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IDENTIFICATION OF β-CAROTENE RICH MAIZE INBREDS USING PCR – BASED ASSAY FOR *crtRB1*-3'TE ALLELE

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ABSTRACT
Carotene hydroxylase (*crtRB1*) is one of the important gene in the carotenoid biosynthetic pathway, significantly associated with variation for endosperm carotenoids in maize. There are three polymorphic sites within *crtRB1 viz.*, 5'TE, InDel4 and 3'TE. 3'TE (Transposable element) polymorphism of *crtRB1* gene has three alleles, among which 543bp allele (allele1/favourable allele) alone can double the β-carotene concentration in maize endosperm. Estimation of β-carotene requires HPLC which is very costly and laborious method. Favourable allele of *crtRB1*-3'TE gene is associated with higher accumulation of β-carotene and selection of this allele holds immense promise in reducing large scale phenotypic assays Therefore, screening maize inbreds for favourable allele of *crtRB1*-3'TE (PCR based assay) is an alternative for HPLC (High Performance Liquid Chromatography). To identify β-carotene rich maize inbreds we screened seventy inbred lines using PCR – based assay for *crtRB1*-3'TE gene specific marker and identified four inbreds (MGU 23379, MGU 23207, BAJIM 12-11 and CM 150) possessing allele1.

KEYWORDS: Maize, *crtRB1*-3'TE, PCR – based assay, β-carotene.

INTRODUCTION

Malnutrition has become a major health problem and affects millions of people worldwide. Among them, Vitamin A deficiency (VAD) affects over 250 million people and accounts for 70% of childhood deaths globally (Vignesh, 2012; Vignesh *et al*., 2012). Among all cereals, maize exhibits tremendous natural variation for provitamin A carotenoids (Menkir *et al*., 2008). Quantifying the provitamin A carotenoid using high performance liquid chromatography (HPLC) is expensive and time consuming. PCR-based assay can help in identifying genotypes with high β-carotene (Muthusamy *et al*., 2014; Muthusamy *et al*., 2015). Three genes (*psy*, *lcyE* and *crtRB1*) in the carotenoid biosynthesis pathway play a crucial role for the accumulation of carotenoids in maize kernel (Sagare *et al*. 2015). *crtRB1* is the vital gene with three polymorphic sites *viz*., 5'TE, InDel4 and 3'TE, significantly associated with variation for kernel carotenoids in maize (Yan *et al*., 2010). PCR based SSR markers for these polymorphisms pave the way for rapid improvement of provitamin A content in maize kernel (Zhang *et al.*, 2012). 3'TE (Transposable Element) polymorphism of *crtRB1* gene has three alleles (Yan *et al*. 2010), among which 543bp allele (allele1/favourable allele) alone can double the β-carotene concentration in maize kernel (Babu *et al*., 2013). Selection of maize inbreds with enhanced seed β-carotene content is possible by selecting for *crtRB1* allele (Dhyaneswaran, 2012). Therefore, the current study has been taken up to screen the maize germplasm for *crtRB1*-3'TE allele to identify β-carotene rich genotypes.

MATERIALS & METHODS

Plant materials

Seventy Maize germplasm lines (Table 1) were obtained from Indian Institute of Maize Research (IIMR), New Delhi, Maize Research Centre (MRC), Hyderabad, Institute of Biotechnology, Hyderabad and Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora and were sown at MRC, Hyderabad.

DNA isolation and PCR amplification of *crtRB1*-3'TE gene

DNA isolation was carried out using standard CTAB method of Plant genomic DNA isolation from three weeks old young seedlings of each of the inbred. Functional markers for *crtRB1*-3'TE gene used were, forward primer (F)- 5'ACACCACATGGACAAGTTCG3' and the reverse primers (R1) - 5'ACACTCTGGCCCATGAACA C3' and (R2) - 5'ACAGCAATACAGGGGACCAG 3' (Yan *et al*. 2010). PCR was carried out with genomic DNA using functional DNA markers in thermocycler. The reaction was carried out in 10 µl volumes, which contains 2 µl maize genomic DNA, 0.3 µl of F, R1 and R2 primers each, 1 µl dNTPs, 1 µl Taq buffer and 1 units of Taq polymerase. PCR amplification was carried out using the standard cycle conditions as given by Yan *et al*., 2010. The amplified products were resolved on 3.0% metaphor agarose gel and were scored for presence of allele1 (favourable allele) of *crtRB1*-3'TE gene.

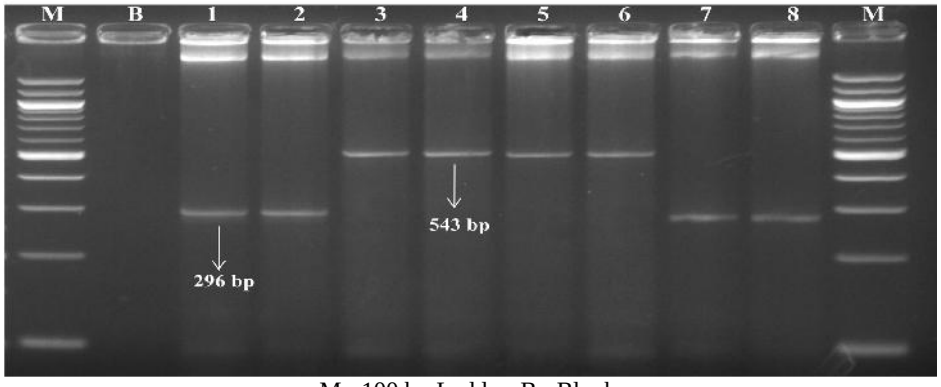
TABLE 1: List of the maize germplasm lines

Sr. No.	Inbred line	Sr. No.	Inbred line	Sr. No.	Inbred line
1	HYB-LM 16	25	MGU 23207	49	BML 2073
2	CM139	26	CM150	50	BML 2071
3	HKI 163	27	BQPML 54	51	BML 2602
4	V373	28	BQPML 5244	52	BML 2040
5	V345	29	BQPML 62	53	BML 2036-3
6	BM13	30	BQPML 199-2	54	BML 2035
7	HKI 1323	31	BQPML 63-1-3	55	BML 2020-1
8	LM16	32	BQPML 504-1	56	BML 2009/201
9	LM15	33	BQPML 5222-1	57	BML 2011/3
10	BAJIM 12-15	34	BQPML 5219	58	BML 200-7
11	BAJIM 12-14	35	BQPML 5207-4	59	BQPML 5219
12	HYB-LM 40	36	BQPML 5122	60	BML 5
13	CM-140	37	BQPML 412	61	CM 211
14	CM-143	38	BPCL 12-1	62	CM 202
15	HKI-161	39	BML 2095	63	BAJIM 12-1
16	HKI-193-1	40	BML2106	64	BAJIM 12-6
17	HKI-128	41	BML2094	65	BAJIM 12-14
18	V 351	42	BML 2091	66	BAJIM 12-13
19	CM 151	43	BML2088-3	67	BAJIM 12-12
20	INB-BML 2011-8	44	BML 2087	68	BAJIM 12-11
21	MGU 23379	45	BML 2080	69	BAJIM 12-10
22	MGU 23287	46	BML 2079	70	BAJIM 12-09
23	BML2	47	CBML6		
24	BML15	48	CBML7		

RESULTS & DISCUSSION

Among the seventy inbred screened, four inbreds *viz*., MGU 23379, MGU 23307, BAJIM 12-11 and CM 150 revealed the favourable allele of *crtRB1*-3'TE gene (Figure- 1), while rest (sixty-six inbreds) showed unfavourable alleles. Maize inbreds, MGU 23379, MGU 23307, BAJIM 12-11 and CM 150 are considered as β-carotene rich as they posses allele 1 of *crtRB1*-3'TE. Allele1 of *crtRB1*-3'TE alone can double the β-carotene concentration in maize kernel, irrespective of the genetic constitution of *crtRB1*-5'TE, *crtRB1*-InDel4 and *lcy*E (Babu *et al*., 2013). Estimation of provitamin A carotenoids requires HPLC which is very costly and laborious method. Favourable allele possessing rare genetic variation in β-carotene hydroxylase (*crtRB1*) gene is associated with higher accumulation of provitamin A, especially β-carotene and selection of this allele holds immense promise in reducing large scale phenotypic assays (Muthusamy *et al*., 2015). Previous studies have been reported a strong relation between allele1 of *crtRB1*-3'TE and β-carotene concentration in maize kernel (Pixley *et al*., 2011; Vignesh, 2012; Vignesh *et al*., 2012; Dhyaneswaran, 2012; Babu *et al*. 2013; Muthusamy *et al*. 2014; Muthusamy *et al*., 2015). Therefore, screening maize inbreds for favourable allele1 of *crtRB1*-3'TE (PCR based assay) is an alternative for HPLC (High Performance Liquid Chromatography) to identify β-carotene rich maize inbreds. Thus, in this study the four maize genotypes (MGU 23379, MGU 23207, BAJIM 12-11 and CM 150) identified with favourable allele of *crtRB1*-3'TE are rich in β-carotene. These maize inbreds can be used as β-carotene donor in Masrker assisted backcross breeding programme.

FIGURE 1: Allelic variation at 3' TE of *β-carotene hydroxylase* (*crtRB1*) gene



M : 100 bp Ladder; B : Blank

Lane 1 - 2 and Lane 7 - 8: Inbreds with unfavourable allele (296 bp)

Lane 3 - MGU 23379, Lane 4 - MGU 23207 Inbreds with favourable

Lane 5 - CM 150 and Lane 6 - BAJIM 12-11 allele (543 bp)

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