

# Marker-assisted breeding for introgression of *opaque-2* allele into elite maize inbred line BML-7

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**Abstract** Improvement of quality protein maize (QPM) along with high content of lysine and tryptophan had foremost importance in maize breeding program. The efficient and easiest way of developing QPM hybrids was by backcross breeding in marker aided selection. Hence, the present investigation aimed at conversion of elite maize inbred line BML-7 into QPM line. CML-186 was identified to be a donor variety as it revealed high-quality polymorphism with BML-7 for *opaque-2* gene specific marker *umc1066*. Non-QPM inbred line BML-7 was crossed with QPM donor CML-186 and produced F<sub>1</sub> followed by the development of BC<sub>1</sub>F<sub>1</sub> and BC<sub>2</sub>F<sub>1</sub> population. Foreground selection was carried out with *umc1066* in F<sub>1</sub>, and selected plants were used for BC<sub>1</sub>F<sub>1</sub> and BC<sub>2</sub>F<sub>1</sub> populations. Two hundred plants were screened in both BC<sub>1</sub>F<sub>1</sub> and BC<sub>2</sub>F<sub>1</sub> population with *umc1066* for foreground selection amino acid modifiers. Foreground selected plants for both *opaque-2* and amino acid modifiers were screened for background selection for BML-7 genome. Recurrent parent genome (RPG) was calculated for BC<sub>2</sub>F<sub>1</sub> population plants. Two plants have shown with RPG 90–93% in two generation with back cross population. Two BC<sub>2</sub>F<sub>2</sub> populations resulted from marker recognized BC<sub>2</sub>F<sub>1</sub> individuals subjected toward foreground selection followed by tryptophan estimation. The tryptophan and lysine concentration was improved in all the plants. BC<sub>2</sub>F<sub>2</sub> lines developed from hard endosperm kernels were selfed for BC<sub>2</sub>F<sub>2</sub> lines and

finest line was selected to illustrate the QPM version of BML-7, with 0.97% of tryptophan and 4.04% of lysine concentration in protein. Therefore, the QPM version of BML-7 line can be used for the development of single cross hybrid QPM maize version.

**Keywords** QPM · BML-7 · Marker-assisted selection · RPG

## Introduction

Among cereals after wheat and rice, maize is the important food crop in the world. It remained as a major staple food for millions of people in developing and developed nations. Structurally maize grain constitutes a small embryo and large endosperm with 90% of starch and 10% of proteins, respectively. Around 70% of this protein was composed of various alcohol soluble prolamins called zeins (Pirone et al. 2005). The distribution of zeins in maize is distinctive with a predominance of four types namely  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  zein. Nevertheless, the distribution of two essential amino acids, i.e., tryptophan and lysine remained deficient. Consumption of normal maize kernels by Children's and adults generally leads to nutritional disorders like marasmus and kwashiorkor (Vasal 2000). In Africa, Asia, Central, and South America millions of people rely on maize for their daily food supplement. Even high content of maize in diet produces a condition known as wet malnutrition, i.e., the person receives sufficient calories of energy but suffers from malnutrition due to lack of protein content.

The lysine and tryptophan levels were better in quality protein maize (QPM) than the conventional maize, whereas maize *opaque-2* genotype resulted in lower yields due to its soft-chalky kernels, making it more prone to insects and

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fungal attacks. In addition, taste and appearance of kernel dissatisfied the consumers, who ultimately rejected the usage of enhanced-protein varieties in the market. However, by means of mutant *opaque-2(o2)* variety, breeders developed maize with rich in lysine and tryptophan by means of normal kernels in vitreous appearance (Krishna et al. 2012; Ortega and Bates 1983). More precisely, CIMMYT breeders improved high lysine maize from *opaque-2* genotypes by selecting phenotypic characteristics like normal endosperm texture and increased levels of lysine and tryptophan. These modified *opaque-2* maize lines were designated as “Quality Protein Maize” lines (Vasal et al. 1980). Due to advantages like vitreous/hard nature of endosperm, resistance to insect damage, kernel texture, and high yield, there was a huge and significant need to develop QPM hybrids. For this purpose, molecular tools can be used to identify the high lysine and tryptophan lines after screening the potential germplasm that can help the breeder in constructing hybrids using Marker assisted selection. QPM is a genotype in which *opaque-2* mutant gene has been integrated along with associated modifiers or enhancers. Modifier genes help in converting hard endosperm to soft endosperm while enhancers confer higher tryptophan and lysine content. Thus, QPM breeding program requires manipulation of three different genetic systems (Krivanek et al. 2007).

Marker-assisted advanced backcross breeding can greatly accelerate the introgression of *opaque-2* allele into normal maize. For genotype characterization, DNA-based molecular markers hold numerous advantages over the phenotype markers. Simple Sequence Repeat (SSR) markers have efficient role among DNA-based markers because of their good reproducibility, high polymorphic, and reliable nature (Gupta et al. 1996). The SSR loci consist of 2 to 6 base pair tandem repeats. The *opaque-2* trait was expressed in recessive state where mutant kernels have a soft textured starchy endosperm with low density. While the mutation in *opaque-2* alters the endosperm texture by decreasing  $\alpha$ -zeins content which, in turn, increases the lysine content. Breeders have systematically transferred the modifier genes into *opaque-2* germplasm to develop normal looking maize with high lysine. Molecular tools have facilitated the breeders to transfer the *opaque-2* along with modifiers into elite maize inbreds for construction of the hybrid. The present investigation for conversion of elite maize inbred line BML-7 into quality protein maize with high lysine and tryptophan using marker-assisted breeding.

## Materials and methods

The QPM germplasm line CML-186 collected from VPKAS (Vivekananda Parvatiya Krishi Anusandhan Sansthan), Almora. Parental inbred line BML-7 was used

as the recipient for the breeding program. For identification of polymorphism between donor and recipient inbred lines DNA, *opaque-2* gene specific SSR markers viz, *umc 1066*, *Phi057* and *Phi112* were used. Donor and recipients were also screened with six amino acid modifier-linked SSR markers, viz, *mmc0241*, *umc1216*, *phi072*, *bnlg1633*, *bmc1382*, and *phi075*. Simple Sequence Repeats (SSRs) marker information was taken from maize database (<http://www.maizegdb.org>).

## DNA isolation

Leaf sampling was done by taking 2 g leaf from 3-week-old seedlings of each genotype. Sample was wrapped in marked aluminum foil and then frozen in liquid nitrogen before storing in  $-80^{\circ}\text{C}$ . Pre-chilled plant leaves (2 g) at  $-80^{\circ}\text{C}$  were crushed in liquid nitrogen with a mortar and pestle. A pre-heated ( $65^{\circ}\text{C}$ ) extraction buffer (2 ml) containing 100 mM Tris-HCl (pH:8); 1.4 mM NaCl; 20 mM EDTA; 0.1% 2-mercaptoethanol GR (Merck); and 2% CTAB powder was added to crushed leaf material. The mixture was incubated at  $65^{\circ}\text{C}$  for 45 min in water bath (Lab Tech) followed by addition of equal volume of Chloroform:Isoamyl alcohol (24:1) (Thermo Fisher Scientific India Pvt. Ltd) mixture and the homogenate incubated for 15 min at room temperature. Centrifugation was done for 10 min at a speed of 10,000 rpm in a centrifuge machine (Eppendorf) at room temperature. The upper aqueous phase was again removed carefully into a new tube and an equal volume of ice-cold isopropanol was added to this to precipitate the DNA. This mixture was centrifuged at maximum speed for 10 min at room temperature to collect the DNA pellet at the bottom of the tube. The DNA pellet was washed by adding 2 ml 70% ethanol and centrifuged at maximum speed of 10,000 rpm for 2 min. The pellet was dried in a vacuum centrifuge (Speed Vac) and re-suspended in 100  $\mu\text{l}$  sdH<sub>2</sub>O (sterile distilled water).

The isolated DNA sample was quantified with spectrophotometer in the photometric mode which was adjusted with wavelengths set at 260 and 280 nm. Normalization of DNA samples was done to equalize the concentrations of all the DNA samples that were to be run in PCR.

## Polymerase chain reaction (PCR)

DNA sample (50 ng) was used per PCR to which 23  $\mu\text{l}$  master mixture (PCR reaction mix.) was added. The PCR mix was centrifuged at 1000 rpm for 1 min and loaded in a 96-well thermal cycler of PCR (Eppendorf). The program comprises of the initial denaturation at  $94^{\circ}\text{C}$  for 4 min, which was further followed by 35 cycles  $94^{\circ}\text{C}$  for 1 min,

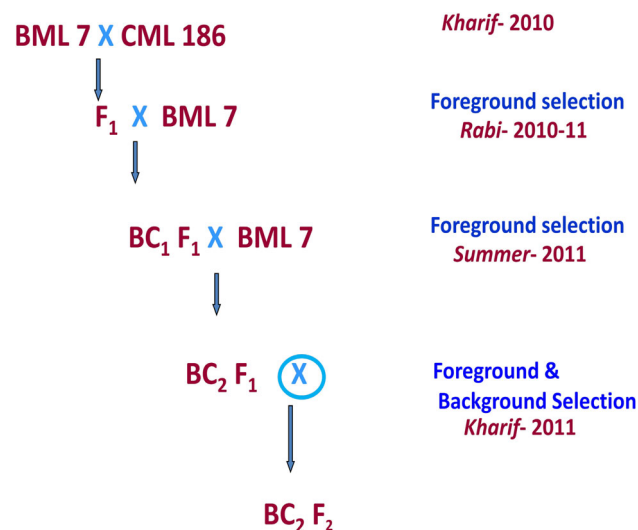
55–63 °C for 30 s. and 72 °C for 1 min. Final extension was carried out at 72 °C for 7 min, and maintained at 4 °C for ever. The amplified products were used for electrophoresis.

### Marker-assisted backcross breeding

The advanced backcross breeding program was carried out at the Maize Research Centre (MRC), ARI, Rajendranagar, and Hyderabad. BML-7 inbred line was used as female parent and QPM line CML-186 used as male parent. Backcross populations were developed from the crosses between the BML-7 × CML-186. Crosses were affected during *Kharif-2010* for F<sub>1</sub> Seed production. F<sub>1</sub> seed was subjected to foreground selection with *opaque-2*-specific SSR marker *umc1066*. These foreground selected F<sub>1</sub> plants were backcrossed with recurrent parent to produce BC<sub>1</sub>F<sub>1</sub> during *rabi-2010*. The BC<sub>1</sub>F<sub>1</sub>s were then backcrossed with recurrent parent (BML-7) to produce BC<sub>2</sub>F<sub>1</sub> during *summer-2010*. BC<sub>2</sub>F<sub>1</sub> plants were selfed to produce BC<sub>2</sub>F<sub>2</sub> plants (Fig. 1).

### Foreground selection

Fore ground selection for *opaque-2* gene at backcross population is carried out with gene-specific marker *Umc1066*. Two hundred plants were screened for foreground selection in each F<sub>1</sub>, BC<sub>1</sub>F<sub>1</sub>, and BC<sub>2</sub>F<sub>1</sub> populations, respectively. Foreground selection for BML-7 × CML-186 cross BC<sub>2</sub>F<sub>1</sub> population with amino acid modifier linked SSR marker *bnlg1633*.



**Fig. 1** Marker-assisted advanced backcross breeding program for conversion of BML-7

### Background selection

Whole-genome background screening was done for recurrent parent BML-7 using 750 SSR markers. Back ground selection was carried out both BC<sub>2</sub>F<sub>1</sub> population with identified 160 SSR markers. The details of SSR markers and their chromosome number were given in Table 1.

### Estimation of tryptophan and lysine content

In biochemical analysis, a random sample of 20 seeds as representative of each ear was taken for tryptophan analysis with three replications. Total protein was estimated for both the parents (BML-7 and CML-186) and selected BC<sub>2</sub>F<sub>2</sub> plants. Tryptophan content was estimated by high-performance liquid chromatography (HPLC) method. HPLC (SCHIMADZU, Tokyo) with reversed-phase C18 column was used for analysis. Lysine content was calculated by a correlation with Tryptophan concentration according to the equation (Babu 2015).

$$\text{Lys} = 0.3601 + (4.0745 \times \text{Trp}).$$

### Results and discussion

Both the parents HPLC results indicated that BML-7 contains lysine and tryptophan 2.2 and 0.41% respectively. CML-186 had lysine and tryptophan content with 4.04, 0.98%, respectively (Table 2). CML-186 was showed good polymorphism with BML-7. Hence, CML-186 was used as donor for BML-7 marker-assisted conversion (Fig. 2).

### F<sub>1</sub> population

Cross was made between BML-7 (Non QPM inbred) and CML-186 (QPM donor) during *kharif*-2010. Recurrent parent BML-7 as a female parent and CML-186 (donor) as male parent to produce F<sub>1</sub> seeds. Recurrent parent BML-7 was used as a female and donor parent CML-186 was used as male parent which is a QPM line to produce F<sub>1</sub> seeds.

Foreground selection on F<sub>1</sub> (BML-7 × CML-186) was carried out with *opaque-2* gene-specific SSR marker *umc1066*. All the F<sub>1</sub> populations exhibited heterozygote for gene of interest (Fig. 3). Since SSR is a co-dominant marker, we could differentiate between heterozygous and homozygous inbred lines. Two bands with equal intensity in a genotype were considered as heterozygous, whereas in case of variable intensities and of superimposed conditions, it was considered as heterogeneous. In heterogeneous condition, band with higher intensity was used for scoring and of lower intensity was rejected.

Transfer of recessive genes by conventional breeding needs further selfing over generations next to every

**Table 1** SSR markers used for background selection of BML-7 genome

S. No	SSR	Chr. No.	S. No	SSR	Chr. No.	S. No	SSR	Chr. No.	S. No	SSR	Chr. No.
1	phi097	1	39	umc1844	3	77	phi452693	6	115	phi061	9
2	bnlg1014	1	40	umc1641	3	78	umc1015	6	116	bnlg1687	9
3	bnlg1179	1	41	bnlg1754	3	79	umc1014	6	117	phi061	9
4	bnlg147	1	42	bnlg1098	3	80	bnlg1136	6	118	umc1519	9
5	umc2204	1	43	umc2176	4	81	umc 1063	6	119	phi016	9
6	phi001	1	44	umc2206	4	82	umc1490	6	120	bnlg1884	9
7	umc1545	1	45	umc1821	4	83	phi070	6	121	bnlg1159	9
8	bnlg2086	1	46	umc1117	4	84	umc1490	6	122	bnlg 1209	9
9	umc2025	1	47	umc1382	4	85	umc1653	6	123	umc1357	9
10	bnlg1661	1	48	umc1031	4	86	umc2059	6	124	bnlg1091	9
11	umc1147	1	49	phi 079	4	87	umc1378	7	125	umc1310	9
12	bnlg1643	1	50	bnlg1137	4	88	bnlg1292	7	126	dupssr 28	9
13	umc1111	1	51	bnlg252	4	89	umc1159	7	127	bnlg1375	9
14	umc2245	2	52	bnlg1937	4	90	umc2392	7	128	bnlg1527	9
15	bnlg1297	2	53	umc1574	4	91	umc1567	7	129	umc1137	9
16	bnlg1297	2	54	umc1631	4	92	bnlg339	7	130	umc1675	9
17	bnlg2042	2	55	umc1308	5	93	umc1295	7	131	umc1293	9
18	umc1108	2	56	umc1423	5	94	umc1708	7	132	umc1380	10
19	bnlg104	2	57	bnlg1006	5	95	bnlg2259	7	133	umc1153	10
20	umc1259	2	58	umc1097	5	96	umc1708	7	134	umc1337	10
21	mmc0402	2	59	bnlg1836	5	97	phi 116	7	135	umc1576	10
22	bnlg1184	2	60	phi096	5	98	bnlg1194	8	136	bnlg210	10
23	umc1042	2	61	umc1389	5	99	umc1913	8	137	bnlg1079	10
24	umc1798	2	62	umc1192	5	100	phi119	8	138	bmc1655	10
25	bnlg1233	2	63	umc1822	5	101	umc1868	8	139	bnlg640	10
26	umc2144	2	64	umc1800	5	102	umc1627	8	140	umc1077	10
27	umc1230	2	65	umc1822	5	103	umc1141	8	141	umc1077	10
28	umc1736	2	66	bnlg278	5	104	umc1005	8	142	umc2163	10
29	umc1394	3	67	umc1646	5	105	phi015	8	143	bnlg1526	10
30	umc2369	3	68	bnlg1043	6	106	umc1272	9	144	bnlg1506	10
31	phi193329	3	69	umc1143	6	107	umc1040	9	145	umc1506	10
32	bmc2136	3	70	umc112	6	108	bnlg1724	9	146	umc1477	10
33	umc1158	3	71	umc2059	6	109	phi028	9	147	bnlg1185	10
34	dupssr 23	3	72	umc2312	6	110	bnlg1810	9	148	umc1993	10
35	umc2271	3	73	umc 1376	6	111	bnlg1401	9	149	bnlg2122	10
36	umc1404	3	74	mmc 024	6	112	umc2213	9	150	umc1344	10
37	umc1286	3	75	umc1656	6	113	umc1507	9	151	umc1038	10
38	umc1320	3	76	umc1656	6	114	bnlg244	9			

backcross. This procedure requires extensively long duration for improving best variety for profitable breeding purposes. Marker-aided background selection may aid in recovering the same genotype of recurrent parent within three generations which would not be achieved by the conventional phenotypic selections which require six-to-seven generations. Hence, MAS centered on SSR markers designed for conversion of normal maize lines into QPM was known to be efficient, simple, rapid, and cost-effective

method as a complementary to prevailing breeding protocols (Dreher et al. 2000).

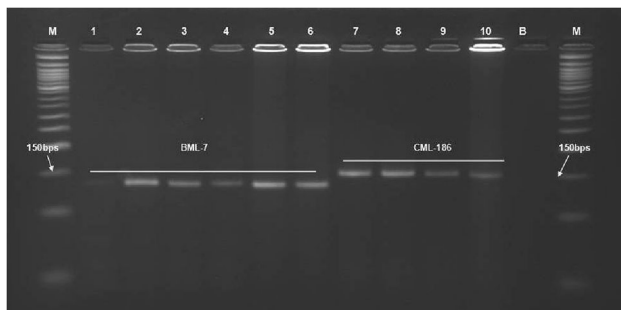
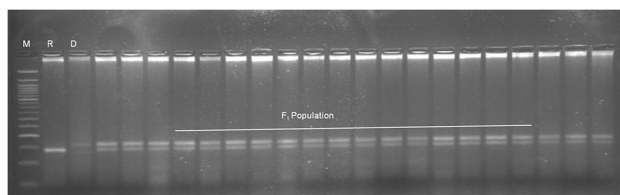
### BC<sub>1</sub>F<sub>1</sub> population

F<sub>1</sub> (BML-7 × CML-186) seeds were sown as per year to row method during *rabi* 2010–11. These F<sub>1</sub> plants were crossed with recurrent parent BML-7 eventually to produce BC<sub>1</sub>F<sub>1</sub> seeds. Two hundred BC<sub>1</sub>F<sub>1</sub> [(BML-7 × CML-

**Table 2** Agronomic trait features of the recurrent parent (BML-7), donor parent (CML-186), and converted QPM version of BML-7 (BC<sub>2</sub>F<sub>2</sub> line)

Agronomic or biochemical trait	BML-7 (recurrent parent)	CML-186 (QPM donor parent)	Developed QPM version of BML-7 (BC <sub>2</sub> F <sub>2</sub> line)
Plant height (cm)	168 ± 8	160 ± 12	167 ± 7
Ear height (cm)	65 ± 4.46	67 ± 3.41	65 ± 4.92
Days to 50% silking	54–56	55–59	53–55
Days to 50% pollen shed	57–60	58–63	58–60
Maturity (range in numbers of days—seed to seed)	95–100	100–105	95–100
Maturity group	Medium duration	Medium duration	Medium duration
Total protein content (%)	8.6 ± 0.43	7.5 ± 0.53	8.7 ± 0.61
Total tryptophan (%)	0.41 ± 0.02	0.98 ± 0.13	0.97 ± 0.24
Total lysine (%)	2.2 ± 0.21	4.04 ± 0.12	4.04 ± 0.16
Kernel hardness	Hard	Hard	Hard
Grain yield (g/plant)	52.84 ± 2.36	58.24 ± 4.58	53.48 ± 1.25

Mean ± standard error

**Fig. 2** Identification of potential QPM donor for non QPM recipient BML-7 by SSR marker *umc1066*. *M* 50 bp ladder, *1–6* BML-7, *7–10* CML-186, *B* blank**Fig. 3** F<sub>1</sub> (CML-186 × BML-7) population screened for foreground selection with *umc 1066*. *M* 50 bp ladder, *R* recurrent parent, *D* donor parent

186) × BML-7] plants were screened for *opaque-2* gene. Out of 200 plants, 98 plants was shown heterozygote condition (Fig. 4).

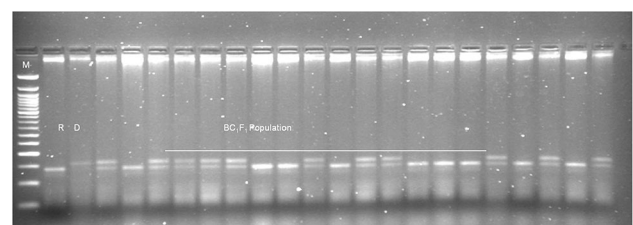
### BC<sub>2</sub>F<sub>1</sub> population

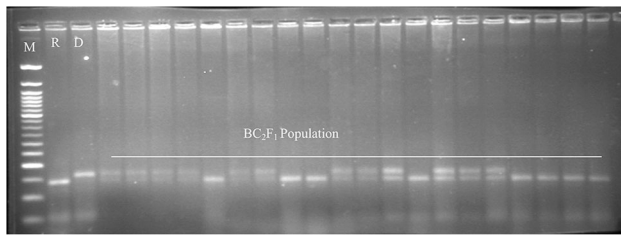
BC<sub>1</sub>F<sub>1</sub> seeds were sown along with recurrent inbred line BML-7 during summer 2011. Foreground selected 98 plants were back crossed with recurrent parent BML-7

(Fig. 5). Two hundred BC<sub>2</sub>F<sub>1</sub> plants were screened with gene specific marker *umc1066*. Out of 200 plants, 90 plants exhibited heterozygous loci (Fig. 6). These results confirmed that SSR markers were trustworthy for opting QPM kernels and may even be extended further for recognizing completely modified QPM kernels. Identified 90 heterozygous plants were screened for amino acid modifier marker *bnlg1633*. Out of 90 plants, 65 plants were shown heterozygous for amino acid modifier marker *bnlg1633* (Fig. 5). Though a huge number of SSR loci were identified and used for parental polymorphism, sufficient polymorphic loci were not detected, as both parental lines were isogenic but then differ in tryptophan content (Babu 2015).

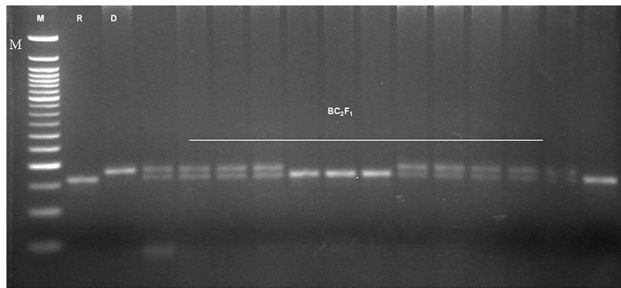
### Whole genome background selection

A total of 750 SSR markers were screened which are distributed all over the 10 chromosomes for polymorphism between BML-7 (Recipient) and CML-186 (Donor) for background selection. Out of 750 SSR markers, 151 SSR

**Fig. 4** Foreground selection of BC<sub>1</sub>F<sub>1</sub> [(CML-186 × BML-7) × BML-7] population with *umc 1066* (*M* 50 bp ladder, *R* recurrent parent, *D* donor parent)



**Fig. 5** Foreground selection of  $BC_2F_1$  ( $BC_1F_1 \times BML-7$ ) population with *umc 1066* (*M* 50 bp ladder, *R* recurrent parent, *D* donor parent)



**Fig. 6** Foreground selection of  $BC_2F_1$  ( $BC_1F_1 \times BML-7$ ) population with amino acid modifier *bnlg1633* (*M* 50 bp ladder, *R* recurrent parent, *D* donor parent)

markers were shown polymorphism between donor (CML-186) and recipient (BML-7) (Table 1).

It has been revealed from simulation studies that cumulative sum of markers per non-carrier chromosome was essential from primary generations. In the course of applied MAS program, a great attention has to be taken to avoid sampling error and exaggerated approximations of recurrent parent genome connected with less integer of marker data points (Hospital et al. 1992). Out of 65  $BC_2F_1$  plants, three plants had shown 90.47, 92.8, 5 and 90.48% Recurrent Parent Genome (RPG); three plants had shown 86.9, 85.71, and 88.9% RPG and remaining plants have shown 80–85% RPG. Plants which exhibited >80% RPG were selfed, to produce  $BC_2F_2$  plants. The whole-genome background selection was to improve quickly maximum ratio of recurrent parent genome (RPG) on non target loci through DNA-based markers that were dispersed equally all over the genome (Hospital et al. 1992; Frisch et al. 1999; Visscher 1996; Young and Tanksley 1989). Simulation studies have showed that application of background selection in the present and later generation along with that of foreground selection in every back cross generations may, perhaps, be efficient (Frisch et al. 1999; Ribaut and Hoisington 2002). In the current investigation, twofold generation-based marker breeding program where whole-genome background selection is on non-target loci remained functional merely in the  $BC_2$  generation. In this study, we selected markers aligned on chromosomes, especially SSR consensus map of maize genome accessible in public domain (<http://www.maizegdb.org>).

Marker-assisted breeding (MAB) is one of the most predictable and frequently cited tools used for indirect selection in breeding programs (Semagn et al. 2006). The rapid inbred line conversion approaches bring together the noteworthy features of both marker-assisted and phenotypic selection methods like fixing the target trait from large segregating generation and recovery of maximum extent of recurrent parent genome (RPG) within two back cross generations providing abundant scope for possible desired agronomic and biochemical traits. Recurrent genotype recovery can be improved with the help of molecular markers. By the advent of molecular marker technology in third generation, detection of PCR amplified products by SNPs and fluorescent centered dyes, viz, Taqman probe and molecular beacons may assist and enhance the power and efficiency of genotyping much better in the present and future generations (Salvi et al. 2001; Tyagi et al. 1998). Results unveiled 4.04% of lysine and 0.97% of tryptophan content by  $BC_2F_2$  plants. Hence, QPM version of BML-7 can be used as parent for single cross hybrids, while duration of time required for a conventional breeding program can be highly reduced with the approach of marker-assisted selection.

#### Compliance with ethical standards

**Conflict of interest** Authors state no conflict among them.

#### References

- Babu PK (2015) Mapping QTLs for opaque2 modifiers influencing the tryptophan content in quality protein maize using genomic and candidate gene-based SSRs of lysine and tryptophan metabolic pathway. *Plant Cell Rep* 34:37–45
- Dreher K, Khairallah M, Pandey S (2000) Is marker assisted selection cost effective compared to conventional plant breeding methods? The case of Quality Protein Maize. International Consortium on Agricultural Biotechnology Research (ICABR). 'The economics of Agricultural Biotechnology'. Held in Revello, Italy, 24–28
- Frisch M, Bohn M, Melchinger AE (1999) Comparison of selection strategies for marker-assisted backcrossing of a gene. *Crop Sci* 39:1295–1301
- Gupta PK, Balyan HS, Sharma PC, Ramesh B (1996) Microsatellites in plants: a new class of molecular markers. *Curr Sci* 70:45–54
- Hospital F, Chevalet C, Mulsant P (1992) Using markers in gene introgression breeding programs. *Genetics* 132:1199–1210
- Krishna MSR, Chinnababunaik V, Sokka Reddy S (2012) Assessment of genetic diversity among the QPM lines using SSR markers. *Afr J Biotech* 11(98):16427–16433
- Krivanek AF, De Grote H, Gunaratna NS, Diallo AO, Friesen D (2007) Breeding and disseminating quality protein maize (QPM) for Africa. *Afr J Biotech* 6:312–324
- Ortega EI, Bates LS (1983) Biochemical and agronomic studies of two modified hard-endosperm *opaque-2* maize (*Zea mays* L.) populations. *Cereal Chem* 60:107–111
- Pirana R, Hartings H, Lauria M, Rossi V, Motto M (2005) Genetic control of endosperm development and of storage products accumulation on maize seeds. *Maydica* 50:515–530

- Ribaut JM, Hoisington D (2002) Stimulation experiments on efficiencies of gene introgression by back crossing. *Crop Sci* 42:557–565
- Salvi ND, George L, Eapen S (2001) Plant regeneration from leaf base callus of turmeric and random amplified polymorphic DNA analysis of regenerated plants. *Plant Cell*. 66:113–119
- Semagn K, Bjornstad A, Ndjiondjop MN (2006) An overview of molecular markers methods for plants. *Afr J Biotechnol* 5:2540–2568
- Tyagi S, Bratu DP, Kramer FR (1998) Multicolor molecular beacons for allele discrimination. *Nat Biotechnol* 16:49–53
- Vasal SK (2000) Quality protein maize story. Proceedings of workshop on improving human nutrition through agriculture. The role of international agricultural research. IRRI, Los Baños, pp 1–16
- Vasal SK, Villegas E, Bjarnason M, Gelaw B, Goertz P (1980) Genetics modifiers and breeding strategies in developing hard endosperm *opaque2* materials. In: Pollmer WG, Phipps RH (eds) Improvement of quality traits of maize for grains and silage use. Nijhoff, The Hague, pp 37–73
- Visscher PM (1996) Proportion of the variation in genetic composition in backcrossing programs explained by genetic markers. *J Hered* 87:136–138
- Young ND, Tanksley SD (1989) Analysis of the size of chromosomal segments retained around Tm-2 locus of tomato during back cross breeding. *Theor Appl Genet* 77:353–359