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Introgression of the low phytic acid locus (*lpa2*) into elite maize (*Zea mays* L.) inbreds through marker-assisted backcross breeding (MABB)

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Abstract Phytic acid (PA) is an important antinutritional component in maize that affects the availability of major micro-nutrients like di- and multivalent mineral cations like iron (Fe) and zinc (Zn). The long-term consumption of maize as a staple food crop leads to micronutrient malnutrition especially iron and zinc deficiency in the human population. In addition, it also acts as a storehouse of a major part of mineral phosphorous (P), approximately 80% of the total P stored as phytate P is not available to monogastric animals like humans and poultry birds, and it gets excreted as such, leading to one of the major environmental pollution called eutrophication. Of the various low phytic acid (*lpa*) mutants, *lpa2-2* generated through mutagenesis reduces PA by 30%. BML 6 and BML 45, the parents of the popular maize hybrid DHM 121 with high PA were selected to introgress *lpa2-2* through marker-assisted backcross breeding (MABB). The percent recurrent parental genome (RPG) in the selected BC_2F_2 plants ranged from 88.68 to 91.04% and 90.09–91.51% in the genetic background of BML 6 and BML 45, respectively. Based on the highest percentage of RPG, best five BC_2F_2 plants, *viz*., #3190, #3283, #3230, #3263 and #3292 with RPG 88.68–91.04% in the genetic background of BML 6 and #3720, #3776, #3717, #3828 and #3832 with RPG 90.09–91.51% in the genetic background of BML 45 were advanced to BC_2F_3. The newly developed near-isogenic lines (NILs) possessed low phytate content (2.37 mg/g in BML 6 and 2.40 mg/g in BML 45) compared to 3.59 mg/g and 3.16 mg/g in recurrent parents BML 6 and BML 45, respectively thereby reducing the phytate by an average of 34 and 24 per cent, respectively. These newly developed progenies were similar to their recurrent parents for various morphological traits. These inbreds assume great significance in alleviating Fe and Zn deficiencies in worldwide.

Keywords Inorganic phosphorus · Maize · Marker assisted backcross breeding · Near-isogenic lines · Phytic acid

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Introduction

The diverse uses of maize (*Zea mays* L.) as food, feed and fodder draw attention to its nutritional importance. Maize is largely used to meet the energy requirement of animals and human beings. In addition to energy, it also serves as a source of several micro-nutrients like minerals and vitamins (Rouf Shah et al. 2016). However, the bioavailability of some of the major essential micro-nutrients like iron, zinc, magnesium and phosphorous gets hindered due to the presence of phytic acid (PA) in maize grain (Raboy 2020). Thus, PA is considered an antinutrtional factor in maize. Besides maize, PA [myo-inositol-1, 2, 3, 4, 5, 6-hexakisphosphate {InsP(6)}] has been considered as major antinutritional in many other crops like barley, wheat, soybean etc. The strong negative charge due to phosphate backbone leads to chelation of positively charged di- and multi-valent cations that affect their bioavailability. The long-term consumption of maize as a staple food crop either by monogastric animals (poultry, fish, and swine) and human beings which lack phytase enzyme leads to micro-nutrient malnutrition in the population (Brinch-Pedersen et al. 2002). On the contrary, the phosphate backbone of the PA does not get released into the digestive system and it gets excreted as such leading to environmental pollution called eutrophication.

The discovery of *opaque* mutant followed by its biochemical analysis has opened up an opportunity to improve the nutritional value of maize but that could not able to address the reduction of PA content to improve the micro-nutrient status and availability of phosphorous. However, efforts were initiated around the late 1980s or early 1990s to create novel variants with low-phytic acid (LPA) trait through mutagenesis in different crops (Raboy et al. 2000; Wilcox et al. 2000,). In maize, it was successfully demonstrated that the transfer of LPA mutants, viz., *lpa1*, *lpa2* and *lpa3* which were generated through mutagenesis, into different genetic backgrounds have reduced the PA by 66%, 50% and 50%, respectively as compared to that of wild-type kernels (Raboy 2002, 2007; Shi et al. 2005). The *lpa2-2* mutants showed a 30% reduction in PA content and a threefold increment of inorganic phosphate (P_i) when compared to the wild type (Shi et al., 2003).

The availability of linked molecular markers to LPA mutants can be effectively used to transfer LPA mutant alleles into elite parental inbred lines of popular maize hybrids through marker-assisted backcross breeding (MABB) approach. In the present study, the parental lines (BML 6 and BML 45) of maize hybrid DHM 121, widely cultivated in north eastern plains zone (NEPZ) and central western zone (CWZ) of India, having high PA and low P_i content were chosen to introgress the *lpa2* allele through MABB. The study is more relevant to address the nutritive value of the hybrid, to increase the bioavailability of P_i and other nutritionally important micro-nutrient mineral cations like Fe and Zn by reducing the PA content. The near-isogenic lines (NILs) with low PA developed through MABB were evaluated for low phytate content as well as for agronomic performance to identify the NILs with low phytate along with comparable performance with that of recurrent parents namely BML 6 and BML 45.

Materials and methods

Plant materials

Well-adapted tropical maize inbred lines, BML 6 and BML 45 with superior agronomic performance were used as recurrent parents to transfer low phytate traits through MABB. BML 6 and BML 45 are the male parent and female parent of widely cultivated (in NEPZ and CWZ of India) maize hybrid DHM 121, a medium duration (seed to seed 90–95 days) single cross maize hybrid released and notified for commercial cultivation in north-estern plains zone and central-western zone of India with yield level of 6 t/ha across two zones. The LPA mutant maize inbred, LPA 2 carrying *lpa2* gene, obtained from ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora was used as donor line. The pedigree and other details of the genetic material used in the present study are given in Table 1. The selected NILs developed through MABB carrying the *lpa2* gene were evaluated for agronomic performance as well as for PA and P_i contents.

Deoxyribonucleic acid (DNA) extraction and polymerase chain reaction (PCR) analysis

The leaf samples from 12–15 days old maize seedlings were collected from the experimental field. The modified Cetyltrimethyl ammonium bromide (CTAB)

Table 1 Details of genetic material used in the present study

S.No	Inbred	Pedigree	Grain types
1	BML 6	SRRL 65-B96-1–1-2- #—2–2-1-⊗-1–1-⊗b-⊗b	Normal, yellow
2	BML 45	Derived from cross NH 6240 × BH 1620	Normal, yellow
4	LPA 2	Low phytate mutant line	Normal, yellow

protocol (Dellaporta et al. 1983) was used for DNA isolation. *lpa2* gene specific marker and a set of polymorphic simple sequence repeats (SSRs) selected from maize GDB (www.maizegdb.org) were used for PCR analysis. PCR reaction mixture for amplification was 20 µl, which consisted of (i) 4.0 µl PCR buffer, (ii) 11.2 µl dd H_2O (Molecular biology grade), (iii) 1 µl each of genomic DNA (20 ng/µl) and (iv) 1.0 µl Forward & Reverse primers each (10 pmol/reaction) (v) 0.2 µl Taq polymerase (vi) 0.4 µl deoxynucleotide triphosphates (dNTPs) (200 µM) and (vii) 1.2 µl $MgCl_2$ (1.5 mM). The amplified products were resolved in 4% metaphor gel at 120 V for 1.5 to 2 h, and alleles were scored manually using a DNA ladder (50 bp).

Foreground selection for *lpa2* allele

In the current study, a co-dominant SSR marker 'umc2230' closely linked to *lpa2* locus (0.4 cM) showed polymorphism between the donor and recurrent parents and it was used for foreground selection for the *lpa2* allele. The forward and reverse primer sequences used for PCR amplification of umc2230 were 5'-AACGCGACGACTTCCACAAG-3' and 5'-ACACGTAATGTCCCTACGGTCG-3', respectively. The forward and reverse primers were used to screen the low phytate plants in the backcross population by amplifying genomic DNA fragments through PCR.

Background selection using SSR markers

A set of 450 SSR markers distributed throughout the maize genome and covering all the 10 chromosomes at the regular intervals were selected from the maize genome database (www.maizegdb.org). These markers were subjected to a polymorphism study to identify the polymorphism between the donor and recurrent parents. Around 100 identified polymorphic markers were selected and used in background selection in BC_1F_1, BC_2F_1, and BC_2F_2 generation to identify the progenies with high recurrent parental genome (RPG). The list of background markers used is shown in Supplementary Table 1.

Marker-assisted backcross breeding (MABB) program

Recurrent parents, BML 6 and BML 45, and donor parent (LPA 2) were raised during *rabi* 2015–16 at Winter Nursery Centre, ICAR-Indian Institute of Maize Research (ICAR-IIMR), Rajendranagar, Hyderabad, Telangana, India ($17^0$32′64″70 N, $78^0$39′84″92E). F_1 crosses between recurrent and donor parents were developed. The F_1s were raised along with parents during *Kharif* 2016 at the experimental site of Unit Office, ICAR-Indian Institute of Maize Research (IIMR), Pusa Campus, New Delhi, India ($28^0$63′96″09 N, $77^0$15′11″64E). The F_1s were confirmed both with SSR marker linked to the gene of interest, umc2230 for the presence of the desired allele, and also with random SSR marker for hybridity. The confirmed F_1 plants in each cross were selectively backcrossed with their respective recurrent parents to generate BC_1F_1 seed. The BC_1F_1 populations were raised along with the parents during November-March, a winter (*rabi*) season of 2016–17. A molecular marker linked to the *lpa2* locus was used to identify and select BC_1F_1 plants heterozygous at *lpa2* (umc2230) locus, (foreground selection). BC_1F_1 plants carrying the desired gene in heterozygous conditions were screened by using polymorphic SSR markers to identify and select the BC_1F_1 plants with a higher percent of recurrent parent genome (background selection). The BC_1F_1 plants selected based on the foreground and background selection were backcrossed with their respective recurrent parent to produce BC_2F_1 generation. The BC_2F_1 populations were raised during June-October, a rainly (*kharif*) season of 2017 and the plants heterozygous at *lpa2* were identified and the background selection was performed in the selected BC_2F_1 plants to identify the plants with the highest RPG. The BC_2F_1 plants with the highest RPG were selfed to produce BC_2F_2 generation. The BC_2F_2 plants were raised during *rabi* 2017–18 at WNC, ICAR-IIMR, Hyderabad. The BC_2F_2 plants homozygous for *lpa2* were identified

and selected based on foreground selection. The selected BC_2F_2 plants which are homozygous for *lpa2* were screened using polymorphic markers to identify plants with the highest RPG. The BC_2F_2 plants with the highest RPG and also homozygous for *lpa2* were advanced to BC_2F_3 and BC_2F_4 and maintained through self-pollination.

Biochemical estimation of phytic acid (PA) and inorganic phosphate (P_i)

The PA and P_i were estimated using a calorimetry method by following the protocol as described by Lorenz et al. (2007). The reagents required for PA and P_i estimation were different and prepared separately (Supplementary Information 1). The seed sample of 10 g each of the three analytical replicates of each plant was drawn randomly from the BC_2F_3 seeds harvested from BC_2F_2 plant carrying *lpa2* allele in homozygous condition to estimate PA and P_i of each genotype. The BC_2F_2 plants were grown under optimum growing conditions by following recommended agronomic practices. Ten milligrams (mg) maize flour from each of the above ~ 10 g stock was taken as a sub-sample and placed in 2 mL centrifuge tubes, 200 µL of 0.65 M hydrochloric acid (HCl) was added to the tubes. The mixture was kept for ~ 12-h incubation at room temperature on a shaker/rocker. After the incubation, the tubes were centrifuged at 3000 rpm for 20 min. For PA estimation, 30 µL extract was taken out after centrifugation into a 96-well microplate, then 200 µL diluted (1:4) wades reagent was added to each well. For P_i estimation, another 30 µL extract was taken out in a separate 96-well microplate to which 130 µL deionized water and 100 µL Chen's reagent were added to each well. The sodium phytate (HiMedia, GRM6226) and potassium dihydrogen phosphate (Merck, 104,873 Supelco) were used for the preparation of PA and P_i control standards respectively. The three analytical replicates of control standards of PA and P_i were also prepared as described above and placed in the assigned wells of respective 96-well microplates. For measurement of PA and P_i, the 96-well plates containing control standards and samples of different genotypes were kept for 15–20 min, the OD_{490} and OD_{820} nm were recorded for PA and P_i estimation, respectively using BioTek Epoch 2 Microplate Spectrophotometer (BioSPX B.V., LA Abcoude, The Netherlands).

The standards curves drawn using optical density (OD) values obtained against the respective known concentrations of PA and P_i standards were used to estimate the PA and P_i content in the samples, respectively. The standard curves were linear in the respective assays and the PA or P_i of samples (x) was estimated by substituting the values in the respective linear equation of PA and P_i standards curve, $y = ax + b$ where y is the OD value of the sample, a is the slope, b = constant or intercept.

Evaluation for agronomic performance

The agronomic evaluation was carried out at the experimental site of ICAR-IIMR, Pusa Campus, New Delhi. The NILs evaluated in the present experiment were at BC_2F_3 generation, the agronomic evaluation was conducted along with their respective recurrent parents during *Kharif* 2018 in a randomized complete block design (RCBD) with three replications. Each plot contained four rows of 3 m length with the plant to plant and between row spacing of 20 cm and 70 cm, respectively. The recommended crop production practices were followed and optimum stress-free production condition was provided to ensure proper growth and development of plants.

NILs were evaluated along with their respective recurrent parents and observations were recorded on 18 agronomic traits which included germination percentage (GP), days to 50% anthesis (DA), days to 50% silking (DS), anthesis-silking interval (ASI), leaf width (LW), ear height/placement (EH), plant height (PH), tassel length (TL), ear length without husk (EL), ear diameter without husk (ED), ear circumference (EC), number of kernel rows (KR), number of kernels per rows (KpR), 1000 kernel weight (TKW), shelling percentage (SP), ear to plant ratio (EtoPR), barrenness (Barr) and grain yield (GY).

Data analysis

Agronomic data generated during *Kharif* 2018 were subjected to statistical analysis using SAS 9.2 (SAS version 9.2 software packages; SAS Institute, Inc.; Cary, NC) software for the calculation of the coefficient of variation (CV), Honest Significant Difference (HSD), standard error (SE) and analysis of variance (ANOVA). The graphical representation of background recovery of recurrent parent genome was

generated using the GGT ver. 2.0 (an acronym for Graphical Genotypes).

Results

Validation of foreground marker for *lpa2* allele

The PCR of SSR marker umc2230, tightly linked to the gene determining low phytate content resulted in the amplicon size of 150 bp and 155 bp, in recurrent parents (BML 6 and BML 45) and donor parent, respectively (Fig. 1). The umc2230 marker is located in the maize genome at 0.4 cM away from the *lpa2* gene on the short arm of chromosome 1. For foreground selection, umc2230 which showed polymorphism between the donor (LPA 2), and recipient parents (BML 6 and BML 45) was used as a foreground marker at every step in MABB to identify plants carrying the *lpa2* allele and also differentiate the zygosity condition.

Marker-assisted introgression of *lpa2* gene

The gene determining low phytate content, *lpa2* was introgressed from the donor parent to the genetic background of the recurrent parents BML 45 and BML 6, the female and male parental inbred lines, respectively of commercially released single cross hybrid maize, DHM 121 through MABB to develop low phytate maize.

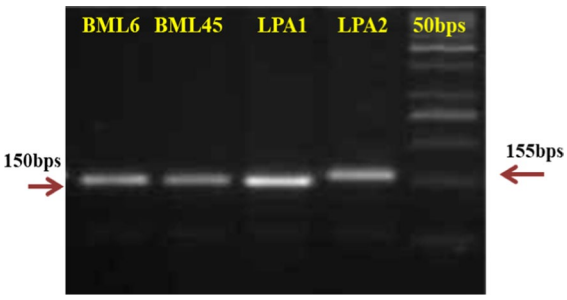


Fig. 1 Parental polymorphism for *lpa2* allele using SSR marker, umc2230

Confirmation of F_1s

Polymorphism between recurrent and donor parent was confirmed and validated using the SSR marker umc2230, linked to *lpa2* gene (0.4 cM). Thus, umc2230 is used as a foreground marker to identify and confirm that the plants carrying *lpa2* allele in F_1 and also in different generations during MABB. The F_1 plants of crosses namely BML 45/LPA 2 and BML 6/LPA 2 were screened using umc2230. The PCR amplification showed that the F_1 plants of the above crosses were heterozygous with a banding pattern of 155/150 bp (Supplementary Fig. 1). The confirmed F_1 plants were also screened using unlinked SSR markers to doubly confirm the heterozygous at selected SSR marker loci. The confirmed F_1 hybrid plants of each cross were used as female parents to backcross with their respective recurrent parents as males to develop the BC_1F_1 population.

Foreground and background selection

The true F_1 plants namely #1 (1, BML 6/LPA 2) and #4 (4, BML 45/LPA 2) were selected based on conformity of heterozygosity at *lpa2* locus and backcrossed with their respective recurrent parents namely BML 6 and BML 45, respectively to generate BC_1F_1. The number of BC_1F_1 plants from F_1 plants was 168 (1, BML 6/LPA 2) and 324 (4, BML 45/LPA 2). The numbers of plants heterozygous at *lpa2* locus were 76 and 169 in BML 6/LPA 2 and BML 45/LPA 2 crosses, respectively (Fig. 2A and 3A). The remaining BC_1F_1 progenies showed recurrent parent allele (150/150), homozygous for wild type allele, *LPA2*. Based on the relative resemblance with recurrent parents in morphological traits, ten heterozygous BC_1F_1 plants were used for background selection to identify plants with higher RPG % recovery. The number of polymorphic SSR markers selected for background selections was 100 and 99 between BML 6 and LPA 2 and BML 45 and LPA 2, respectively. The number of polymorphic markers on each chromosome ranged from 6 (Chromosome 9) to 16 (Chromosome 1) between BML 6 and LPA 2 and 4 (Chromosome 9 and 10) to 19 (Chromosome 1) between BML 45 and LPA 2. The highest number of polymorphic markers were located on Chromosome 1 as the target gene, *lpa2* is located on this chromosome. Since the Chromosomes in maize are arranged in descending order,

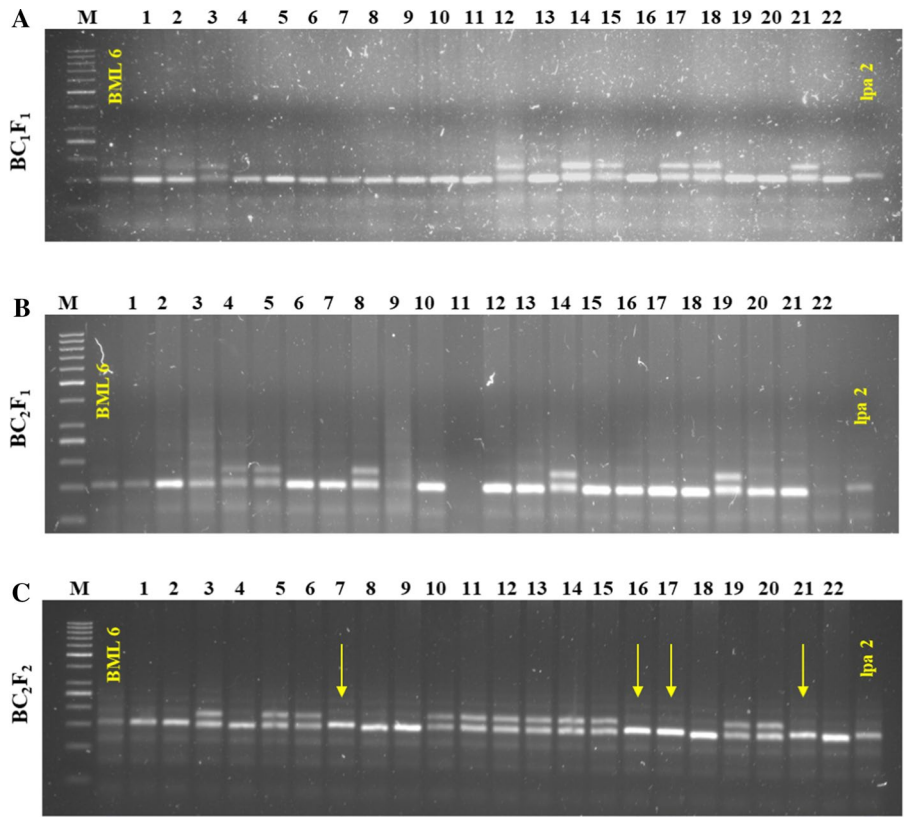


Fig. 2 Foreground selection for *lpa2* allele in different backcross generations in BML6 genetic background using SSR marker umc2230. A- BC_1F_1, B- BC_2F_1, C-BC_2F_2 generation

longest Chromosome was given number 1 while the shortest Chromosome was given number 10, the higher number of polymorphic markers were located on Chromosome 1 while the lower or few polymorphic markers were located on Chromosome 9 and/or 10. The graphical representation of the polymorphic markers used in backcross generation was given in Supplementary Fig. 2. The PCR amplified products of each polymorphic SSR marker in each plant were scored as AA (amplicon size corresponds to recurrent parent allele) and AB (amplicon size corresponds to both recurrent as well as donor allele) (Fig. 4A and B). The RPG % in each plant was estimated and it was ranged from 74.06–79.72% in BC_1F_1s derived from BML 6/LPA 2 cross whereas 74.53–80.19% in BML 45/LPA 2 derived BC_1F_1s. It is important to mention here is that the the estimation of RPG % on Chromosome with few polymorphic markers could be an overestimate, especially when the markers are unevenly distributed and the distance between the adjacent polumorphic markers is higher, due to higher probability of double crossing overs (or higher order). The pictorial representation of the RPG recovery of chromosome number 1 of BML 6/LPA 2 and BML 45/LPA 2 crosses in BC_1F_1 generation is shown in Fig. 5A and B, respectively. Based on the RPG recovery across all 10 chromosomes, the BC_1F_1 plants, #117 (79.72% RPG) of BML 6/LPA 2 cross and #353 (80.19% RPG) of BML 45/LPA 2 crosses with the highest RPG were selected and backcrossed with their respective recurrent parents to develop BC_2F_1 population.

The number of BC_2F_1 plants raised was 218 (BML 6/LPA 2) and 165 (BML 45/LPA 2). The foreground selection performed in the BC_2F_1 generation is similar to that of BC_1F_1. The number of plants heterozygous at *lpa2* locus was 72 and 55 in BML 6/LPA 2 and BML 45/LPA 2 derived BC_2F_1 population, respectively (Figs. 2B and 3B). Whereas the size of the amplicons in the remaining plants was similar to that of wild type allele *LPA2*. Similar to BC_1F_1 generation, ten BC_2F_1 plants, heterozygous at *lpa2* locus were selected, based on relative phenotypic resemblance with their respective recurrent parents for background selection. The background selection in BC_2F_1 generation was carried out using

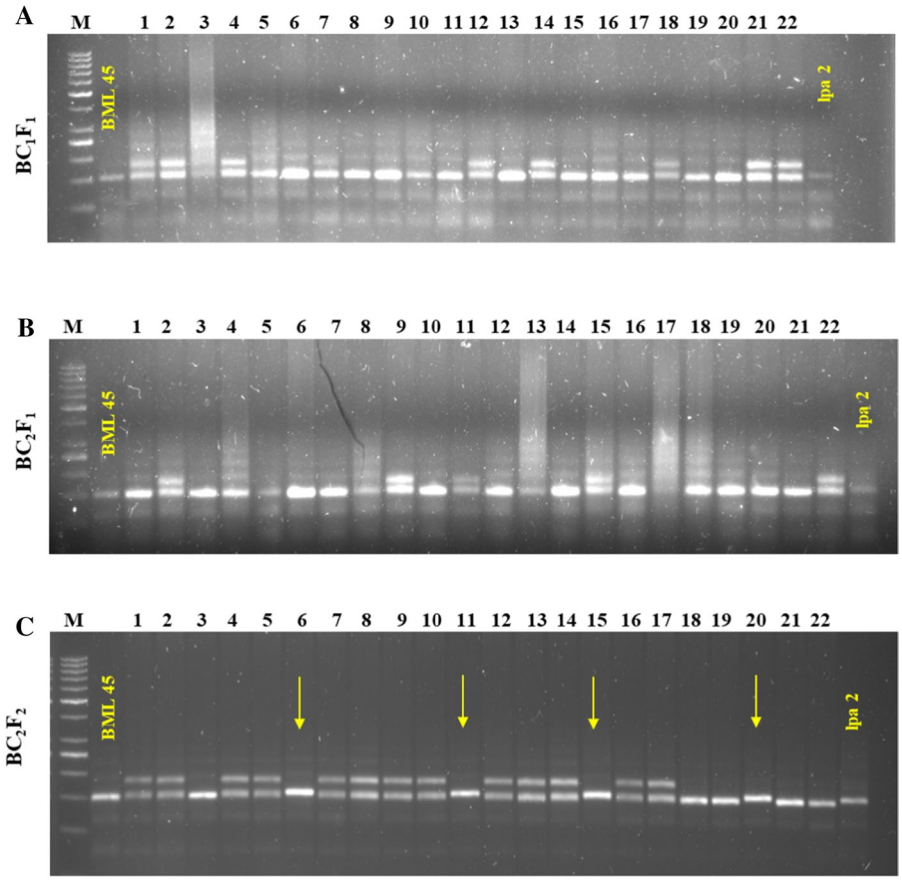


Fig. 3 Foreground selection for *lpa2* allele in different backcross generations in BML45 genetic background using SSR marker umc2230. A- BC_1F_1, B-BC_2F_1, C-BC_2F_2 generation

99 polymorphic SSR markers covering all 10 chromosomes. The number of polymorphic markers on each chromosome varies from 5 (Chromosome 9) to 15 (Chromosome 1) between BML 6 and LPA 2; 3 (Chromosome 10) to 19 (Chromosome 1) between BML 45 and LPA 2. The PCR amplicons of each polymorphic SSR molecular markers in each individual were scored as AA (recurrent parent allele) and AB (heterozygous containing both recurrent as well as donor allele) (Fig. 4C and D). The per cent recovery of RPG in BC_2F_1 generation ranged from 86.32–89.62% and 84.91–89.15% in BML 6/LPA 2 and BML 45/LPA 2 derived backcrosses respectively. The BC_2F_1 plants viz., #2734 [2734, {117, BML 6*/(1, BML 6/LPA 2)}/*BML 6], #2741 [2741, {117, BML 6*/(1, BML 6/LPA 2)}/*BML 6], #1996 [1996, {353, BML 45*/(4, BML 45/LPA 2)}/*BML 45], and #2006 [2006, {353, BML 45*/(4, BML 45/LPA 2)}/*BML 45] with highest RPG were selected and advanced through self-pollination to develop BC_2F_2 population. The RPG % in selected BC_2F_1 plants was 89.62 (# 2734) 89.15 (# 2741), 88.68 (# 1996), and 89.15 (# 2006). The pictorial representation of RPG recovery of chromosome number 1 of BML 6 and BML 45 crosses in BC_2F_1 generation is shown in Fig. 5A and B, respectively.

The number of plants raised in BC_2F_2 generation, derived from BML 6/LPA 2 and BML 45/LPA 2 crosses were 241 and 192, respectively. The number of BC_2F_2 progenies that were homozygous for the *lpa2* allele in the genetic background of BML 6 and BML 45 was 46 and 33, respectively. The homozygous plants showed a similar banding pattern (155/155) as that of the donor line (LPA 2) for the low phytate allele. Whereas the number of plants that were homozygous for wild-type allele, *LPA2* (150/150) was 51 and 42 in the genetic background of BML 6 and BML 45, respectively; the banding pattern was similar to that of recurrent parents (Figs. 2C and 3C). The PCR amplicons banding pattern in the remaining plants was heterozygous (155/150). Based on relative morphological resemblance with their

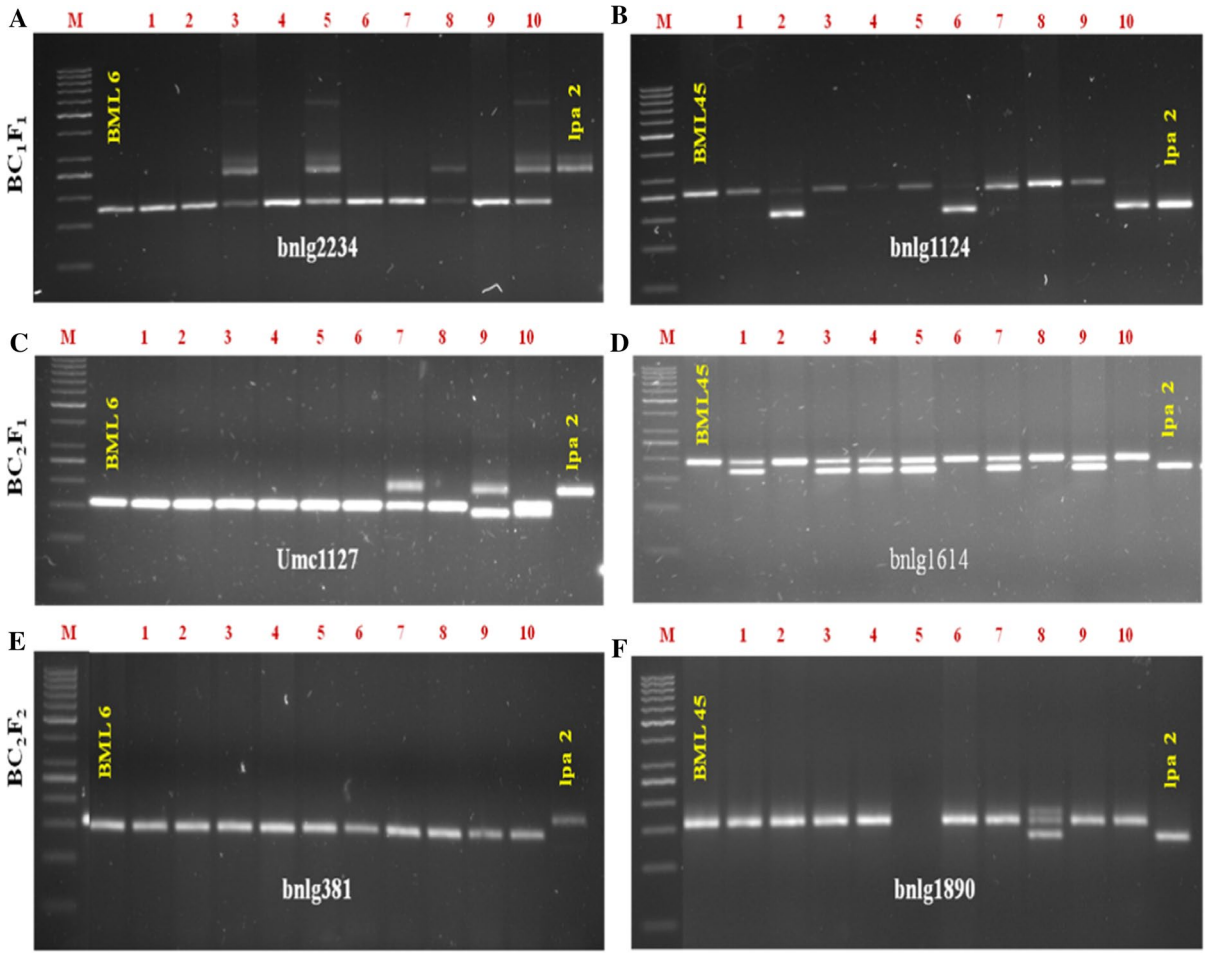


Fig. 4 Background screening of different backcross generations with low phytate trait *(lpa2)* using SSR markers. A&B- BC_1F_1; C&D- BC_2F_1; E&F-BC_2F_2 generation

respective recurrent parents, ten homozygous BC_2F_2 plants were selected for background selection to identify BC_2F_2 plants with the highest RPG content. The number of polymorphic SSR markers chosen for background selection in BC_2F_2 generation derived in the genetic background of BML 6 and BML 45 were 101 and 100, respectively. The polymorphic markers covered all 10 chromosomes and the number of polymorphic markers on each chromosome varies from 5 (Chromosome 9) to 15 (Chromosome 1) between BML 6 and LPA 2; 4 (Chromosome 9 and 10) to 18 (Chromosome 1) between BML 45 and LPA 2. The PCR amplicons of each of the SSR markers in each BC_2F_2 individual plant were scored as AA (recurrent parent allele), and AB (contain both recurrent as well as donor allele), and BB (donor parent allele) (Fig. 4E and F). The RPG % in the selected BC_2F_2 plants ranged from 88.68–91.04 and 90.09–91.51 in the genetic background of BML 6 and BML 45, respectively. Based on the background selection, the best five BC_2F_2 plants viz., #3190, #3283, #3230, #3263, and #3292 in the genetic background of BML 6 and #3720, #3776, #3717, #3828, and #3832 in the genetic background of BML 45 with highest RPG were selected and advanced or maintained through self-pollination to develop BC_2F_3 stage NILs. The pictorial representation of recurrent parent genome recovery of chromosome number 1 of BML 6 and BML 45 crosses in BC_2F_2 generation is shown in Fig. 5A and B, respectively. The stabilized NILs carrying *lpa2* gene, determining the low phytate content would be further used in the hybridization program to

reconstitute the original hybrid DHM 121 with low phytate content.

Estimation of PA and P_i in BC_2F_3 stage NILs carrying *lpa2* allele

The qualitative and quantitative estimation of PA and P_i was done for parents (LPA 2, BML 6, and BML 45) and selected BC_2F_3 progenies which are homozygous for the *lpa2* allele. The PA and P_i were estimated as mentioned in materials and methods. The PA and P_i estimation was done to confirm the expression of introgressed *lpa2* allele with low phytate phenotypes in the genetic background of recurrent parents.

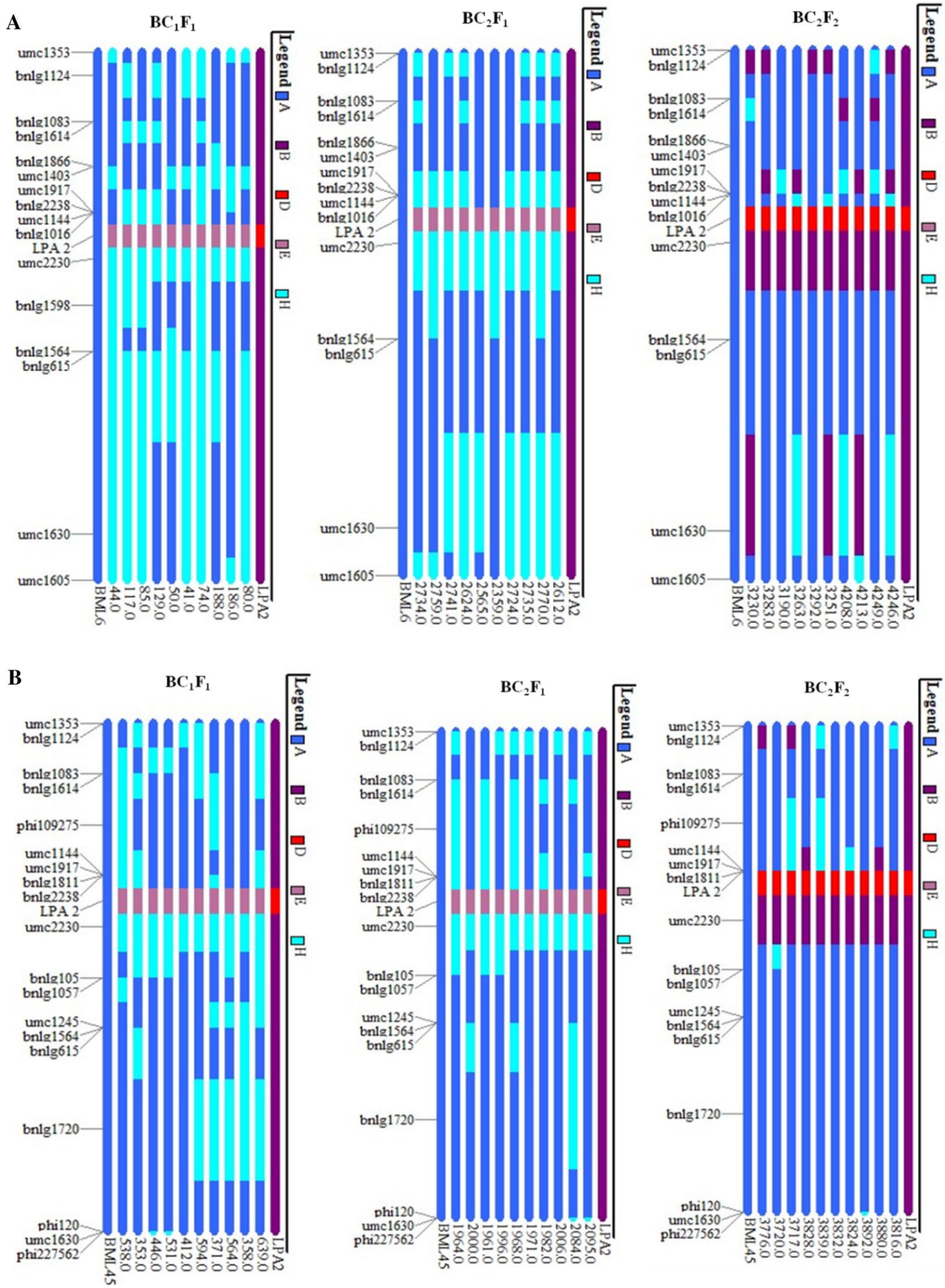
The PA and P_i levels in recurrent parents were relatively high as compared to donor parents. This was confirmed using the high inorganic phosphorous (HIP) assay which is a quick, easy and inexpensive method to differentiate high, low, or intermediate phytate lines. In the HIP assay, kernels having high phytate content produced light blue colour, and kernels with low phytate content produce a dark blue colour, whereas intermediate phytate content kernels produce a medium blue colour. This colour differentiation helps to differentiate phytic acid levels among various lines. In marker-assisted backcross breeding, the HIP assay was used to confirm *lpa2* allele introgression in BC_2F_3 seeds (Fig. 6). This figure shows that some BC_2F_2 lines with *lpa2* allele introgression produce dark blue colour which is comparable to that of our donor mutant line and these lines were selected as low phytate near-isogenic lines.

Quantitative estimation of phytic acid and inorganic phosphate was analyzed by taking the readings at OD_{490} and OD_{820} nm, respectively. The estimation was performed in the *lpa2* donor line, recurrent parents and newly developed low phytate BC_2F_3 lines. The analysis revealed that PA and P_i levels varied among the selected lines (Table 2). The donor line which is homozygous for mutant *lpa2* allele having a minimum level of PA (1.72 ± 0.118 mg/g) and maximum level of P_i (1.22 ± 0.49 mg/g), whereas recurrent parents which are homozygous for the wild type *LPA2* allele having maximum PA (BML 6: 3.59 ± 0.12 mg/g, BML 45: 3.16 ± 0.1 mg/g) and a minimum P_i (BML 6: 0.65 ± 0.49 mg/g, BML 45: 0.51 ± 0.47 mg/g). The newly developed low phytate BC_2F_3 lines which are homozygous for *lpa2* alleles contains lower levels of PA (ranges from 1.67 ± 0.118 to 2.84 ± 0.12 mg/g in NILs of BML 6 and 1.8 ± 0.1 to 3.01 ± 0.1 mg/g in NILs of BML 45) and higher levels of P_i (ranges from 0.15 ± 0.49 to 1.01 ± 0.49 mg/g in NILs of BML 6 and 0.3 ± 0.47 to 1.48 ± 0.47 mg/g in NILs of BML 45) when compared to their respective recurrent parents. Among the NILs developed, #3190 (1.67 ± 0.118 mg/g) of BML 6 and #3720 (1.8 ± 0.1 mg/g) of BML 45 were statistically on par with that of donor parents and #3230 (2.02 ± 0.118 mg/g), #3283 (2.07 ± 0.118 mg/g) of BML 6 and #3828 (2.13 ± 0.1 mg/g), #3776 (2.17 ± 0.1 mg/g) of BML 45 were significantly low levels of PA when compared to recurrent parents. The NILs viz., #3190 (1.01 ± 0.49 mg/g) of BML 6 and #3720 (1.38 ± 0.47 mg/g) of BML 45 have the highest levels of P_i when compared to recurrent parents and are comparable with that of the donor parent. The results indicate that MAB breeding has successfully introgressed the low phytate allele from donor parent to recurrent parents.

Agronomic evaluation of NILs with low phytate content

The agronomic evaluation of near-isogenic lines (NILs), carrying *lpa2* allele in the genetic background of BML 6 and BML 45 along with their respective recurrent parents namely BML 6 and BML 45, respectively was done to compare agronomic performance of NILs with their respective recurrent parents and identify NILs with comparable agronomic performance to their recurrent parents. The observations were recorded on 18 agronomically important traits and all the traits are quantitative. The descriptive statistics for agronomic traits in this study are shown in Tables 3 and 4.

The results of the agronomic evaluation indicated that the NILs developed in the genetic background of BML 6 showed no significant differences in eight (ASI, EC, ED, EtoPR, KR, SP, TKW, and Barr) out of 18 traits studied. All the eight traits with no significant differences contribute directly or indirectly to final grain yield. Out of 10 NILs, two NILs viz., LPABML 6–5 for two traits (DA and EH); LPABML 6–9 for EH differed significantly with that of recurrent parent BML 6. Whereas the rest of the eight NILs did not differ significantly from that of the recurrent parent in any of the traits. If LPABML 6–5 excluded then the DA of NILs were ranged from 56


A
BC1F1
BC2F1
BC2F2
Legend
A
B
D
E
H
umc1353
bnlg1124
bnlg1083
bnlg1614
bnlg1866
umc1403
umc1917
bnlg2238
umc1144
bnlg1016
LPA 2
umc2230
bnlg1598
bnlg1564
bnlg615
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umc1605
BML6
LPA2
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BC2F2
umc1353
bnlg1124
bnlg1083
bnlg1614
phi109275
umc1144
umc1917
bnlg1811
bnlg2238
LPA 2
umc2230
bnlg105
bnlg1057
umc1245
bnlg1564
bnlg615
bnlg1720
phi120
umc1630
phi227562
BML45
LPA2

◂**Fig. 5** A Recurrent parent genome recovery of chromosome number 1 in BC_1F_1, BC_2F_1 and BC_2F_2 generations using SSR markers in BML6 with *lpa2* allele, respectively. B Recurrent parent genome recovery of chromosome number 1 in BC_1F_1, BC_2F_1 and BC_2F_2 generations using SSR markers in BML45 with *lpa2* allele, respectively. A: BML 45 specific allele, B: LPA 2 allele, D: LPA 2 in the homozygous state, E: LPA 2 in the heterozygous state, H: Heterozygote

(LPABML 6–3) to 62 (LPABML 6–4, LPABML 6–8, LPABML 6–10) which did not differ significantly with the recurrent parent BML 6 (61). Similarly, the DS among the NILs derived in the genetic background of BML 6 varied between 60 (LPABML 6–3) to 70 (LPABML 6–6), with no significant difference with recurrent parent BML 6 (66).

Similarly, NILs developed in the genetic background of BML 45 were compared with the recurrent parent BML 45. The results indicated that out of 18 traits, three traits namely ASI, EH, and SP did not show any significant differences. However, when compared with the recurrent parent BML 45, none of the traits showed any significant difference between NILs and recurrent parent BML 45. Thus all the traits are comparable between NILs and recurrent parent BML 45. Out of 10 NILs one NIL, LPABML 45–6 has differed significantly with recurrent parent BML 45 for one trait kernel per row (KpR). The present study could able to identify eight and ten NILs that are comparable with the recurrent parents BML 6 and BML 45, respectively in 17 of the 18 traits studied.

Grain yield being a very complex trait, almost all the NILs derived in the genetic background of BML 6 and BML 45 were comparable with their recurrent parent BML 6 and BML 45, respectively. Even though almost all NILs except two (LPABML 6–6 and LPABML 6–7) derived in the genetic background of BML 6 differed significantly from that of the recurrent parent but they were numerically superior over the recurrent parent BML 6 which is desirable. Whereas in the case of NILs developed in the genetic background of BML 45, out of 10 NILs selected based on the foreground and background selection three NILs namely LPABML 45–6, LPABML 45–8, and LPABML 45–10 were significantly inferior over the recurrent parent BML 45.

The DHM 121 hybrid was re-constituted by making crosses between ten NILs each of BML 45 and BML 6. The total number DHM 121 versions evaluated were 61 and primary data was generated on 30 morphological traits excluding six ancillary traits like ASI, barrenness, ear to plant ration, ear height to plant height ratio, shelling percentage, and germination percentage, which were calculated from the primary data. The 30 morphological traits data includes seven yield contributing traits like number of kernel rows, number of kernels per row, ear length, ear diameter, thousand kernel weight, days to anthesis, and days to silking, and grain yield. Based on the agronomic evaluation data, 53 versions of DHM 121 out of 61 versions of DHM 121 involving NILs showed superior yield over the original hybrid DHM 121 (data not shown).

Percentage of germination is another important trait that requires special attention, especially in low phytate maize. The results obtained in the present study have shown that none of the NILs developed in the genetic background of BML 6 and BML 45 differed significantly for germination which is very much required.

In summary, through MABB it was possible to transfer low phytate traits successfully from donor to recipient parents. The biochemical and agronomic performance for most of the agronomic traits were comparable with that of recurrent parents. Thus the newly developed NILs are not only similar to that of the recurrent parents but also having low phytate content which is very much useful to reconstitute DHM 121 hybrid with low phytate content.

Discussion

Phytic acid is an important anti-nutritional factor in maize that needs to be reduced without affecting the agronomic performance of the genotype. Presently, few LPA mutants are available in maize which can be effectively used for rapid conversion of elite inbred lines of maize into LPA maize through MABB. The mutant alleles affecting the PA have been mapped and the tightly linked molecular markers are available in the public domain. In the present study, SSR marker umc2230, tightly linked to the *lpa2* allele was used as a foreground marker to introgress into elite inbred lines BML 6 and BML 45 through MABB. The SSR marker umc2230 located 0.4 cM away from the *lpa2* was polymorphic between the donor and recurrent parents. The tightly linked molecular marker is expected to show polymorphism between the donor

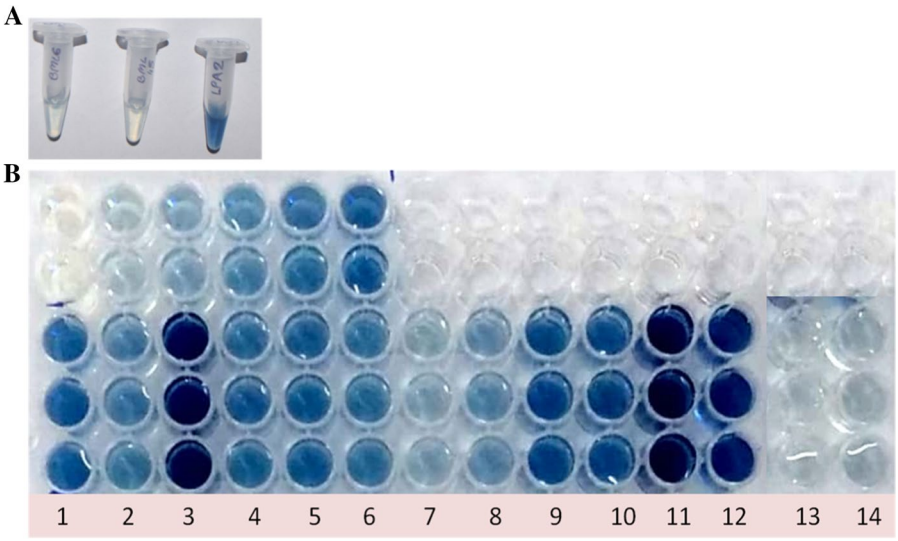

Fig. 6 Qualitative estimation of inorganic phosphorus (P_i) content of selected BC_2F_3 seeds with *lpa2* allele. (A) Donor and recurrent parents (B) Row 1–2 – Standards; Lane 1 to 5 – NILs of BML 6 Lane 6 to 10 – NILs of BML 45; 11-LPA 1; 12—LPA 2; 13-BML 6; 14-BML 45

Table 2 Phytic acid (PA) and inorganic phosphorus (P_i) (mg/g) content of newly developed near-isogenic lines (NILs) along with their parents (BML 6, BML 45, LPA 2)

S. No	Plant No	PA	P_i	Plant No	PA	P_i
1	LPABML 6–1	2.02^{d}	0.46^{de}	LPABML 45–1	2.17^{e}	0.80^{d}
2	LPABML 6–2	2.07^{d}	0.31^{f}	LPABML 45–2	1.80^{f}	1.38^{a}
3	LPABML 6–3	1.67^{e}	1.01^{b}	LPABML 45–3	2.18^{e}	0.65^{ef}
4	LPABML 6–4	2.22^{d}	0.32^{f}	LPABML 45–4	2.13^{e}	0.74^{de}
5	LPABML 6–5	2.22^{d}	0.38^{ef}	LPABML 45–5	2.24^{de}	0.72^{de}
6	LPABML 6–6	2.51^{c}	0.31^{f}	LPABML 45–6	2.44^{cd}	0.30^{i}
7	LPABML 6–7	2.48^{c}	0.15^{g}	LPABML 45–7	2.46^{c}	0.58^{fg}
8	LPABML 6–8	2.84^{b}	0.20^{g}	LPABML 45–8	2.68^{b}	0.90^{c}
9	LPABML 6–9	2.54^{c}	0.48^{d}	LPABML 45–9	2.76^{b}	0.80^{d}
10	LPABML 6–10	2.52^{c}	0.45^{de}	LPABML 45–10	3.01^{a}	0.46^{h}
11	BML 6	3.59^{a}	0.65^{c}	BML 45	3.16^{a}	0.51^{gh}
12	LPA 2	1.72^{e}	1.22^{a}	LPA 2	1.72^{f}	1.22^{b}
	General Mean	2.37	0.49	General Mean	2.40	0.76
	Mean SS	0.81^{**}	0.31^{**}	Mean SS	0.59^{**}	0.32^{**}
	p-Value	< .00	< .00	*p*-Value	< .00	< .00
	CV (%)	6.1	12.0	CV (%)	5.1	7.6
	SE(d)	0.12	0.049	SE(d)	0.099	0.04
	LSD at 5%	0.25	0.10	LSD at 5%	0.20	0.09

Means with at least one letter common are not statistically significant using TUKEY's Honest Significant Differe;
** = significant at *P*-value 0.01

and recurrent parents due to differences in the phytic acid content between the donor and recurrent parents. Similar polymorphism between the donor and recurrent parents while transferring *lpa2* gene from the mutant line into tropical germplasm was observed in other studies as well (Sureshkumar et al. 2014a; Tamilkumar et al. 2014).

The background selection using molecular markers is crucial while executing MABB for rapid conversion of elite lines with an improved trait (Singh and Singh, 2015). Thus, the polymorphic SSR molecular markers unlinked to *lpa2* are essential to accelerate the recovery of the RPG. Background selection was performed in progenies selected based on foreground selection in each backcross generation to identify the plants with the highest RPG in introgressed lines.

The present study integrated both phenotypic and genotypic selection as a part of the cost-cutting

Table 3 The descriptive statistics of near-isogenic lines derived in the genetic background of BML 6 with low phytate traitfor agronomic traits

S. No.	Genotype	GP (%)	DA (days)	DS (days)	ASI (days)	LW (cm)	PH (cm)	EH (cm)	TL (cm)	EL (cm)	ED (cm)
1	BML 6	73^{ab}	61^{bc}	66^{ab}	6	6.6^{a}	111^{abc}	64^{ab}	23^{abc}	12^{ab}	4
2	LPABML 6–1	66^{ab}	58^{bc}	65^{ab}	7	7.5^{a}	129^{a}	74^{a}	25^{abc}	14^{a}	4
3	LPABML 6–2	75^{a}	59^{bc}	64^{ab}	5	6.4^{a}	113^{abc}	59^{abcd}	24^{abc}	14^{a}	4
4	LPABML 6–3	57^{ab}	56^{c}	60^{b}	4	7.8^{a}	126^{ab}	65^{ab}	30^{abc}	14^{a}	4
5	LPABML 6–4	78^{a}	62^{ab}	64^{ab}	2	7.8^{a}	108^{bc}	65^{ab}	31^{a}	12^{ab}	4
6	LPABML 6–5	60^{ab}	66^{a}	69^{a}	3	7.0^{a}	112^{abc}	47 ^{cd}	24^{abc}	12^{ab}	3
7	LPABML 6–6	66^{ab}	61^{ab}	70^{a}	9	6.1^{a}	107^{bc}	51^{bcd}	22^{c}	11^{ab}	3
8	LPABML 6–7	80^{a}	61^{ab}	66^{ab}	5	6.2^{a}	115^{abc}	52^{bcd}	31^{a}	12^{ab}	3
9	LPABML 6–8	80^{a}	62^{ab}	67^{ab}	5	6.8^{a}	115^{abc}	58^{abcd}	30^{ab}	10^{ab}	4
10	LPABML 6–9	77^{a}	57^{bc}	63^{ab}	5	6.3^{a}	101^{c}	44^{d}	23^{bc}	12^{ab}	3
11	LPABML 6–10	67^{ab}	62^{ab}	68^{ab}	6	6.1^{a}	116^{abc}	61^{abc}	24^{bc}	14^{a}	3
12	General Mean	71	60	66	5	6.8	114	58	26	12.4	4
13	Mean SS	791**	24**	26**	10	1.2**	197**	238**	40**	31**	1.5
13	*p*-Value	0.00	<.00	0.00	0.06	0.00	0.00	<.00	<.00	0.00	0.09
14	CV(%)	21	2.4	3.7	40	7.3	4.8	7.7	9	22	26
15	SE(d)	10.8	1.2	2.0	1.7	0.4	4.5	3.7	1.9	1.9	0.7

S. No.	Genotype	EC (cm)	KR (no.)	KpR (no.)	TKW (g)	SP (%)	EtoPR (no.)	Barr (no.)	GY (kg/ha)
1	BML 6	12	14	23^{abc}	170	65	0.8	0.23	768^{f}
2	LPABML 6–1	13	14	25^{ab}	225	69	0.5	0.50	1070de
3	LPABML 6–2	12	14	25^{ab}	197	61	0.6	0.37	1050de
4	LPABML 6–3	13	14	29^{a}	242	59	0.6	0.43	1183^{d}
5	LPABML 6–4	13	15	23^{abc}	245	79	0.8	0.30	1291^{d}
6	LPABML 6–5	11	13	16^{abc}	175	74	0.9	0.10	1050de
7	LPABML 6–6	10	10	18^{abc}	122	66	0.5	0.70	661^{f}
8	LPABML 6–7	10	11	16^{abc}	115	78	0.5	0.50	2778^{a}
9	LPABML 6–8	12	15	17^{abc}	200	77	0.5	0.60	631^{f}
10	LPABML 6–9	8	10	12^{bc}	155	62	0.5	0.53	1606^{c}
11	LPABML 6–10	10	11	15^{abc}	192	64	0.7	0.70	974^{e}
12	General Mean	11	13	20	185	69	0.6	0.45	1278
13	Mean SS	13	17	116**	8272	1005	0.1	0.1	1,407,614**
13	*p*-Value	0.18	0.24	0.00	0.06	0.12	0.06	0.08	<.00
14	CV(%)	26	27	23	33	39	42	50	3.8
15	SE(d)	2.3	2.8	3.54	49	19	0.2	0.19	39

Means with at least one letter common are not statistically significant using TUKEY's Honest Significant Difference

Germination percentage (GP),days to 50% anthesis (DA), days to 50% silking (DS), anthesis-silking interval (ASI), leaf width (LW), ear height/placement (EH), plant height (PH), tassel length(TL), ear length without husk (EL), ear diameter without husk (ED), ear circumference (EC), number of kernel rows (KR), number of kernels per rows (KpR), 1000 kernel weight (TKW), shelling percentage (SP), ear to plant ratio (EtoPR), barrenness (Barr) andgrain yield (GY)

strategy in the MABB. It was possible to reduce the number of plants in different backcross generations through selecting the plants carrying the gene of interest. In the present case (*lpa2*), based on morphological resemblance with the recurrent parents followed by background selection using molecular markers has reduced the number of PCR reactions substantially. In addition, the breeder can give attention to morphological traits of a recurrent parent in the introgressed individuals to select and reconstitute the recurrent

Table 4 The descriptive statistics of near-isogenic lines derived in the genetic background of BML 45 with low phytate traitfor agronomic traits

S. no.	Genotype	GP (%)	DA (days)	DS (days)	ASI (days)	LW (cm)	PH (cm)	EH (cm)	TL (cm)	EL (cm)	ED (cm)
1	BML 45	60^{a}	62^{ab}	65^{a}	3	5.8^{ab}	90^{ab}	38	24^{a}	10^{ab}	3^{b}
2	LPABML 45–1	78^{a}	57^{b}	60^{a}	3	6.0^{ab}	100^{ab}	42	24^{a}	11^{a}	4^{a}
3	LPABML 45–2	93^{a}	61^{ab}	63^{a}	2	5.0^{ab}	84^{ab}	34	24^{a}	9^{ab}	3^{ab}
4	LPABML 45–3	84^{a}	59^{ab}	62^{a}	3	4.6^{b}	95^{ab}	39	22^{a}	10^{ab}	3^{ab}
5	LPABML 45–4	68^{a}	61^{ab}	64^{a}	3	5.9^{ab}	92^{ab}	42	24^{a}	7^{ab}	2^{ab}
6	LPABML 45–5	64^{a}	58^{ab}	61^{a}	3	5.3^{ab}	101^{ab}	43	23^{a}	11^{a}	4^{a}
7	LPABML 45–6	69^{a}	66^{a}	71^{a}	5	6.0^{a}	108^{a}	34	28^{a}	10^{ab}	4^{b}
8	LPABML 45–7	71^{a}	61^{ab}	65^{a}	4	5.5^{ab}	77^{b}	39	19^{a}	10^{ab}	3^{ab}
9	LPABML 45–8	64^{a}	64^{ab}	67^{a}	3	6.4^{a}	101^{ab}	44	32^{a}	9^{ab}	3^{ab}
10	LPABML 45–9	62^{a}	61^{ab}	62^{a}	1	5.4^{ab}	103^{ab}	41	23^{a}	11^{a}	4^{a}
11	LPABML 45–10	87^{a}	61^{ab}	69^{a}	8	5.8^{ab}	97^{ab}	32	24^{a}	8^{ab}	3^{ab}
12	General Mean	73	61	64	3	5.6	95	39	24.2	10	3.1
13	MeanSS	523^{*}	20^{**}	37^{*}	11	0.8^{**}	387^{*}	51	34^{*}	21^{**}	2.4^{**}
13	*p*-Value	0.01	0.00	0.02	0.08	0.00	0.01	0.17	0.04	0.00	0.00
14	CV(%)	18	3.93	5.6	67	7.06	11.53	14.4	15.4	25.4	22.7
15	SE(d)	10	1.9	2.9	1.9	0.3	8.9	4.6	3.1	1.9	0.5

S. no.	Genotype	EC (cm)	KR (no.)	KpR (no.)	TKW (g)	SP (%)	EtoPR (no.)	Barr (no.)	GY (kg/ha)
1	BML 45	10^{ab}	13^{ab}	17^{a}	230^{ab}	80	0.8^{ab}	0.2^{ab}	1400^{bc}
2	LPABML 45–1	11^{a}	12^{ab}	17^{a}	225^{ab}	62	0.7^{ab}	0.3^{ab}	787^{cd}
3	LPABML 45–2	10^{ab}	11^{ab}	15^{ab}	245^{ab}	73	1.0^{a}	0.0^{b}	1564^{ab}
4	LPABML 45–3	11^{a}	12^{ab}	17^{a}	185^{ab}	85	0.8^{ab}	0.3^{ab}	1050^{bcd}
5	LPABML 45–4	7^{ab}	8^{ab}	12^{ab}	250^{ab}	72	0.6^{ab}	0.5^{ab}	1188^{bcd}
6	LPABML 45–5	11^{a}	14^{a}	20^{a}	258^{a}	66	1.0^{a}	0.0^{b}	2041^{a}
7	LPABML 45–6	12^{a}	13^{ab}	16^{a}	262^{a}	53	0.07^{b}	0.9^{a}	1382^{bc}
8	LPABML 45–7	10^{ab}	11^{ab}	17^{a}	233^{ab}	55	0.8^{ab}	0.2^{ab}	1040^{bcd}
9	LPABML 45–8	10^{ab}	12^{ab}	17^{a}	243^{ab}	63	0.5^{ab}	0.5^{ab}	732^{d}
10	LPABML 45–9	12^{a}	12^{ab}	22^{a}	247^{ab}	67	0.6^{ab}	0.4^{ab}	1485^{ab}
11	LPABML 45–10	9^{ab}	9^{ab}	12^{ab}	235^{ab}	53	0.5^{ab}	0.5^{ab}	767^{d}
12	General Mean	10.3	12	16.5	238	66.2	0.68	0.33	1221.7
13	MeanSS	20^{**}	25^{**}	75^{**}	$11{,}746^{**}$	1531	0.2^{**}	0.2^{**}	$872{,}633^{**}$
13	*p*-Value	0.00	0.00	0.00	0.00	0.1	0.00	0.00	< .00
14	CV(%)	24	25.5	24.1	27	50	30.8	63.2	15.80
15	SE(d)	1.9	2.2	3.01	47	23	0.17	0.16	141

Means with at least one letter common are not statistically significant using TUKEY's Honest Significant Difference Germination percentage (GP),days to 50% anthesis (DA), days to 50% silking (DS), anthesis-silking interval (ASI), leaf width (LW), ear height/placement (EH), plant height (PH), tassel length(TL), ear length without husk (EL), ear diameter without husk (ED), ear circumference (EC), number of kernel rows (KR), number of kernels per rows (KpR), 1000 kernel weight (TKW), shelling percentage (SP), ear to plant ratio (EtoPR), barrenness (Barr) andgrain yield (GY)

parent. Thus, after foreground selection, ten plants that were heterozygous for *lpa2* gene with relatively more resemblance towards recurrent parents were selected in each backcross generation for background selection. The number of SSR markers used for background selection in each backcross generation varied from 99–100 covering all the ten chromosomes. In each of the backcross populations developed in the genetic background of BML 6 and BML 45, one plant among the 10 selected plants with the highest RPG was backcrossed with the respective recurrent parent to develop the next backcross generations namely

BC_2F_1, BC_2F_2 etc. The percent recovery of RPG in BC_1F_1, BC_2F_1 and BC_2F_2 generation was higher by approximately 5–10% against the average recovery in each of backcross generations derived in the genetic background of BML 6 and BML 45. Similar studies of accelerated development of NILs through the transfer of one or two genes in different crops for different traits has been achieved (Naidoo et al. 2012; Elilis and Pantalone 2009; Arunakumari et al. 2016).

The utility of MAS is more pronounced if the phenotyping of trait under transfer is laborious and time-consuming or requires destructive sampling. PA is determined by recessive gene *lpa2,* PA being biochemical compound that requires its estimation through a biochemical procedure. In such cases, selfing after every backcrossing is a must, especially in crops like maize where tillering is not observed and also multiple ears are not produced in all the genotypes to attempt both selfing and bacrossing on the same plant. Further, PA being a biochemical trait, a destructive sampling of biochemical estimation of PA delays the duration required for generation advancement further. The application of MABB has reduced time substantially in the present case. The successful application of MAS to transfer recessive genes have been practiced in other crops including maize (Naidoo et al. 2012; Bhatt et al. 2018; Prasanna et al. 2020).

The graphical genotyping has shown the pictorial representation of chromosomes with varying percentages of donor genome in the introgressed lines and also the recurrent parent genome. The background selection has reduced substantially the chromosomal region of the donor genome on the carrier chromosome which has been reflected in the graphical genotyping. The background selection has been applied for reduction of linkage-drag in other crops as well including maize (Herzog and Frisch 2011; Hospital 2001, ; Joshi and Nayak 2010). The population size in different backcross generations is also an important factor for rapid conversion of lines to improve simply inherited traits. In the present study, approximately 150–200 plants in each backcross generation have been generated to increase the probability of recombinants with the highest percentage of recurrent parent genome. There are studies that employed different populations size in maize, and the recovery of recurrent parent genome reported is 91–93% in BC_3F_2 generation by Sureshkumar et al. (2014a), 92.15% in BC_2F_1 generation by Naidoo et al. (2012).

The NILs were evaluated for PA and P_i content. The effect of the background genome on the expression of any trait for that matter varies across crops and traits. Previously, several studies have reported different levels of trait expression among the NILs with relatively non-significant differences in the RPG (Sureshkumar et al. 2014a; Tamilkumar et al. 2014). The effect of the background genome is also observed in the present investigation. The NILs with > 90% RPG did show significant variation in the PA and P_i content. Among the NILs developed in the genetic background of BML 6, PA and P_i content showed relatively more variability than the variation observed for RPG content. Similarly in the genetic background of BML 45 also similar variation in the PA and P_i content was observed which requires further studies to identify and understand the effects of specific genomic regions affecting the PA and P_i content in the NILs. The possible reason for variation in the PA content in NILs could be several, one of the reasons could be genetic background effect due to variation in the number and types of transcription factors involved in different intermediate steps in the biochemical or metabolic pathways involved in PA synthesis and accumulation across different NILs. There could be less number of markers to recover all possible or full genome in the NILs; the number of markers required depends on the type of genotype, variation in the recombination frequency across genotypes, and many other unknown reasons. This is due to recovery of different combinations of genomic regions in different NILs. The original mutant lines carrying *lpa2* has shown approximately 50% reduction in the PA content than that of its corresponding wild-type genotype (Raboy 2002; Shi et al. 2005). The introgression of the *lpa2* gene into BML 6 and BML 45 also reduced the PA content in the introgressed genotype up to ~ 53 and 43%, respectively. However, the range of variability in reduction of PA in the NILs developed in the genetic background of BML 6 and BML 45 was 53.48% and 43.03%, respectively. Thus MABB has successfully demonstrated that it is possible to reduce the PA content in the introgressed lines comparable to that of original mutant lines.

It is also reported that the P_i content also increases by 2–threefold with a corresponding reduction in the PA content (Shi et al. 2003; Badone et al. 2012). In

the present study, it is also observed that the P_i content also increased proportionately by 2–3 folds in the NILs developed in the genetic background of BML 6 and BML 45. Similar observations on increased P_i content were also reported previously by Shi et al. 2003; Badone et al. 2012. It is desirable to increase the P_i content which has advantages in terms of increased availability of phosphorous and also other mineral elements.

In general, it is considered that genotypes with LPA show reduced germination and agronomic performance (Oltmans et al. 2005; Anderson and Fehr 2008,; Raboy 2002). Previously several literatures have published that the MABB has led to reconstitute the recurrent parents with comparable agronomic and other traits (Shi et al. 2007; Sureshkumar et al. 2014b; Elilis and Pantalone 2009). The NILs of BC_2F_3 generations were evaluated for germination and other agronomic performance to assess the effect of low PA on the overall agronomic performance. The NILs were assessed for 18 agronomic traits, which are considered important for the identity of the recurrent parents, yield performance, and adaptation. Some traits, mostly measurable quantitative traits including yield showed significant differences between NILs. One of the important reason is the presence of interaction between genotype and environement (G×E). The differential inetaction of each NILs with environment is due to different percentage of RPG in different NILs. The variation in the RPG% in NILs can interact differentially with environment which will lead to differences in the quantitative traits. Further, random molecular markers were used for background selection which might not able to recover all the genomic regions which determine traits of economic significance like yield. Thus, significant variation was observed in NILs for yield and other yield related traits. Contrary to the general perception, the NILs showed comparable performance with that of respective recurrent parents in most of the traits including germination. Previously it was reported that 50% and 94% of the backcross derived lines for low phytate content (CX1834-1–6 (low-phytate line) and B019 (normal cultivar)) in soybean were comparable to that of recurrent parent for field emergence and yield, respectively (Spear and Fehr (2007). However, despite the cause of reduced seedling emergence in low phytate lines, it has been agreed that other agronomic traits are not diminished in low phytate lines (Scaboo et al. 2009; Maupin et al. 2011; Spear and Fehr, 2007). Raboy et al. (1985) also stated that reduced phytic acid quantity does not show any adverse effect on soybean seeds germination.

Thus the results indicated that foreground selection and background selection proves effective in transferring simply inherited traits without losing any of the essential traits of recurrent parents. The integration of morphological selection has also played an important role to select the plants with resemblance to that of recurrent parents which is quite an important role to recover minor morphological traits which many times not possible to observe.

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Authors' contributions Conceptualization and investigation of work by CGK and YKR. The experiments and studies are conducted by YKR, SSG. Part of the experimental work was also supported by AK, P J, H K Y, and S S. The data analysis was done by HK MS and SK. The first draft of the manuscript was written by YKR and CGK. The manuscript was imporved by SR, AS, JCS, RNG, RKP, FH, and AKD. All authors read and approved the manuscript.

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Declarations

Conflict of interests All the authors declare no competing interests.

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