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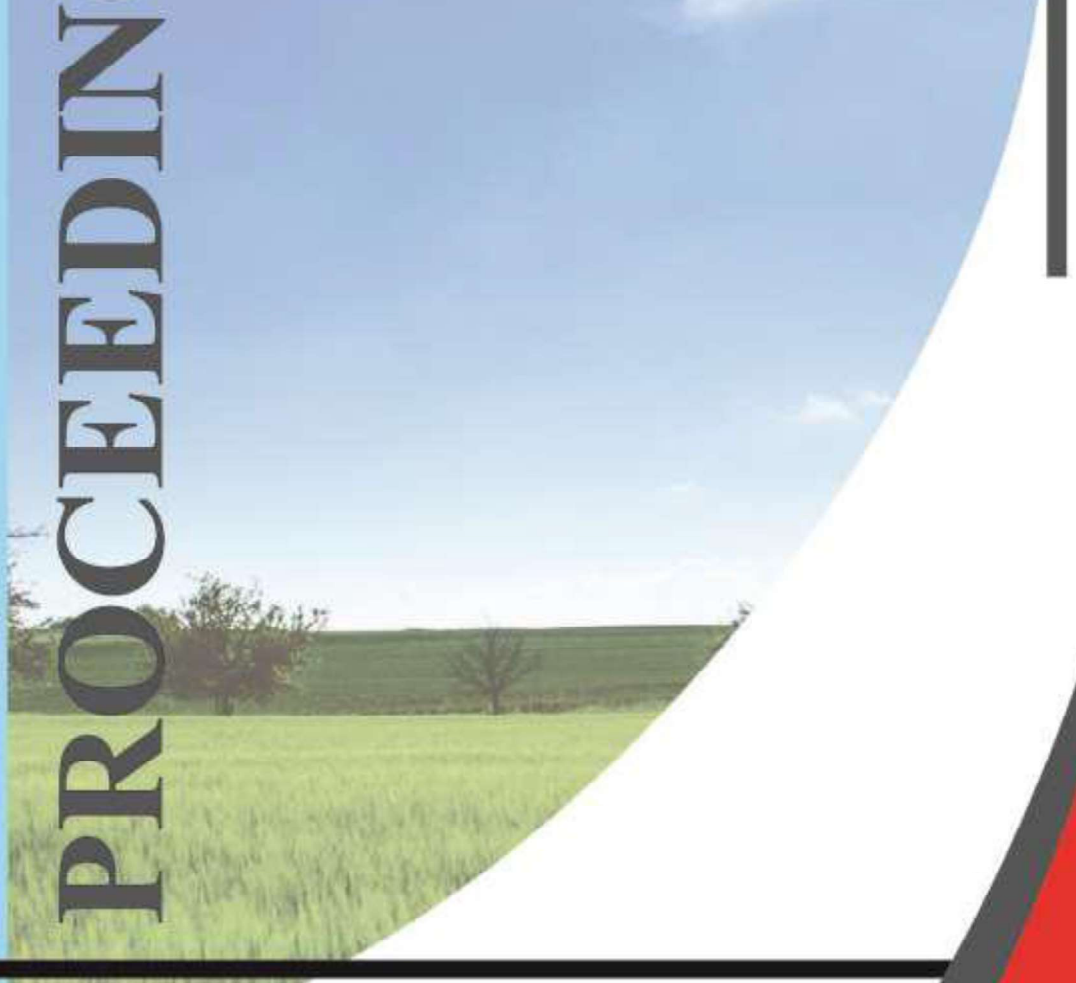

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MARKER ASSISTED SELECTION FOR β-CAROTENE RICH MAIZE: TWO-LEVEL SELECTION PROCEDURE IN BC_2 GENERATION

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Abstract

This paper is a part of the breeding program conducted at the Maize Research Institute "Zemun Polje" (MRI), with the aim to convert the standard maize to β-carotene rich genotypes adapted to temperate regions using the marker assisted selection. When SSR marker specific to the *crtRB1* gene has been tested on parental lines, distinct polymorphism was observed between the donor and recurrent parents. The same marker has also been validated in BC_1 generation when heterozygous individuals were clearly distinguishable from the homozygous dominants. The results presented herein refer to two-level selection procedure in BC_2 generation. First, BC_2 plants were analyzed with *crtRB1*-specific molecular marker to separate heterozygotes from dominant homozygotes. Percentage of heterozygous plants was approximately 50%, which was in accordance with the expected Mendelian ratio of 1:1. Second, the selected heterozygotes were screened with 30 polymorphic SSR markers distributed throughout the maize genome to identify genotypes with the highest recovery of recurrent parent's genome (RPG). The RPG values among three analyzed parental lines and their respective progenies ranged from 85-99%. For each line separately, RPG values were: 86-97% (RP_1), 90-95% (RP_2) and 85-99% (RP_3). Plants with RPG above 95% were selfed to produce BC_2F_2 generation in which homozygous recessive individuals would be identified. Those genotypes will be subjected to biochemical and phenotypic evaluation to confirm their nutritional and agronomical superiority. Finally, these β-carotene enriched lines, as well as the resulting improved hybrids, can be used in the biofortification programs.

Keywords: *β-carotene, crtRB1 gene, maize, marker assisted selection*.

Introduction

Vitamin A deficiency (VAD) is a public health problem and the leading cause of preventable childhood blindness, anaemia and weakened host resistance to infection, which can increase the severity of infectious diseases and risk of death (WHO, 2009). Since vitamin A cannot be synthesized inside the human body and it needs to be provided through diet, breeding for increasing provitamin A (ProVa) carotenoids in staple crops would be a viable strategy to minimize the adverse effects of VAD (Bouis *et al*., 2011; Muthusamy *et al.,* 2015).

One of the most important breeding objective is improving the ProVA content of maize grain. Two genes, *lcyE* on chromosome 8 and *crtRB1* on chromosome 10, have the most significant effect on ProVA concentrations in maize grain. According to Babu *et al*. (2013), *crtRB1* gene had a much larger effect on ProVA concentration than *lcyE*. Three polymorphisms were detected in *crtRB1* that were significantly associated with the β-carotene concentration in maize kernels (Yan *et al.,* 2010), of which *crtRB1* 3′TE favourable allele alone caused two to tenfold variations in the β-carotene concentration among the inbreds (Babu *et al.,* 2013; Muthusamy *et al.,* 2014). The 3′TE (transposable element) polymorphism of the gene that spans the 6th exon and the 3′-UTR (untranslatedregion) generates three allele sassociated with

altering β-carotene accumulation: *allele 1* (543 bp; without TE insertion), *allele 2* (296 bp+875 bp; with 325 bp TE insertion) and *allele 3* (296 bp+1221 bp+1880 bp; with 1250 bp TE insertion). *Allele 1* of the *crtRB1* gene is favourable and increases the level of β-carotene, whereas *allele 2* and *allele 3* cause unfavourable effects (Yan *et al.* 2010).

The accelerated development of β-carotene enriched maize using marker assisted selection (MAS) has been reported by Muthusamy *et al.*, (2014). As concluded by these authors, this breeding strategy holds immense promise as it precisely selects desirable plants and eliminates large scale biochemical estimation in the segregating generations. These β-carotene enriched lines, as well as the resulting improved hybrids, can be used in the biofortification programs alleviating VAD worldwide.

This paper is a part of the breeding program conducted at the Maize Research Institute "Zemun Polje" (MRI) with the aim to convert the standard maize to β-carotene rich genotypes adapted to temperate regions using the marker assisted selection. When SSR marker specific to the *crtRB1* gene has been tested on parental lines, distinct polymorphism was observed between the donor and recurrent parents (Kostadinović *et al.,* 2018). The same marker also clearly distinguished the heterozygous individuals from the homozygous dominants in BC_1 generation. The main objective of this research was the two-level selection procedure in BC_2 generation. First, BC_2 plants were analyzed with *crtRB1*-specific molecular marker to separate heterozygotes from dominant homozygotes. Second, the selected heterozygotes were screened with a set of polymorphic SSR markers distributed throughout the maize genome to identify genotypes with the highest recovery of recurrent parent's genome (RPG).

Material and Methods

Plant material

Three MRI commercial inbred lines adapted to the local environmental conditions in Serbia were used as the recurrent parents (RP_1, RP_2 and RP_3). Due to their excellent combining abilities, these lines are components of the leading MRI hybrids. As a donor of the favourable allele of *crtRB1* gene, i.e. β-carotene increased content, a line provided by the International Maize and Wheat Improvement Center (CIMMYT) was used.

The conversion process is given in Figure 1. F_1 plants were backcrossed onto reccurent parent line to generate BC_1 progeny. The BC_1 plants that were heterozygous for the *crtRB1* locus were selected for backcrossing. The results of this paper relate to the two-level selection procedure carried out in BC_2 generation (emphasized in Figure 1). First, BC_2 plants were analyzed with *crtRB1*-specific molecular marker to separate heterozygotes from dominant homozygotes. Second, the selected heterozygotes were screened with 30 polymorphic SSR markers distributed throughout the maize genome to identify genotypes with the highest recovery of recurrent parent's genome (RPG). Those plants were selfed to produce BC_2F_2 seeds.

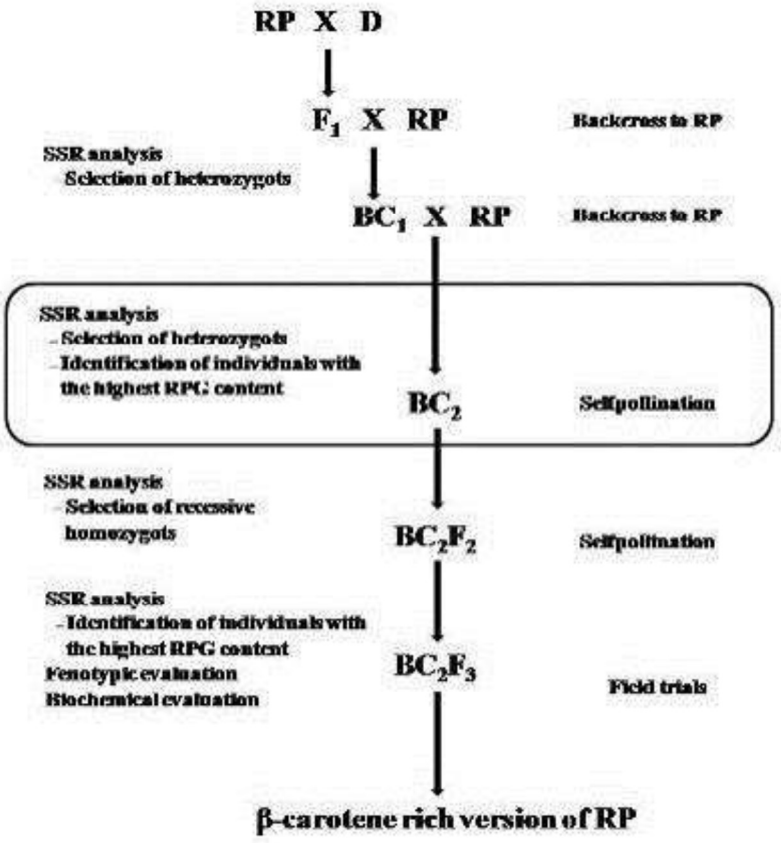

Figure 1. Schematic presentation of MAS for conversion of standard maize to β-carotene rich maize line (RP-recurrent parent, D-donor line, RPG-recurrent parent's genome)

DNA extraction

Genomic DNA was isolated from the four-weeks-old plants applying modified Dorokhov and Klocke protocol (1997). The DNA was quantified using biospectrometer (BioSpetrometer kinetic, Eppendorf) and diluted to a working concentration of 20 ng/μL.

Foreground selection

The set of primers presented in Table 1. has already shown codominant polymorphism between the donor and recurrent parents (Kostadinović *et al.,* 2018). Therefore, it was used in foreground selection for the *crtRB1* gene.

Table 1. The set of primers used as a foreground selection marker for the *crtRB1* gene

Primer	Sequence
crtRB1-3′TE-F	5′-ACACCACATGGACAAGTTCG-3′
crtRB1-3′TE-R1	5′-ACACTCTGGCCCATGAACAC-3′
crtRB1-3′TE-R2	5′-ACAGCAATACAGGGGACCAG-3′

Polymerase chain reaction was carried out in 20 μL reaction volume containing: DreamTaq™ Green PCR Master Mix (2X) (Thermo Scientific™, USA), 0.25 μM primers and 20 ng DNA template.

Background selection

Simple sequence repeat (SSR) analysis was done with 30 polymorphic SSR markers spanning over the whole genome, selected from the maize database (www.maizegdb.org) (Table 2). PCR was carried out in 25 µL reaction volume containing: DreamTaq™ Green PCR Master Mix (2X) (Thermo Scientific™, USA), 0.5 µM primers and 20 ng DNA template.

Table 2. The list of SSR markers used in background selection

	Name	Bin	Repeat	Sequence (forward and reverse)
1	umc1282	1.00	(AT)6	5'-TACACTACACGACTCCCAACAGGA-3'
				5'-GCGAGGGTTCTTTCCATAGAGAAT-3'
2	umc2230	1.05	(AGC)5	5'-ACGCGACGACTTCCACAAG-3'
				5'-ACACGTAATGTCCCTACGGTCG-3'
3	umc2047	1.09	(GACT)4	5'-GACAGACATTCCTCGCTACCTGAT-3'
				5'-CTGCTAGCTACCAAACATTCCGAT-3'
4	umc2129	2.07	(CGC)5	5'-ACGTGGTCATCACTCACCGC-3'
				5'-AAGGAGGAGCGTTCTCGTGG-3'
5	umc1265	2.02	(TCAC)4	5'-GCCTAGTCGCCTACCCTACCAAT-3'
				5'-TGTGTTCTTGATTGGGTGAGACAT-3'
6	umc1535	2.05	(AT)7	5'-GGCAGAGAGATGAAAAAGAATGGA-3'
				5'-CAAGGCACCCACACACATACATA-3'
7	bnlg1456	3.05	AG(15)	5'-CTCTAGGTGGTTAAGATTAACTCATT-3'
				5'-TTCATGAGGACCGTGTTGAA-3'
8	umc1273	3.08	(AAG)4	5'-GTTCGCTGCTGCTTCTTATATGCT-3'
				5'-AATTGGCGCAGGCTATAGACATTT-3'
9	bnlg1257	3.09	AG(28)	5'-CGGACGATCTTATGCAAACA -3'
				5'-ACGGTCTGCGACAGGATATT-3'
10	umc1418	4.08	(GGAAG)4	5'-TCACACACACACTACACTCGCAAT-3'
				5'-GAGCCAAGAGCCAGAGCAAAG-3'
11	bnlg1784	4.07	AG(13)	5'-GCAACGATCTGTCAGACGAA-3'
				5'-TTGGCATTGGTAATGGGTCT-3'
12	umc2360	4.08	(GCC)4	5'-TAGCAGCTAGCTTCAGTCACAGGC-3'
				5'-CAGATCGGACTACTGGTGGCTAAG-3'
13	bnlg1046	5.03	AG(39)	5'-TGAGCCGAAGCTAACCTCTC-3'
				5'-GATGCAAAGGAGGTTCAGGA-3'
14	umc2373	5.04	(GCT)4	5'-ACCCAAGTGAGGTGAAGTGAAGC-3'
				5'-TATGGTACAGGCACAGCAGCAGTA-3'
15	umc1792	5.08	(CGG)5	5'-CATGGGACAGCAAGAGACACAG-3'
				5'-ACCTTCATCACCTGCAACTACGAC-3'
16	umc1006	6.02	(GA)19	5'-AATCGCTTACTTGTAACCCACTTG-3'
				5'-AGTTTCCGAGCTGCTTTCTCT-3'
17	umc1887	6.03	(CGA)4	5'-CTTGCCATTTTAATTTGGACGTTT-3'
				5'-CGAAGTTGCCCAAATAGCTACAGT-3'
18	umc2375	6.06	(GCG)4	5'-GCCGTACTGATGTGATGGTCC-3'
				5'-TCTGACATTGTCCTCTTGACCAAA-3'
19	umc1324	7.03	(AGC)5	5'-ATCCATCATCATCATCATTGCTTG-3'
				5'-ATGTCATCATGTACCAGGTGTTGG-3'
20	umc1695	7.00	(CA)8	5'-CAGGTAATAACGACGCAGCAGAA-3'
				5'-GTCCTAGGTTACATGCGTTGCTCT-3'

21	umc1782	7.04	(GAC)4	5'-CGTCAACTACCTGGCGAAGAA-3' 5'-TCGCATACCATGATCACTAGCTTC-3'
22	bnlg1782	8.05	AG(13)	5'-CGATGCTCCGCTAGGAATAG -3' 5'-TGTGTTGGAAATTGACCCAA-3'
23	phi080	8.08	AGGAG	5'-CACCCGATGCAACTTGCGTAGA-3' 5'-TCGTCACGTTCCACGACATCAC-3'
24	umc2355	8.03	(CCT)5	5'-CTACTCCCCGAAGCCGTCTAAG-3' 5'-CGGGTTGTTGTTGGAGTAGGAC-3'
25	umc2393	9.00	(ACG)7	5'-CAACTCGATCCAGACCACACATAG-3' 5'-CTCTTGGTTGTTTGTTTCCTTGCT-3'
26	umc1040	9.01	(CT)11	5'-CATTCACTCTCTTGCCAACTTGA-3' 5'-AGTAAGAGTGGGATATTCTGGGAGTT-3'
27	umc1492	9.04	(GCT)4	5'-GAGACCCAACCAAAACTAATAATCTCTT-3' 5'-CTGCTGCAGACCATTTGAAATAAC-3'
28	umc1827	10.04	(GAC)6	5'-GCAAGTCAGGGAGTCCAAGAGAG-3' 5'-CCACCTCACAGGTGTTCTACGAC-3'
29	umc1506	10.05	(AACA)4	5'-AAAAGAAACATGTTCAGTCGAGCG-3' 5'-ATAAAGGTTGGCAAAACGTAGCCT-3'
30	bnlg1839	10.07	(AG)24	5'-AGCAGACGGAGGAAACAAGA-3' 5'-TCTCCCTCTCCCTCTTGACA-3'

The PCR and polyacrylamide gel electrophoresis, both in foreground and background selection, were performed as explained in Kostadinović *et al.,* (2018). In foreground selection, the size of the amplification products was determined comparing with the 100 bp molecular weight ladder. In background selection, SSR profiles were converted into a binary matrix based on the presence (1) or the absence (0) of a specific allele. Genetic similarity (GS) was calculated in accordance with Dice (1945): GSij = 2a/2a+b+c; where: a is the number of fragments present in both variety i and j (1,1), b is the number of fragments present in i and absent in j (1,0), c is the number of fragments absent in i and present in j (0,1). Marker data analyses were performed using statistical NTSYSpc2 program package (Rohlf, 2000).

Results and Discussion

The two-level marker assisted selection in BC_2 generation implied the application of molecular markers both for precise transfer of genomic regions of interest (foreground selection) and the recovery of the recurrent parent's genome (background selection). The first goal was identification of heterozygous plants with molecular marker specific for the *crtRB1* gene. Out of 180 analyzed plants (60 plants per line), 87 (48%) were identified as heterozygous and 93 (52%) as dominant homozygous, which was in accordance with the expected Mendelian ratio of 1:1 in backcross generations. Out of these 87 heterozygous individuals, 30 originated from RP_1, 28 from RP_2 and 29 from RP_3. Figure 2 shows the codominant nature of *crtRB1*-3′TE marker that can distinguish homozygous and heterozygous individuals. Lanes 3, 4, 5, 6, 7, 12 and 13 were heterozygous and lanes 8, 9, 10, 11 and 14 were dominant homozygous individuals.

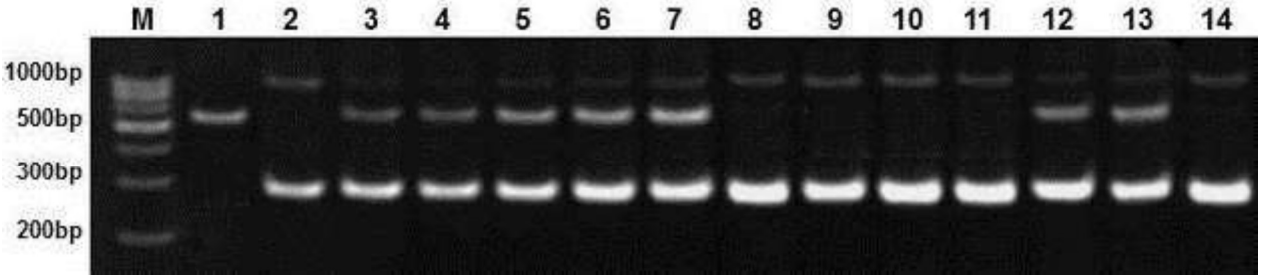

Figure 2. SSR profile of BC_2 individual plants detected with the *crtRB1*-specific marker. M:100bp DNA ladder, 1-donor parent, 2: recurrent parent, 3-14: BC_2 individuals.

Once the heterozygotes were identified, our second goal was to determine genetic similarity among these individuals and their recurrent parents with SSR markers distributed over the maize genome. The 30 SSRs was chosen for this analisys based on previously shown polymorphism (Kostadinovic *et al.,* 2018), since monomorphic markers that are not able to distinguish the two parental genotypes bear no value in selection work (Miah *et al.,* 2015). Total number of alleles detected with 30 informative markers was 44 for RP_1, 43 for RP_2 and 50 for RP_3, average being 1.47, 1.54 and 1.72, respectively. These results are similar to those previously reported in maize inbreds. In the study by Kanagarasu *et al.* (2013), the average number of alleles was 2.3, in Lopes *et al.* (2014) 2.6, while in Kostadinović *et al.* (2018) 2.5. Somewhat higher values in these studies can be explained by the higher number of analyzed genotypes and their diversity, as well as the number of markers used in the analysis (Mehta *et al.*, 2017).

Genetic similarity values between three analyzed parental lines and their corresponding BC_2 progenies, calculated using Dice coefficient, ranged from 0.85 to 0.99 (85-99% RPG). As explained in Muthusamy *et al.*, 2014, the variable proportion of RPG among BC_2 progenies is due to fixation of different proportion of recurrent parent alleles among the heterozygous plants. For each line separately, RPG values were: 86-97% (RP_1), 90-95% (RP_2) and 85-99% (RP_3). Average values for the RPG content ranged from 92 to 94%. Similar results have been reported in Muthusamy *et al.*, (2014), where RPG values ranged from 83.1% to 93.7%. Babu *et al.*, (2005), got the average RPG of 78.4% and they selected three plants with the highest values (93-96%) for developing the next generation. Thakur *et al.,* (2014) reported average RPG values from 90.1-97.2%, while Singh and Ram (2014) reported 82.5-98.5%.

In our study, a great acceleration of recipient genome recovery was achieved. Theoretically, the proportion of the RPG after *n* generations of backcrossing is given by $(2^{n+1} - 1)/2^{n+1}$ (Collard *et al.*, 2005). For BC_2 generation, that value should be 87.5%. In our case, 80% of progenies had RPG above this theoretical value. Another significant accomplishment were progenies with 99% of the RPG, the value theoretically achieved in BC_6 generation. These results confirmed that the combined approach of phenotypic and marker assisted selection reduces the time and economize the resources involved in the development of the favourable genotypes.

Plants with the highest RPG were self-pollinated to produce BC_2F_2 generation which will be subjected to the foreground selection before flowering to identify homozygous recessive individuals. Those genotypes will be subjected to biochemical and phenotypic evaluation to confirm their nutritional and agronomical superiority. Finally, these β-carotene enriched lines, as well as the resulting improved hybrids, can be used in the biofortification programs.

Conclusions

Being the codominant marker, *crtRB1*-3′TE clearly distinguished heterozygous individuals

from the homozygous dominants. That enables its utility in foreground selection for the *crtRB1*gene in our MAS program. Selected polymorphic SSR markers were employed in background selection to detect the recovery of the recurrent parent's genome. The RPG content of the BC_2 generation was between 85-99%. Progenies with the highest RPG values were selfed to produce BC_2F_2 generation for marker assisted introgression of the favourable allele of *crtRB1* into the local maize genotype. This study confirmed the efficiency of molecular markers both for foreground and background selection.

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References

Babu, R., Nair S.K., Kumar A., Venkatesh S., Sekhar J.C., Singh N.N., Srinivasan G., Gupta H.S. (2005). Two-generation marker-aided backcrossing for rapid conversion of normal maize lines to quality protein maize (QPM). Theor Appl Genet 111: 888-897.

Babu, R, Rojas N.P., Gao S., Yan J., Pixley K. (2013). Validation of the effects of molecular markerpolymorphisms in *lcyE* and *crtRB1* on provitamin A concentrations for 26 tropical maize populations. Theor Appl Genet 126: 389-399.

Bouis, H.E., Hotz C., McClafferty, B., Meenakshi, J.V., Pfeiffer, W.H. (2011). Biofortification: A new tool to reduce micronutrient malnutrition. Food and Nutrition Bulletin, 32, S31-40.

Collard B.C.Y, Jahufer M.Z.Z, Brouwer J.B, Pang E.C.K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica, 142: 169-196.

Dice L.R. (1945): Measures of the amount of ecologic association between species. Ecology, 26: 297-302.

Dorokhov, D.B., Klocke, E.A. (1997). A Rapid and Economic Technique for RAPD Analysis of Plant Genomes. Genetika, 33(4): 443-450.

Gupta, H.S., Babu R., Agrawal P.K., Mahajan V., Hossain F., Thirunavukkarasu N. (2013). Accelerated development of quality protein maize hybrid through marker-assisted introgression of *opaque-2* allele. Plant Breed 118: 77-82.

Kanagarasu, S., Nallathambi, G., Ganesan, K. N., Kannan, S., Shobhana V.G., Senthil N. (2013). Determination of genetic polymorphism among indigenous and exotic maize inbreds using microsatellite markers. African Journal of Biotechnology, 12(39): 5723-5728.

Kostadinović M., Ignjatović-Micić D., Vančetović J., Ristić D., Obradović A., Stevanović M., Mladenović Drinić S. (2018). Parental polymorphism analysis in marker assisted selection for β-carotene rich maize. Proceedings of the IX International Agricultural Symposium "Agrosym 2018", Jahorina, Bosnia and Herzegovina, October 4 - 7, 333-338.

Lopes, A. D., Scapim, C. A., Mangolin, C. A., Machado, M. F. P. S. (2014). Genetic Divergence Among Sweet Corn Lines Estimated by Microsatellite Markers. *Genetics and Molecular* Research, 13(4): 10415-10426.

Mehta, B., Hossain, F., Muthusamy, V., Baveja, A., Zunjare, R., Jha, S. K., Gupta, H. S. (2017). Microsatellite-based genetic diversity analyses of *sugary1*-, *shrunken2*- and

double mutant- sweet corn inbreds for their utilization in breeding programme. Physiology and Molecular Biology of Plants, 23(2): 411-420.

Miah G., Rafii M.Y., Ismail M.R., Puteh A.B., Rahim H.A., Latif M.A. (2015). Recurrent parent genome recovery analysis in a marker-assisted backcrossing program of rice (*Oryza sativa* L.) Comptes Rendus Biologies, 338: 83-94.

Muthusamy, V., Hossain F., Thirunavukkarasu N., Choudhary M., Saha S., Bhat J.S., Prassana M.B., Gupta H.S. (2014). Development of β-carotene rich maize hybrids through marker-assisted introgression of *β-carotene hydroxylase* allele. PLoS ONE 9(12): e113583. doi:10.1371/journal.pone.0113583.

Muthusamy V., Hossain F., Thirunavukkarasu N., Saha S., Gupta H.S. (2015). Allelic variations for *lycopene-ε-cyclase* and *β-carotene hydroxylase* genes in maize inbreds and their utilization in *β*-carotene enrichment programme. Cogent Food & Agriculture 1: 1-8.

Rohlf F.J. (2000): NTSYS-pc Numerical Taxonomy and Multivariate Analysis System version 2.1. Owner's manual.

Singh, R., Ram, L. (2014). DNA Aided Introgression of *Opaque-2* Allele for Development of Quality Protein Maize. International Journal of Science and Research, 3(11): 3006-3011.

Thakur, V.V., Tiwari, S., Tripathi, N. (2014). Studies on Genome Recovery Percentage for *Opaque2* Introgressed in Backcross Population for Marker Assisted Selection of Quality Protein Maize. Trends in Biosciences, 7(15): 1897-1901.

World Health Organization. Global prevalence of vitamin A deficiency in populations at risk 1995-2005. WHO Global Database on Vitamin A Deficiency. Geneva, World Health Organization, 2009.

Yan J., Kandianis B.C., Harjes E.C., et al. (2010). Rare genetic variation at *Zea mays crtRB1* increases b-carotene in maize grain. Nat Genet 42: 322-327.