82) under controlled-pollination (Table 2). DMRQPM-56 and DMRQPM-28-3 scored 2.49 and 2.36 respectively, but their experimental cross showed poor cumulative index (1.46) under controlled pollination mode indicating negative complementation for kernel vitreousness.

DMRQPM-60 x DMRQPM-17-4 showed high cumulative index (2.06) under controlled pollination, while the open-pollinated ears recorded only 0.89 (Table 2). Similarly, in the case of inbred lines, DMRQPM-60 revealed better kernel texture under controlled-pollination compared to those from open-pollination. In contrast, DMRQPM-45 x DMRQPM-60 showed a high index (2.34) in the open-pollinated set, while under controlled pollination, the index was only 0.99.

Better modification of the kernels under controlled-pollination mode compared with the open-pollination in certain QPM crosses could be due to the accumulation and/or complementation of favourable alleles for endosperm modification in these genotypes, while pollen coming from other genotypes (under open–pollination) could dilute the effects of such genes. Cases in which the QPM genotypes exhibited better kernel vitreousness under open-pollination than the same from controlled-pollination, could be due to the modifier genes present in the parental lines of these hybrids were not optimum, while open-pollination probably led to the accumulation of favourable alleles through pollen from other QPM genotypes in the trial.

The overall mean of cumulative indices for the genotypes in the diallel set were 1.62 (controlled pollination) and 1.75 (open-pollination). The t-test indicated that there were no significant effects of pollination mode on the individual kernel modification attributes as well as for the cumulative indices. This suggests that xenia effects might not have significant influence on kernel modification *per se* in the QPM genotypes.

Correlation between endosperm modification and crown modification in the diallel set was found to be positive and significant only in case of open-pollination ($r = 0.38$). Similar trend was also observed between endosperm modification and ear appearance ($r = 0.32$). However, positive and significant correlations were observed among crown modification and ear appearance in both open- and control-pollination modes ($r = 0.74$ and 0.88, respectively). Positive significant correlation between the three kernel attributes is the indicative of the presence of a common set of

Table 2. Kernel modification attributes of the QPM genotypes in the diallel analysis

S. No.	Genotypes	EM		CM		EA		CI	
		C	O	C	O	C	O	C	O
1	DMRQPM-56	1.46^{II}	2.19^{II}	0.03^{I}	0.15^{II}	2.00^{II}	2.33	2.49	1.94
2	DMRQPM-60	3.02	2.84	0.04^{II}	0.39	2.00^{II}	4.00	1.90	0.91
3	DMRQPM-401	3.13	2.98	0.16	0.03^{I}	2.00^{II}	1.66^{II}	1.56	2.03
4	DMRQPM-28-3	1.56^{II}	2.47^{II}	0.06	0.15^{II}	2.00^{II}	3.00	2.36	1.63
5	DMRQPM-403	1.39^{I}	2.94	0.07^{II}	0.03^{I}	1.33^{I}	1.33^{I}	2.65	2.21
6	DMRQPM-17-4	3.21	2.73	0.05	0.08^{II}	2.00^{II}	2.00^{II}	1.77	1.91
7	DMRQPM-45	3.12	2.11^{I}	0.03^{II}	0.19	2.00^{II}	2.66	1.91	1.81
8	DMRQPM-56 x DMRQPM-60	1.61^{2}	2.35	0.18	0.27	2.00^{2}	2.66	2.01	1.49
9	DMRQPM-60 x DMRQPM-56	2.09	1.67^{2}	0.25	0.22	2.33	3.00	1.58	1.91
10	DMRQPM-56 x DMRQPM-401	1.87	2.46	0.23	0.37	2.66	4.00	1.59	1.04
11	DMRQPM-401 x DMRQPM-56	2.13	1.48^{1}	0.25	0.44	3.66	4.33	1.04	1.26
12	DMRQPM-56 x DMRQPM-28-3	2.87	2.65	0.18	0.03^{2}	2.33^{3}	2.00	1.46	2.08
13	DMRQPM-28-3 x DMRQPM-56	2.42	2.15	0.13	0.16	2.33	2.00	1.81	2.08
14	DMRQPM-56 x DMRQPM-403	1.64^{2}	2.08	0.15	0.09	2.33^{3}	2.00	2.01	2.24
15	DMRQPM-403 x DMRQPM-56	1.15^{1}	2.09	0.04^{3}	0.14	1.66^{2}	2.33	2.68	2.11
16	DMRQPM-56 x DMRQPM-17-4	1.04^{1}	2.05^{3}	0.21	0.21	2.66	2.66	1.93	1.92
17	DMRQPM-17-4 x DMRQPM-56	1.72^{3}	2.09	0.16	0.23	3.00	3.00	1.59	1.65
18	DMRQPM-56 x DMRQPM-45	1.89	1.69^{1}	0.14	0.21	2.00^{2}	2.33	2.06	2.13
19	DMRQPM-45 x DMRQPM-56	1.88	3.01	0.29	0.09	2.66	2.33	1.37	1.59
20	DMRQPM-60 x DMRQPM-401	4.05	2.77	0.36	0.15	3.66	2.33	0.29	1.76
21	DMRQPM-401 x DMRQPM-60	3.34	2.81	0.17	0.19	3.33	3.00	0.88	1.45
22	DMRQPM-60 x DMRQPM-28-3	2.39	2.21	0.09	0.45	2.00	2.66	2.03	1.20
23	DMRQPM-28-3 x DMRQPM-60	3.44	2.83	0.23	0.05	2.00^{3}	2.00	1.30	1.97
24	DMRQPM-60 x DMRQPM-403	3.09	2.90	0.03^{3}	0.10	2.00^{2}	2.00	1.91	1.87
25	DMRQPM-403 x DMRQPM-60	3.18	2.87	0.13	0.41	2.66	3.00	1.44	1.06
26	DMRQPM-60 x DMRQPM-17-4	1.67^{3}	3.19	0.13	0.31	2.33^{3}	3.33	2.06	0.89
27	DMRQPM-17-4 x DMRQPM-60	2.98	3.08	0.48	0.17	3.66	2.33	0.53	1.44
28	DMRQPM-60 x DMRQPM-45	3.20	2.96	0.17	0.27	2.00^{2}	3.00	1.47	1.17
29	DMRQPM-45 x DMRQPM-60	1.75	2.80	0.36	0.00^{1}	3.66	1.33^{2}	0.99	2.34
30	DMRQPM-401 x DMRQPM-28-3	3.25	2.84	0.11	0.21	2.66	2.66	1.43	1.55
31	DMRQPM-28-3 x DMRQPM-401	4.22	3.14	0.50	0.39	2.66	3.66	0.62	0.68
32	DMRQPM-401 x DMRQPM-403	3.29	2.99	0.01^{1}	0.02^{2}	2.00^{2}	1.33^{2}	1.95	2.09
33	DMRQPM-403 x DMRQPM-401	4.15	3.44	0.78	0.76	3.66	4.33	0.20	0.18
34	DMRQPM-401 x DMRQPM-17-4	3.51	3.35	0.27	0.26	2.66	2.66	0.88	1.07
35	DMRQPM-17-4 x DMRQPM-401	3.31	2.91	0.47	0.09	2.66	2.00	0.78	1.87
36	DMRQPM-401 x DMRQPM-45	2.74	3.10	0.23	0.11	2.00^{2}	2.00	1.59	1.61
37	DMRQPM-45 x DMRQPM-401	2.92	2.50	0.11	0.03^{2}	2.33	2.00	1.68	2.15
38	DMRQPM-28-3 x DMRQPM-403	1.74	2.47	0.22	0.10	2.00^{2}	2.00	1.88	1.98
39	DMRQPM-403 x DMRQPM-28-3	2.11	2.50	0.24	0.04^{3}	2.00^{3}	1.66^{3}	1.72	2.19
40	DMRQPM-28-3 x DMRQPM-17-4	3.01	2.56	0.03^{3}	0.00^{1}	1.33^{1}	1.00^{1}	2.20	2.48
41	DMRQPM-17-4 x DMRQPM-28-3	1.25^{2}	2.62	0.04^{1}	0.02^{2}	1.33^{1}	1.33^{1}	2.82	2.28
42	DMRQPM-28-3 x DMRQPM-45	2.25	2.17	0.02	0.02	1.33^{1}	1.66^{3}	2.49	2.40
43	DMRQPM-45 x DMRQPM-28-3	3.03	1.98	0.04^{2}	0.02^{2}	2.00^{3}	1.66^{3}	1.95	2.62
44	DMRQPM-403 x DMRQPM-17-4	2.65	2.02^{2}	0.03^{3}	0.07^{3}	2.00^{2}	1.66^{3}	2.11	2.48
45	DMRQPM-17-4 x DMRQPM-403	3.32	2.76	0.53	0.17	3.66	2.33	0.40	1.70
46	DMRQPM-403 x DMRQPM-45	2.48	2.73	0.08	0.22	2.00^{2}	3.00	1.98	1.45
47	DMRQPM-45 x DMRQPM-403	2.38	1.73^{3}	0.17	0.05	2.33	1.66^{3}	1.59	2.55
48	DMRQPM-17-4 x DMRQPM-45	2.73	2.80	0.14	0.08	2.00^{2}	2.00	1.81	1.97
49	DMRQPM-45 x DMRQPM-17-4	3.05	3.01	0.28	0.15	2.66	2.33	1.05	1.54
51	Shakti-1 (QPM check)	3.32	3.66	0.35	0.45	2.66	3.00	-	-

EM: Endosperm modification; CM: Crown modification; EA: Ear appearance; CI: Cumulative Index; O: Open- pollination; C: Controlled-pollination; I, II, III: DMRT ranking among inbred lines; 1, 2, 3: DMRT ranking among experimental crosses

endosperm modifier genes influencing the spatial distribution and packaging of protein bodies in the crown as well as endosperm.

Successful breeding approaches are the direct consequence of gene effects prevalent in the breeding population under consideration. The relative importance of additive and non-additive effects is the indication of gene effects. The value of an inbred line depends on its ability to produce superior hybrids in combination with other inbreds [19]. Combining ability is one of the most important areas in breeding programme and it has a significant impact on inbred line evaluation and population improvement in maize breeding [20, 21]. Analysis of experimental crosses in a full diallel set, including reciprocals, would not only give an idea of extent of complementation of endosperm modifiers in the QPM inbred lines, but also the possible dosage effects of these modifier genes considering the triploid endosperm.

ANOVA for combining ability showed that both additive and non-additive components of variance played a significant role for controlling all the three kernel mofication attributes *viz*. endosperm modification, crown modification and ear appearance in both pollination modes. Variation due to *gca* effects and *sca* effects for the three analyzed attributes were significant, thereby suggesting the importance of both additive and non-additive gene effects. For endosperm modification, both additive and non-additive gene effects were observed to be almost equally distributed under controlled-pollination mode. On the other hand, for both crown modification (V_D/V_A = 11.15) and ear appearance (V_D/V_A = 3.01), preponderance of non-additive variance (particularly dominance variance) was noted. However, under open-pollination mode, additive variance (V_D/V_A = 0.50) appeared more important for endosperm modification, while additive and non-additive variances were almost equally distributed (V_D/V_A = 1.19) for crown modification but non-additive variance was found to be high (V_D/V_A = 4.83) for ear appearance.

Thus, the results of the diallel analyses showed the importance of both *gca* and *sca* components on all the three traits: endosperm modification, crown modification and ear appearance, thereby indicating the role of both additive and dominance gene effects in the QPM germplasm. Vasal *et al*. [6, 22] reported the significance of only *gca* effects (additive component), while the *sca* effects (non-additive component) on endosperm hardness were found to be non-significant. Similar trends were also observed by several workers [6, 9, 12, 13, 22-24].

Combining ability analysis based on Griffing's model revealed that under controlled-pollination, DMRQPM-56 was the best general combiner (–0.76), with good endosperm modification (Table 3)**.** For crown modification, DMRQPM-28-3 (–0.05), DMRQPM-45 (–0.04) and DMRQPM-56 (–0.02) were found to be the good general combiners. Lowest negative *gca* effects and low means were observed in DMRQPM-28-3 (–0.36), DMRQPM-403 (–0.15) and DMRQPM-45 (–0.15) for ear appearance. In general, DMRQPM-403, DMRQPM-56 and DMRQPM-28-3 exhibited desirable *gca* effects for all the three kernel modification attributes. However, under open-pollination, DMRQPM-56 (–0.42) for endosperm modification, DMRQPM-28-3 (–0.05), DMRQPM-45 (–0.05) and DMRQPM-17-4 (–0.03) for crown modification, and DMRQPM-403 (–0.26), DMRQPM-45 (–0.21) and DMRQPM-17-4 (–0.21) for ear appearance were found to be the best general combiners.

DMRQPM-56 x DMRQPM-403, DMRQPM-403 x DMRQPM-56, DMRQPM-401 x DMRQPM-60, DMRQPM-401 x DMRQPM-45 and DMRQPM-28-3 x DMRQPM-17-4 revealed the best specific combining ability effects (Table 3) under controlled-pollination, considering all the three kernel modification attributes. However, DMRQPM-45 x DMRQPM-403, DMRQPM-45 x DMRQPM-60 and DMRQPM-28-3 x DMRQPM-17-4 were the best specific combiners under open-pollination.

Considering all the three attributes under study, DMRQPM-403, DMRQPM-56 DMRQPM-28-3 and DMRQPM-45 were found to be highly promising. Hohls *et al*. [12] earlier identified RO465(M), RO460(M) and RO452(M) as the best general combiners for endosperm hardness, while Bhatnagar *et al*. [13] identified T x X124 as an excellent general combiner for the same trait. Vasal *et al*. [6, 22] reported Pool 31 QPM, Pool 33 QPM and Population 69 QPM as having desirable *gca* effects for endosperm modification.

In many cases, reciprocal cross differences with respect to kernel modification were found to be prominent. For instance, under controlled-pollination, DMRQPM-17-4 x DMRQPM-28-3 showed higher *sca* effect for endosperm modification than its reciprocal crosses (Table 3). Similarly, DMRQPM-401 x DMRQPM-45 revealed better ear appearance compared to that of the reciprocal cross. Interestingly, DMRQPM-403 x DMRQPM-17-4 showed no reciprocal cross difference for kernel modification under controlled-pollination, but significant reciprocal cross difference under open-pollination. The same cross combination showed positive

significant *sca* effects (0.50) under controlled-pollination, and negative significant *sca* effects (–0.35) under open-pollination. In general, fewer cross-combinations displayed reciprocal cross differences for endosperm modification and crown modification under open-pollination compared to controlled-pollination. The reciprocal cross difference for kernel modification could be attributed to the dosage effects of the endosperm modifier genes due to the triploid nature of the endosperm [8]. This observation is in agreement with those made in some earlier studies [1, 9, 23, 25]. However, Bjarnason *et al*. [24] found no significant maternal or paternal effects for endosperm modification in a diallel study. Though maternal or cytoplasmic effects on kernel modification were not largely reported, Wessel-Beaver and Lambert [10] identified cytoplasmic effects as the plausible reason for reciprocal cross differences. Reciprocal cross differences for kernel modification may not be seen in all QPM crosses. Nevertheless, observations from the present study highlight the importance of selecting suitable male and female QPM parents in hybrid breeding programmes.

Studies undertaken so far on the combining ability for kernel modification in QPM were primarily based on experiments undertaken in open-pollination mode although the trials were spatially/temporally isolated from the normal (non-QPM) maize. Based on such experiments, earlier workers [6, 13, 22, 26] analyzed the combining ability for endosperm modification along with yield and yield-related traits in QPM. The present study was the first to analyze the possible effects of both the pollination modes (open-pollination vs. controlled-pollination) on the kernel modification attributes, including the estimation of combining ability and the gene effects.

The study also revealed that DMRQPM-56 was an excellent general combiner under controlled-pollination for both the endosperm modification and crown modication, while the same genotype was found to be a poor combiner for crown modification and ear appearance under open-pollination. Similar trend was observed for DMRQPM-403 (for endosperm modification) where it was a promising line under controlled pollination while it performed poorly under open-pollination. In contrast, DMRQPM-17-4 was found to be an excellent combiner for crown modification under open-pollination mode, but it performed poorly under controlled pollination. Differences in *sca* effects were also prominent in both the pollination modes. As kernel modification attributes are not maternal traits and governed by the interaction of genes (coming from both the parents) after fertilization, thus controlled-pollination is the best way to examine the performance of a set of lines rather than analyzing through random pollination, where the source of pollen is unknown. This study also clearly indicates that experiments on QPM genotypes using exclusively open-pollination mode could lead to biased estimates of additive and non-additive variance and in turn, might affect proper selection of parental lines, and responses to selection or genetic gain during the selection process. Not only this, choosing of parents on the basis of general combining ability is also dependent on pollination mode as selection on the basis of open-pollination may undermine utility of an inbred line which is otherwise promising under controlled-pollination. It may also lead to the selection of an otherwise poor combiner under controlled-pollination.

In conclusion, the present study revealed (i) the genetic variability in the Indian QPM lines with regard to kernel modification attributes, (ii) the need for accumulation of kernel modifier genes in the Indian QPM inbred lines, (iii) the potential of QPM inbreds like DMRQPM-28-3, DMRQPM-56, DMRQPM-403 with respect to kernel modification; and (iv) significant influence of the mode of pollination (open- *vs*. controlled-pollination) on the kernel modification attributes of the QPM genotypes. Both additive and dominance gene effects were found to be important for endosperm modification. Reciprocal cross differences for kernel modification were noticed, indicating the dosage effects of the endosperm modifier genes. Through a comparison of kernel modification in QPM ears using two pollination modes (open- *vs*. controlled-pollination), the study for the first time clearly demonstrated that for proper analysis of combining ability and for understanding the relative significance of genetic variance components with respect to kernel modification, experiments based on controlled-pollination could be more reliable than open-pollination.

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Table 3. The *gca* and *sca* effects of selected parental lines and crosses showing desirable kernel modification attributes in maize

Genotypes\	Controlled-pollination			Open-pollination		
Parents	EM	CM	EA	EM	CM	EA
P_1	-0.76**	-0.02*	0.04	-0.42**	0.03**	0.27*
P_4	-0.06	-0.05**	-0.36**	-0.07	-0.05**	-0.23*
P_5	-0.14**	0.00	-0.15*	0.03	-0.01	-0.26*
P_6	0.05	0.02*	0.07	0.14	-0.03**	-0.21*
P_7	0.05	-0.04**	-0.15*	-0.10	-0.05**	-0.21*
SE (g_i)	0.03	0.008	0.06	0.06	0.01	0.08
SE ($g_i - g_j$)	0.05	0.01	0.09	0.09	0.02	0.12

Crosses	F_1	RF_1	rd	F_1	RF_1	rd	F_1	RF_1	rd	F_1	RF_1	rd	F_1	RF_1	rd	F_1	RF_1	rd
$P_1 \times P_2$	-0.16*	0.24*	+*	0.05*	0.04*	NS	-0.43**	0.17	NS	-0.29	-0.34	NS	-0.02	-0.02	NS	-0.19	0.17	NS
$P_1 \times P_3$	-0.45**	0.13	+*	-0.01	0.01	NS	0.41*	0.50**	NS	-0.45**	-0.49*	NS	0.16**	0.03	-*	1.21**	0.17	-*
$P_1 \times P_4$	0.90**	-0.23*	-*	0.04*	-0.03	-*	0.29	0.00	NS	0.32*	-0.25	-*	-0.06	0.07	+*	-0.43*	0.00	NS
$P_1 \times P_5$	-0.27**	-0.25**	NS	-0.06**	-0.05*	NS	-0.26	-0.33	NS	-0.10	0.00	NS	-0.07	0.02	NS	-0.24	0.17	NS
$P_1 \times P_6$	-0.48**	0.34**	+*	0.01	-0.02	NS	0.36*	0.17	NS	-0.22	0.02	NS	0.06	0.01	NS	0.38	0.17	NS
$P_2 \times P_3$	0.27**	-0.36**	-*	-0.01	-0.10**	-*	0.60**	-0.17	-*	-0.20	0.02	NS	-0.12**	0.02	+*	-0.38	0.33	+*
$P_2 \times P_4$	0.20*	0.52**	+*	0.02	0.07**	NS	-0.19	0.00	NS	-0.13	0.31	NS	0.05	-0.20**	-*	-0.19	-0.33	NS
$P_2 \times P_5$	0.50**	0.05	-*	-0.11**	0.05*	+*	-0.07	0.33	NS	0.13	-0.01	NS	0.03	0.16**	+*	0.00	0.50*	NS
$P_2 \times P_6$	-0.50**	0.65**	+*	0.09**	0.18**	+*	0.38*	0.67**	NS	0.28	-0.05	NS	0.03	-0.07	NS	0.28	-0.50*	-*
$P_2 \times P_7$	-0.34**	-0.72**	-*	0.11**	0.09**	NS	0.43**	0.83**	NS	0.25	-0.08	NS	-0.05	-0.14**	NS	-0.38	-0.83**	NS
$P_3 \times P_4$	0.58**	0.48**	NS	0.08**	0.19**	+*	0.31*	0.00	NS	0.22	0.15	NS	0.12**	0.09*	NS	0.71**	0.50*	NS
$P_3 \times P_7$	-0.43**	0.09	+*	-0.07**	-0.06**	NS	-0.40*	0.17	+*	0.06	-0.30	NS	-0.01	-0.04	NS	-0.48*	0.00	NS
$P_4 \times P_6$	-0.43**	-0.88**	-*	-0.12**	0.00	+*	-0.73**	0.00	+*	-0.05	0.03	NS	-0.08*	0.01	NS	-0.79**	0.17	+*
$P_4 \times P_7$	0.09	0.39**	NS	-0.08**	0.01	+*	-0.19	0.33	+*	-0.34*	-0.10	NS	-0.05	0.00	NS	-0.29	0.00	NS
$P_5 \times P_6$	0.50**	0.34**	NS	0.08**	0.25**	+*	0.55**	0.83**	NS	-0.35*	0.37*	+*	0.00	0.05	NS	0.07	0.33	NS
SE(s_{ij}) or E(r_{ij})	0.08	0.09		0.02	0.02		0.15	0.17		0.15	0.18		0.04	0.04		0.20	0.23	
SE(s_{ij} - s_{kl}) or SE($r_{ij} - r_{kl}$)	0.11	0.13		0.03	0.03		0.20	0.24		0.21	0.25		0.05	0.06		0.27	0.32	

F_1 = direct cross, RF_1 = reciprocal cross, rd = reciprocal cross difference, *, ** = Significant at P = 0.05 & 0.01, respectively; + = better modification toward direct cross, - = better modification toward reciprocal cross; P_1 = DMRQPM-56, P_2 = DMRQPM-60, P_3 = DMRQPM-401, P_4 = DMRQPM-28-3, P_5 = DMRQPM-403, P_6 = DMRQPM-17-4, P_7 = DMRQPM-45

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