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Development of QPM Version of DHM117 Maize Hybrid Using Marker Assisted Selection

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A B S T R A C T

Development of QPM (Quality Protein Maize) with high lysine and tryptophan is foremost important task in enhancing nutritional quality in maize through breeding programme. Marker assisted selection is the most feasible way of developing QPM hybrids in short time. The present investigation deals with conversion of elite normal maize inbred lines BML6 and BML7 (parental lines of DHM117 hybrid) into QPM lines using marker assisted selection. The nutritional quality of maize is enhanced by introgression of the *opaque2 (o2)* gene along with numerous modifiers for kernel hardness. To improve the efficiency of QPM breeding, the utility of three simple sequence repeat (SSR) markers *viz. umc1066, phi057 and phi112* were used in selection and introgression of the *opaqaue2* gene. Polymorphism was detected between recipient parents (BML7 and BML6) and donor parent (CML181) with *umc1066* SSR marker. Foreground selection was exercised in each generation using *opaque2* specific marker *umc1066* while background selection was carried out in BC_1F_1 and BC_2F_1 generations to recover the recurrent parent (RPG) genome using SSR markers distributed across the genome. In BC_2F_1 the recovery of recurrent parent was between 90 to 93% and the plants with highest recovery were selfed to generate advanced generations (BC_2F_2 and BC_2F_3). Kernels were screened for endosperm hardness using light box and kernels showing less than 25% opacity were selected. Rigorous phenotyping was done for plant characters and tryptophan was estimated using colorimetric method. Tryptophan content varied from 0.76% to 0.95% in BC_2F_3 derived population of BML6 and 0.72% to 0.92% in BC_2F_3 derived population of BML7. Normal looking converted inbreds (CBML6 and CBML7) with high tryptophan and high yield were used for reconstitution of the QPM version of DHM117 maize hybrid. Two cross combinations CB6-36 × CB7-28 and CB6-36 × CB7-59 exhibited relatively higher tryptophan content and on par grain yield per plant compared to check DHM 117 which need to be further tested under multi-location trials prior to commercial exploitation.

Introduction

Maize (*Zea mays* L.) is a staple food for millions of people in poor countries around the world and it provides 15% of the world's protein and 20% of the world's calories. This indicates maize's status as a paramount crop in the context of global nutrition. But, maize kernels lack the essential amino acids lysine and tryptophan. Deficiency in these

amino acids causes some of the fatal diseases like pellagra, kwashiorkor etc. where maize is a staple food and also leads to a condition called wet malnutrition.

Maize mutant *opaque2* was discovered by Mertz *et al.,* (1964) with high lysine and tryptophan content than the normal maize kernels. But due to some of its undesirable and pleiotropic effects like soft, chalky kernel and low yields it was ultimately rejected in the market. In order to prevent these undesirable traits of *opaque2* varieties, researchers started to combine the *opaque2* maize with genetic modifiers which improves the vitreousness of the kernels. These modifiers overcome the negative pleotropic effects of the *o2* gene (Ortega and Bates, 1983). Genotypes with *o2* allele and *o2* modifiers with elevated lysine and tryptophan level and without negative effect are termed as quality protein maize (QPM) (Bjarnason and Vasal 1992; Geevers and Lake, 1992).

Three SSR markers viz., *phi 057, phi112* and *umc1066* are discovered that lie within the *opaque2* gene facilitated study and application of the *o2* gene (Mbogori *et al.,* 2006). Breeding for QPM genotypes becomes straight forward with the help of SSRs located within the *o2* locus, which are useful for the QPM breeders (Babu *et al.,* 2005; Danson *et al.,* 2006). Marker Assisted Selection (MAS) is an appropriate technology for traits such as high lysine in maize and can be a cost effective procedure for selecting *o2* locus in breeding populations (Babu *et al.,* 2005 and Gupta *et al.,* 2009).

MAS in combination with conventional breeding techniques can greatly accelerate the introgression of QPM genotype into normal maize. In India, only one QPM hybrid, Vivek Hybrid-9 was released in 2008 (Gupta *et al.,* 2009) using MAS at Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora, Uttaranchal. The present investigation has been taken for conversion of elite maize inbreds BML6 and BML7 into QPM with high lysine and tryptophan using marker assisted breeding and developing QPM version of DHM 117.

Materials and Methods

BML6 female parent and BML7 male parent of popular single cross hybrid DHM 117 were selected as recipients. QPM inbred line CML181 was selected as donor. *opaque2* gene based SSR markers *viz.,umc1066, phi057* and *phi112* (Table 1) were used to check the polymorphism between donor and recipient parents and *umc1066* was used for foreground selection. Flanking markers of *opaque2: bnlg1200* and *bnlg2160* (Table 1) were used for recombinant selection. Six SSR markers *viz, mmc0241, umc1216, phi072, bnlg1633, bmc1382* and *phi075* (Table 1) were used as endosperm and amino acid modifiers.

Parental polymorphism survey between BML6, BML7 (recurrent parents) and CML181 (donor parent) was carried out by Krishna *et al.,* 2011 using 752 genomic SSR markers covering all ten chromosomes. We selected 70 distinct polymorphic markers between BML6 and CML181 and 83 polymorphic markers between BML7 and CML181 were used for background selection. These polymorphic genomic SSR markers were used for background selection.

The field experiments were carried out at the Maize Research Centre (MRC). Genotyping and Biochemical analysis were carried out at Institute of Biotechnology, Rajendranagar, Hyderabad.

The schematic diagram for conversion of BML 6 and BML7 is illustrated in figure 1. PCR programme for genotyping was

standardised (Table 2), the amplified products were resolved on 3.0% metaphor: agarose (1:2) gel and the gel was observed under gel documentation system.

Scoring of alleles was carried out for foreground and background selection. Heterozygous alleles were represented by 'H' and scored as 0.5. Homozygous recipient allele was represented by 'A' and scored as 1 while homozygous donor allele was represented by letter 'B' and scored as 0. Based on the scoring, data was analysed using Graphical Genotypes (GGT2) software (Van Berloo, 2007). RPG was calculated using the formula given by Kumar *et al.,* 2011

$$RPG\ (G) = \frac{[A+1/2H]}{N} \times 100$$

Where,

A = number of markers showing homozygosity for recurrent parent allele

H = number of markers showing heterozygous state for the parental alleles

N = total number of parental polymorphic markers screened.

Light box screening of the kernels was carried out to check the opacity. Tryptophan content of maize kernels was estimated using the method described by Hernandez and Bates (1969).

Phenotypic Data analysis was carried out using Analyses of varience (ANOVA) for opaqueness, tryptophan and lysine in the endosperm and other phenotypic characters under complete randomized design (CRD) using windostat 8.0. Pearson's simple correlation coefficients were calculated using MS office Excel.

Results and Discussion

Parental polymorphism

Distinct polymorphism was observed between the recipient lines (BML 6 and BML 7) and QPM donor (CML 181) with *opaque2* gene specific SSR marker *umc 1066* (Plate 1). *phi057*and *phi112* did not show any polymorphism.

Among six amino acid modifiers screened, *bnlg1633* showed distinct polymorphism between BML6 and CML181 and *mmc0241* shown distinct polymorphism between BML7 and CML 181.

Marker assisted Backcross Breeding programme

Generation of F_1 and molecular confirmation

Crosses were made between recurrent parents with donor during *Kharif* 2010 to generate F_1 seeds. During *Rabi* 2010-11 F_1 crop was raised and true F_1 plants were confirmed using *umc1066* (Plate 2). The confirmed F_1 plants were backcrossed to respective recurrent parents to obtain BC_1F_1 seeds.

Generation of BC_1F_1, foreground, recombinant, amino acid modifiers and background selection

During *Kharif* 2011 BC_1F_1 population was raised with population size of 200 in each cross and foreground selection was done using *umc1066.* Plants showing heterozygous for *opaque2* were identified and subjected to recombinant selection using flanking markers: *bnlg1200* and *bnlg2160* (Plate 3) and also screened for amino acid modifiers: *bnlg1633* for BML6-BC_1F_1 population and *mmc0241* for BML7-BC_1F_1 population (Plate 4). In BML6-BC_1F_1, 43 plants and 64 plants in

BML7-BC_1F_1, were single recombinants and heterozygous. These single recombinants were used for further amino acid modifier and background screening.

For background selection in BML6-BC_1F_1, 70 SSRs were screened (Plate 5) and the recovery of the genome of recipient parent was varied from 60.93% to 83.00%. 15 plants showed recovery in between 80-83% and the remaining has shown less than 80% RPG (Table 3). The plants with >80% RPG were back crossed with BML6 for generating BC_2F_1 population. Similarly, 83 SSRs were used for background selection in BML7-BC_1F_1 and RPG was found in between 58.24% to 83.00%. Ninteen plants showed recovery between 80-83% and remaining plants showed less than 80% RPG (Table 3). The plants with >80% RPG were back crossed with BML7 for generating BC_2F_1 population.

Generation of BC_2F_1, foreground, recombinant, amino acid modifiers and background selection

BML6 and BML7 derived BC_2F_1 seeds were sown during *Rabi 2011-12*.One hundred and forty one plants of each BC_2F_1 populations were selected for foreground selection. In BML6- BC_2F_1 and BML7-BC_2F_1, 60 and 64 plants respectively were with *opaque2* in heterozygous condition (O2o2). Foreground selected BC_2F_1-BML6 plants were screened with flanking marker *bnlg2160* (plate 6) and amino acid modifier marker *bnlg1633*. Foreground selected BC_2F_1 -BML7 plants were screened for flanking marker *bnlg1200*. Double recombinants were not observed in both the BC_2F_1 population. In screening for amino acid modifiers less number of desired allele (donor) and some heterozygous alleles were observed in both BC_2F_1 populations, these heterozygous and donor type allele plants were subjected for background selection.

Background markers which were not recovered in BC_1F_1 generation were used for background screening. Marker data was analyzed and manually scored and recurrent parent genome (RPG) was calculated using GGT2 software. In BC_2F_1 population the recovery of recurrent parent genome ranged between 90-94.5%. Six (p-36, p-36-1, p-38, p-39, p-49 and p-52) and eight (p-11, p-15, p-26, p-28, p-32, p-38, p-59 and p-92) plants from BML6 and BML7 derived populations respectively were identified to generate BC_2F_2 families (Table 4). Kernels with desired characters were screened for hardness under light box.

Generation of BC_2F_2, foreground selection and tryptophan estimation

BC_2F_2 population was raised from selected kernels showing less than 25% opacity during *Kharif* 2012 and foreground selection was done for *opaque2* gene using *umc1066.* 182 BML6-BC_2F_2 and 207 BML7-BC_2F_2 plants were subjected to foreground selection. 44 and 56 individuals exhibited recessive homozygous loci in BML6-BC_2F_2 and BML7-BC_2F_2 populations respectively (Plate 7). Kernels of selected families of BC_2F_2 populations (CB6-36, CB6-39, CB7-11, CB7-28 and CB7-59) with desired characters were screened for hardness under light box (Fig. 2) and tryptophan was estimated using colorimetric method and undesired families were discarded.

Generation of BC_2F3, foreground, recombinant and amino acid modifier selection, tryptophan estimation and phenotyping

BC_2F_3 populations of BML6 and BML7 were generated during *Rabi* 2012-13. Foreground selection, recombinant selection and amino acid modifiers screening was carried out in BC_2F_3. The plants with all desired characters were forwarded for next generation.

Agronomic and biochemical traits recorded in the BC_2F_3 trial were viz., days to 50% tasseling, days to 50% silking, days to maturity, plant height (cm), ear height (cm), ear girth (cm), ear length (cm), number of kernel rows, number of kernels /row, test weight, grain yield/plant (g) and tryptophan concentration (%).

Ears showing similar morphology with recurrent parents in terms of texture, colour of the grains, size of the grains, row arrangements and size of the ears were selected for further advancement. Tryptophan content varied from 0.76% to 0.95% in BML6-BC_2F_3 and 0.72% to 0.92% in BML7-BC_2F_3 population respectively (Table 5). The selected families with all desired characters were selfed to generate BC_2F_4 seeds during *Rabi* 2012-13.

The converted BML6 and BML7 were designated as CB6 and CB7 with their pedigree plant numbers. The selected families were used for crossing program for producing QPM hybrid. The stable expression of these traits was confirmed through BC_2F_3 families, which represented the final converted QPM versions of the original recipient lines.

The expression of genetic modifiers may be affected by maternal influence. Endosperm is a triploid tissue; one may expect maternal influence since two doses of modifying alleles are contributed by the maternal parent and only one by the paternal parent. Reciprocal differences in crosses between soft opaque and modified opaque have been reported by Vasal, 1994.

Other factors such as genetic background and kernel texture can also alter phenotypic manifestation of modifying genes. Flint genetic backgrounds generally exhibit a higher frequency of modified kernels (Vasal, 1994). Therefore, direct and reciprocal crosses between selected families of CB6 and CB7 inbreds were performed to generate F_1 hybrids. In direct cross, CB6-36, CB6-39 as female parent and CB7-11, CB7-28 and CB7-59 as male parent and in reciprocal crosses it was vice-versa for producing QPM hybrid.

The six F_1 hybrids were evaluated during the *Rabi* 2013-14 and *Kharif* 2014 along with five converted inbreds, two normal inbred, one QPM inbred, one standard check hybrid DHM117 and one standard reciprocal check hybrid BML7 X BML6 (Table 6).

Evaluation of QPM version of DHM117 hybrid

During *Rabi* 2013-14 and *Kharif* 2014, all crosses (Table 6) along with 5 parents, 3 check inbreds (BML6, BML7 and CML181) one commercial check, DHM117 and reciprocal cross between BML7 X BML6 were sown in Randomized Block Design (RBD) replicated thrice.

Each entry was sown in a row of five meter length with a spacing of 75 cm between rows and 20 cm between the plants. Necessary plant protection measures were taken to protect the crop from pests and diseases as per the Maize Research Center recommendations, so as to raise a healthy crop.

Converted inbreds (CB6, CB7) and QPM hybrid purity assessment was checked in *Rabi* 2013-14 using gene specific *umc1066* SSR marker. All the QPM hybrids possessed recessive allele of *opaque2*. Phenotypic data was recorded on twelve different characters *viz.*, days to 50 per cent tasseling, days to 50 per cent silking, days to maturity, plant height (cm), ear height (cm), ear length (cm), ear girth (cm), number of kernel rows per ear, number of kernels per row, 100 kernel weights (g), grain yield per plant (g) and tryptophan content (%) in generated hybrids.

Table.1 Markers used in the study

marker	location	Sequence	Reference
Opaque2 specific markers			
umc1066	7.01	F ATGGAGCACGTCATCTCAATGG	www.agron.missouri.edu
		R AGCAGCAGCAACGTCTATGACACT	
phi057	7.01	F CTCATCAGTGCCGTCGTCCAT	
		R CAGTCGCAAGAAACCGTTGCC	
phi112	7.01	F TGCCCTGCAGGTTCACATTGAGT	
		R AGGAGTACGCTTGGATGCTCTTC	
Flanking markers			
bnlg1200	7.01	F CGTCCTCGTTGTTATTCCGT	Babu *et al.*, 2004
		R GTTCCCTCTCCCTCCCTC	
bnlg2160	7.01-02	F GAAGCAACCCATTTTCATCC	
		R AGATTGGATTCCTGCCTCCT	
Amino acid modifiers			
bnlg1633	2.07	F GTACCTCCAGGTTTACGCCA	Wu *et al.*, 2002 Wang and Larkins 2001
		R TCAACTTCTCATGCACCCAT	
bnlg2136	3.04	F TGC TCC TTC TCG AGC ACC	
		R ATG GAC GTA CGG CAG ACT CT	
phi072	4.01	F ACCGTGCATGATTAATTTCTCCAGCCTT	
		R GACAGCGCGCAAATGGATTGAACT	
bnlg1382	5.01	F TTTTCTTTCAAAAATATTCAGAAGC	
		R GCAGGATTTCATCGGTTGTT	
phi119	8.02	F GGG CTC CAG TTT TCA GTC ATT GG	Wu *et al.*, 2002
		R ATC TTT CGT GCG GAG GAA TGG TCA	
phi115	8.03-8.04	F GCT CCG TGT TTC GCC TGA A	Wu *et al.*, 2002
		R ACC ATC ACC TGA ATC CAT CAC A	
bnlg1655	10.03	F ATT AAA ATC TTG CTG ATG GCG	Wu *et al.*, 2002
		R TTC TGT TCC CGC CTG TAC TT	
Endosperm modifiers			
umc1014	6.04	F GAA AGT CGA TCG AGA GAC CCT G	Holing *et al.*, 2008
		R CCC TCT CTT CAC CCC TTC CTT	
umc1216	7.02	F TTGGTTGTTGGCTCCATATTCA	Danson *et al.*, 2006
		R GTTATATGCCCGTGCATTGCTA	
umc1036	7.02	F CTG CTG CTC AAG GAG ATG GAG A	Holing *et al.*, 2011
		R GAC ACA CAT GCA CGA GCA GAC T	

Table.2 PCR programming for three SSR markers used in the present study

Steps follwed in Thermal cycler	**Temperature in degree celcius for one cycle**	**Time for one cycle**
Step 1	94 ºC	5 minutes
Step 2	94 ºC	0.45 minute
Step 3	56 ºC	0.45min
Step 4	72 ºC	1minute
Aabove2-4 steps are repeated for 35 cycles		
Step 5		
Step 6	72ºC hold at 4ºC until ready to load onto gel	10 minutes

Table.3

Population	Population size	Foreground selection			Recombinant selection			No. of polymorphic background markers screened	Range of RPG rate (%)	Mean RPG rate (%)	Individuals with highest RPG	No. of non-recovered background markers
		D	H	R	D	H	R					
BML6-BC_1F_1	200	0	**98**	102	55	23	**20**	**70**	60.93-83.00	78.52	**15**	16
BML7-BC_1F_1	200	0	**103**	97	40	36	**27**	**83**	58.24-83.00	76.20	**19**	17

Table.4

Population	Population size	Foreground selection			Recombinant selection			No. of polymorphic background markers screened	Range of RPG rate (%)	Mean RPG rate (%)	Individuals with highest RPG	No. of non-recovered background markers
		D	H	R	D	H	R					
BML6-BC_2F_1	140	0	**60**	80	20	27	**3**	**16**	87-93.5	89.32	**6**	8
BML7-BC_2F_1	130	0	**64**	76	23	36	**5**	**17**	85-94.5	90.56	**8**	7

Table.5 Tryptophan content of the converted inbreds, normal inbreds and donor

S. No.	BML6 -BC_2F_3	Tryptophan content (%)	S. No.	BML7-BC_2F_3	Tryptophan content (%)
1	36-1	0.95	1	11	0.81
2	36-2	0.86	2	15	0.92
3	38	0.79	3	26	0.74
4	39	0.81	4	28	0.87
5	49	0.76	5	32	0.79
6	52	0.83	6	38	0.81
Control	BML6	0.39	7	59	0.86
Control	BML7	0.42	8	92	0.82
Donor	CML181	0.93			

Table.6 Crosses attempted for making direct and reciprocal hybrids

S.No	Direct	S.No	Reciprocal
1	CB6-36 X CB7-28	1	CB7-11 XCB6-36
2	CB6-36 X CB7-28	2	CB7-11 XCB6-39
3	CB6-36 X CB7-59	3	CB7-28 X CB6-36
4	CB6-39 X CB7-11	4	CB7-28 X CB6-39
5	CB-39 X CB7-28	5	CB7-59 XCB6-36
6	CB-39 X CB7-59	6	CB7-59 XCB6-39
Check	DHM117 (BML6 X BML7)	Check	BML7 X BML6

Table.7 Analysis of variance for randomized block design for yield and yield attributing characters in maize during *Rabi* 2013-14

Character	Mean sum of square		
	Replication (d.f= 2)	Genotypes (d.f=21)	error (d.f=42)
Days to 50% tasseling	1.84	31.56***	1.41
Days to 50% silking	0.173	32.29***	1.09
Days to maturity	2.695*	16.94***	0.77
Plant height(cm)	3.725	2181.64***	2.83
Ear height (cm)	7.501**	737.67**	1.03
Ear length (cm)	0.174	19.19***	0.08
Ear girth (cm)	0.143**	10.60***	0.03
Number of kernel rows per ear	0.002	2.30***	0.04
Number of kernels per row	0.335	193.06***	0.34
100 kernel weight (g)	1.832	183.56***	1.74
Grain yield per plant (g)	23.214	5719.36***	3.41
Tryptophan content (%)	0.000	0.066**	0.00

*significant at 5% level; ** significant at 1% level

Table.8 Analysis of variance for randomized block design for yield and yield attributing characters in maize during *Kharif* 2014

Character	Mean sum of square		
	Replication (d.f= 2)	Genotypes (d.f=21)	error (d.f= 42)
Days to 50% tasseling	0.154	27.80***	0.62
Days to 50% silking	0.466	25.93***	0.58
Days to maturity	9.577**	11.43***	1.27
Plant height(cm)	339.581	2923.94***	351.49
Ear height (cm)	0.009	713.49***	2.10
Ear length (cm)	0.063	18.68***	0.033
Ear girth (cm)	0.031	9.84***	0.024
Number of kernel rows per ear	0.016	2.18***	0.025
Number of kernels per row	0.329	205.48***	0.48
100 kernel weight (g)	0.44	178.39***	0.14
Grain yield per plant (g)	57.041***	5604.50***	3.16
Tryptophan content (%)	0.001***	0.07***	0.00

*significant at 5% level; ** significant at 1% level

Fig.1 Schematic diagram for conversion of BML 6 and BML 7 inbreds to QPM version and reconstitution of QPM hybrid

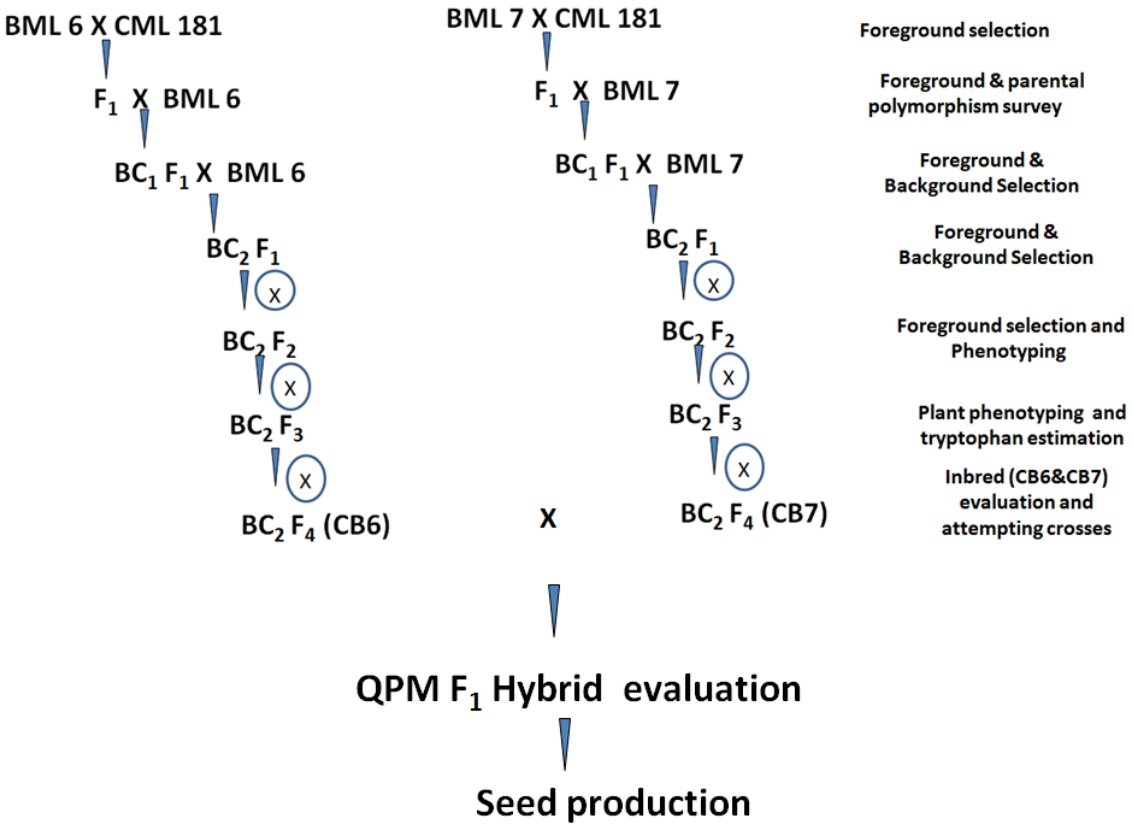


Fig.2 Light box screening for endosperm modification of BML6-BC_2F_2 and BML7-BC_2F_2 individuals

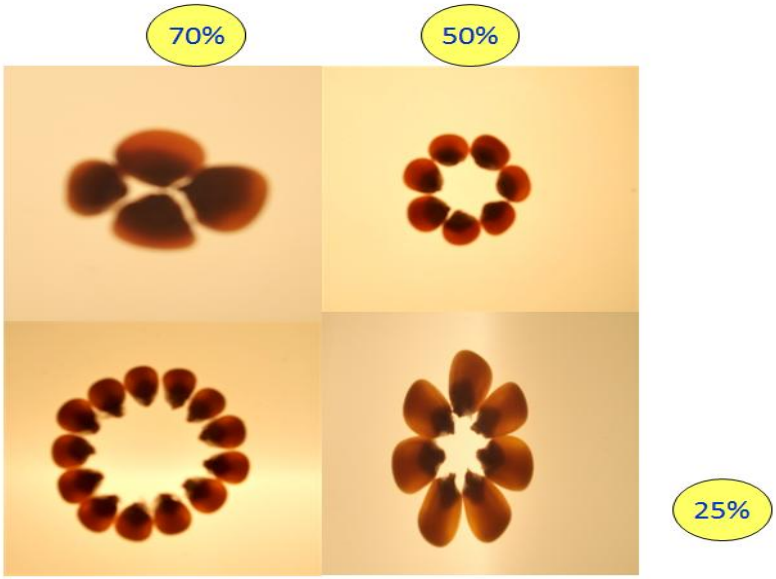
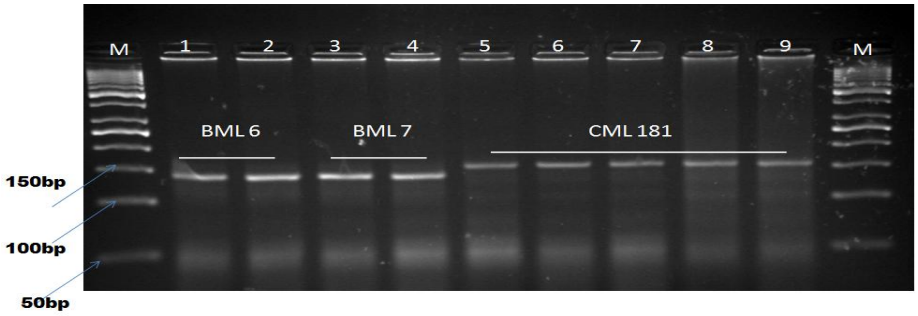
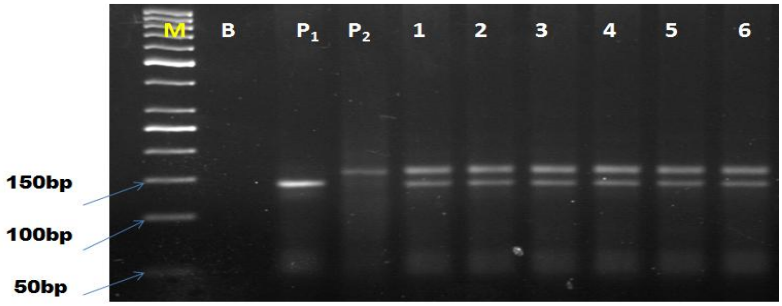


Plate.1 Parental polymorphism for *umc 1066*



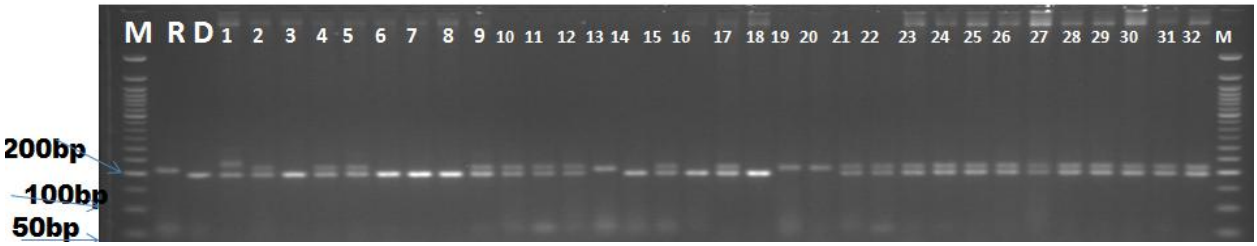
M: 50 bp ladder; 1-2: BML6; 3-4: BML7; 5-9: CML181

Plate.2 F_1 (BML7 X CML181) confirmation by *umc1066* marker



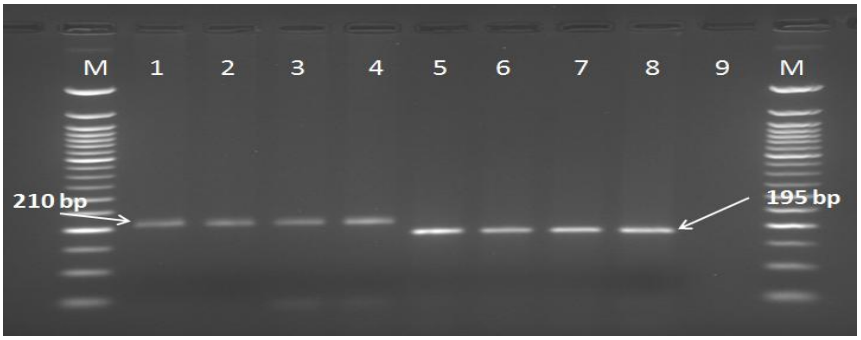
M: 50bp ladder; B: Blank; P_1: BML 7; P_2:CML181; 1-6 F_1 individuals

Plate.3 Recombinant screening of BML6-BC_1F_1 population with *bnlg2160*



M: 50bp ladder; R: BML6; D: CML181; 1-32: BML6-BC_1F_1 population

Plate.4 Screening of BML7-BC_1F_1 population with amino acid modifier *mmc0241*



M: 50 bp ladder; B: Blank; 1-4 : BML 7; 5-8 : CML 181.

Plate.5 Background screening of BML6-BC_1F_1 population with *bmc 1655*

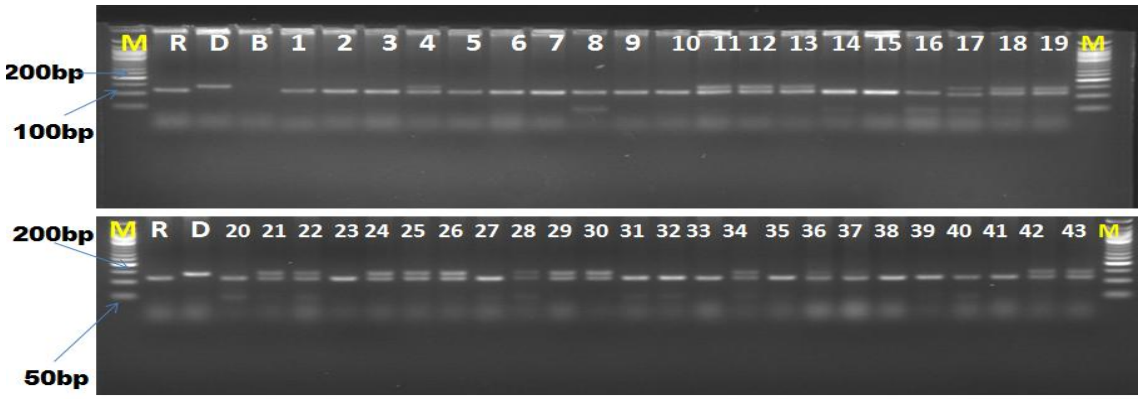
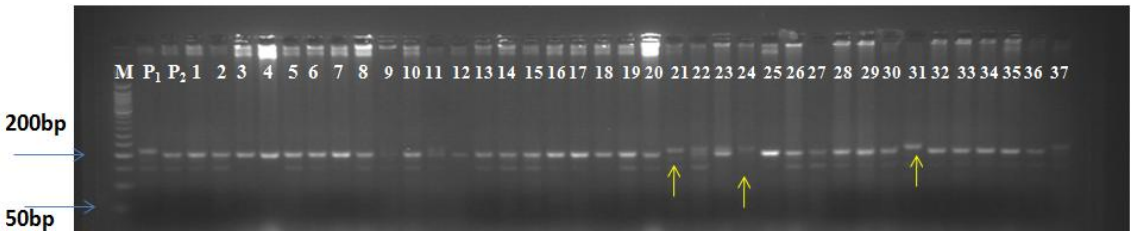
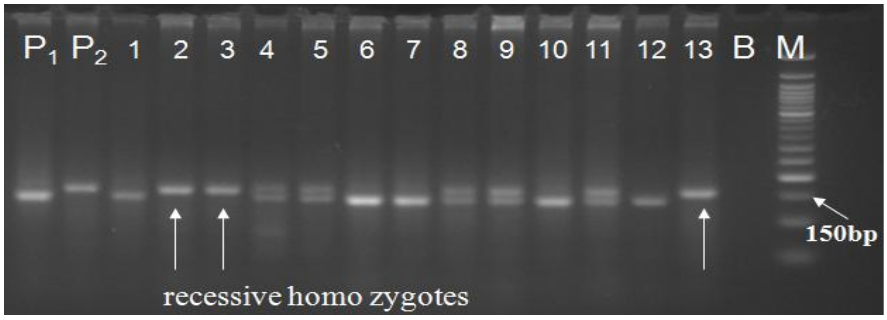


Plate.6 Recombinant screening of BML6-BC_2F_1 population with *bnlg2160*



M: 50bp ladder; P_1: BML 6; P_2: CML181; 1-37: BC_2 F_1 individuals

Plate.7 Foreground screening of BML7-BC_2F_2 population with *umc1066*



M: 50bp ladder; P_1: BML7; P_2: CML181; 1-13: BML7-BC_2F_2 individuals

Hybrid evaluation

The data was recorded on twelve different characters *viz.*, days to 50 per cent tasseling, days to 50 per cent silking, days to maturity, plant height (cm), ear height (cm), ear length (cm), ear girth (cm), number of kernel rows per ear, number of kernels per row, 100 kernel weights (g), grain yield per plant (g) and tryptophan content (%).(Unpublished).

Analysis of variance for *Rabi* 2013-14

The analysis of variance of the parents and the hybrids for yield and yield attributing characters are presented in Table 7. The mean sum of squares for genotypes (parents and hybrids) was highly significant for all the traits studied. While, the mean sum of squares for replications was found to be non-significant for all the traits except for days to

maturity, ear height and ear girth. There by indicating the existence of sufficient variability in the material studied (Fisher and Yates, 1967).

Analysis of variance for *Kharif* 2014

The analysis of variance of the parents and the hybrids for yield and yield attributing characters are presented in Table 8.

The mean sum of squares for genotypes (parents and hybrids) was highly significant for all the traits studied.

While, the mean sum of squares for replications was found to be non-significant for all the traits except for days to maturity, grain yield per plant and tryptophan content. There by indicating the existence of sufficient variability in the material studied (Fisher and Yates, 1967).

Grain yield per plant (g)

Among the QPM hybrids, maximum grain yield per plant was recorded by CB7-59 X CB6-36 (171.34 g) and was significantly superior when compared to the remaining hybrids for this trait. When compared with the standard check DHM 117, six hybrids were on par for grain yield per plant, but remaining six hybrids were recorded significantly superior mean values over DHM117(Unpublished).

Tryptophan content (%)

Among the hybrids, maximum kernel tryptophan content was recorded by CB6-36 X CB7-11 (0.88) and was significantly superior when compared to the remaining QPM hybrids for this trait. When compared with the standard check DHM 117, all hybrids were recorded significantly superior mean values over DHM 117.

Marker assisted selection for opaque 2 gene

In this study, distinct polymorphism could be observed between the normal and QPM inbred lines with two based SSR markers namely umc1066 and phi112. The QPM donor CML181 is an excellent Mexican donor according to Vivek *et al.,* 2007. The SSR marker umc1066, which is located within the middle of the opaque 2 gene. This umc1066 marker shown an excellent allelic polymorphism between donor inbred and recipient inbreds. This marker enables their potential utility in our marker assisted selection study. *umc1066* marker is capable of discriminating homozygotes from heterozygotes and is a good co-dominant marker similar type of results was reported in the earlier studies(Babu *et al.,* 2005; Ignjatovic-Micic *et al.,* 2009 and Singh *et al.,* 2012). Identification of heterozygotes and dominant homozygotes in the early stage prior to pollination in the backcross program.

Based on the earlier studies by Lee (1995), Riabut *et al.,* (2002), Babu *et al.,* 2005 and Gupta *et al.,* 2009, fore ground selection is for discriminating recurrent allele, donor allele and heterozygote, recombinant selection for reducing linkage drag with the help of closest flanking markers and background selection is for selecting high recurrent parent genome proportion in the population. Flanking marker analysis was done for reduction of the proportion on the carrier chromosome around the targ*et al.*,lele (linkage drag) and reduction of the donor genome on the non-carrier chromosomes to the maximum extent. In this study, we used closest flanking SSR markers near to *opaque 2* gene *bnlg1200* and *bnlg 2100*. It was already reported in the earlier study (Babu *et al.,* 2005)

Reduction of the proportion of the donor genome on the carrier chromosomes to the

maximum extent. In this study, we used flanking markers bnlg1200 and bnlg2100 based on the earlier studies by Babu *et al.,* 2005 and Ribaut *et al.,* 2002, it could be generalized that where a target gene is introduced for the first time presumably from either wild or unadapted germ plasm, flanking markers as close as 2cM is considered the ideal option, while in the transfer of the same target gene in subsequent phases from elite into elite line. In this study, although the donor QPM inbred line CML 181(F) is an elite line with proven combining ability, with orange kernels with 0.96% tryptophan content and belongs to late maturity group.

Background selection for recovery of maximum recurrent genome percentage (RPG): The objective of the whole genome selection is to recover rapidly maximum proportion of recurrent parent genome at non-target loci through background polymorphic markers distributed throughout the genome (Young and Tanksley 1989, Babu *et al.,* 2005 and Gupta *et al.,* 2015).

In the present investigation we fallowed a two generation marker based breeding program in which whole genome background selection at non-target loci was applied in the BC_1 and BC_2 generations. In our study a total of 153 polymorphic SSR markers used for background selection and our results was co inside with earlier reports.

Based on the marker aided background analysis, an individual with high proportion of RPG needs to be chosen and forwarded for next generations. We selected the first three individuals with highest proportion of recurrent parent genome for developing further BC_2F_2 families. These could be several modifications to the procedure we have fallowed in this study depending up on the requirements and objectives of each breeding scheme.

Phenotyping

The opaque 2 allele is recessive and the endosperm modifiers are polygenic with, no reliable molecular markers identified for kernel modification. Some of the SSR markers reported endosperm modifier and amino acid modifier were used in the present study. Phenotypic screening of the individual kernels under transmitted light and selection of kernels that have less than 25% opaqueness is the most convenient and efficient strategy employed in all the QPM breeding programs (Vasal *et al.,* 1993). We preferred kernels with less than 25%b opaqueness over 25-50% and more than 50% opaqueness due to the semi soft nature of endosperm and susceptibility to store grain pest and ear rots.

The tryptophan analysis of the kernel was carried out with Hernandez and Bates (1969) protocol. Phenotypic selection was exercised in the marker assisted opaque2 homozygous individuals in the three BC_2F_2 families for desirable yield and yield attributing characters such as days to 50% flowering, plant height, ear height, number of kernel rows, number of kernels per row test weight, tryptophan content etc.

The stable expression of these opaque2 traits could be confirmed through BC_2F_3 families; which reported the final converted QPM version of CB6 and CB7 showed 46% superiority with tryptophan concentration of above 0.83% in endosperm protein. Direct and reciprocal crosses between selected families of CB6 and CB7 inbreds were prformed to generate F_1 hybrids. In direct cross, CB6-36, CB6-39 as female parent and CB7-11, CB7-28 and CB7-59 as male parent and in reciprocal crosses it was vice-versa for producing QPM hybrid. Two cross combinations CB6-36 × CB7-28 and CB6-36 × CB7-59 exhibited relatively higher tryptophan content and on par grain yield per

plant compared to check DHM 117 which need to be further tested under multi-location trials prior to commercial exploitation.

The most important goal of QPM research has been to reduce malnutrition in target countries through direct human consumption, even though the impact as of now, has been great perceived. It is expected that greater impact will accrue out of development and dissemination of improved QPM hybrids worldwide.

The converted QPM inbreds (CBML6 and CBML7) developed in the present study provides an ideal platform for stacking number of nutritionally important traits such as enhanced Fe and Zn, low phytate (for increased bioavailability of nutrients) and high provitamin A. Considering the pace and the technological developments in genomics and proteomics, molecular breeding will be most leading option in future for stacking nutritionally important traits in maize.

Cross combination CB6-36 X CB7-28 recorded higher yield (170g/plant) which is on par with DHM117 hybrid with increased tryptophan (0.78%) content. New QPM inbreds developed (CB6-36, CB6-30, CB6-39, CB7-11, CB7-28 and CB7-59) are useful for further breeding programs for development of improved QPM hybrids.

We further need to develop high throughput, low cost, easily accessible phenotying/screening tools. Generating awareness among the society and building global and national partners for eliminating malnutrition will be future strengths for biofortified maize. Effective seed production and distribution systems, market strategies, strong partnership among research groups and need based government policies will help in solving the problems of many poor and undernourished people.

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