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Characterization of β-carotene rich MAS-derived maize inbreds possessing rare genetic variation in *β-carotene hydroxylase* gene

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Abstract

Traditional yellow maize kernels though contain enough carotenoids, the concentration of β-carotene is quite low. A rare natural variant of *crtRB1* gene enhances β-carotene in the kernel by blocking its conversion to further components. β-carotene rich versions of inbreds, VQL1, VQL2, V335, V345, HKI161, HKI323 and HKI1105 developed through marker-assisted backcross breeding strategy were characterized for selected DUS-characters, yield and yield, attributing traits. The *crtRB1*-introgressed inbreds displayed high degree of phenotypic resemblance with their respective original parents for majority of the characters. V335 based introgressed inbreds displayed 100% phenotypic similarity to original parent, followed by VQL1 (88.9-100%), HKI161 (88.9-100%), VQL2 (94.4%), V345 (88.9%), HKI323 (83.3-94.4%) and HKI1105 (77.8-83.3%) based introgressed inbreds. Introgressed inbreds having contrast for pigmentation in glume base and silk (with respective recurrent parents) possess great utility for registration and unambiguous identification in the field. These newly developed β-carotene rich maize inbreds hold enormous potential in maize biofortification programme.

Key words: β-carotene, biofortification, *crtRB1*, characterization, maize

Micronutrient malnutrition is a global problem particularly in developing countries, where people rely upon cereal based diets that are mostly deficient in micronutrients [1]. Among micronutrients, the deficiency caused by vitamin A has profound effects [2]. Vitamin A deficiency (VAD) causes a number of disorders like blindness, growth retardation, impaired iron mobilization, depressed immune response, and increased susceptibility to infectious diseases [3, 4]. VAD affects over 190 million pre-school children and 19 million pregnant and/or lactating women globally, mostly in Africa and South Asia [3].

Developing micronutrient rich staple foods using breeding methods, a process referred to as 'biofortification', is a sustainable and cost-effective approach [1, 5]. With wide genetic variation for kernel carotenoids, maize shows potential for vitamin A biofortification compared to other staple food crops. The dominant *phytoene synthase* (*Y1*) allele leads to synthesis of carotenoids and makes the kernel yellow in colour [6]. Though traditional yellow maize possesses high kernel carotenoids; non-provitamin A carotenoids such as lutein, zeaxanthin predominate, and provitamin A carotenoids *viz*., α-carotene, β-carotene and β-cryptoxanthin are quite low (0.25-2.50 µg/g), which is far below the prescribed daily requirement (15 µg/g) for humans [1, 7-10].

A natural variation in β-carotene hydroxylase (*crtRB1*) gene, identified in the temperate maize germplasm, increases provitamin A activity of maize grain by limiting the conversion of β-carotene into further components [11]. The *crtRB1*-favourable allele possessing 3'TE polymorphism (allele 1, 543 bp amplicon) associated with reduced transcript expression of the gene correlate with higher *β*-carotene concentration [12-14]. Recent efforts under the maize biofortification programme at IARI, New Delhi, led to introgression of the favourable allele of *crtRB1* gene in seven elite genetic backgrounds *viz*., VQL1, VQL2, V335, V345, HKI161, HKI323 and HKI1105 using marker-assisted selection (MAS) approach [15]. Characterization of these introgressed inbreds for their morphological characteristics holds significance for their objective utilization in the breeding programme. Further evaluation of these introgressed inbreds for distinctness

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from original inbreds is of prime importance for registration. Therefore, the present investigation was carried to characterize the introgressed inbreds for selected DUS-characters, yield and yield attributing traits.

Materials and methods

The *crtRB1*-based introgressed lines of the seven inbreds *viz*., VQL1, VQL2, V335 and V345 (early maturing) and HKI161, HKI323 and HKI1105 (medium maturing) derived through marker-assisted backcross breeding (MABB) strategy were selected for characterization. These inbreds are parents of two early- [Vivek QPM-9 (VQL1 × VQL2) and Vivek Hybrid-27 (V335 × V345)] and two medium- [HM-4 (HKI1105 × HKI323) and HM-8 (HKI1105 × HKI161)] maturing single cross hybrids adapted to diverse ecological regions of India. The introgressed inbreds have been denoted here with a 'PV' suffix to its original parents. Further 'A', 'B' and 'C' have been used to denote various versions of the inbreds. For example, the introgressed inbreds of VQL1 have been presented as VQL1-PV-A, VQL1-PV-B and VQL1-PV-C. These introgressed inbreds are rich in kernel β-carotene (mean: 14.1 µg/g) as compared to their original recurrent parents (mean: 1.4 µg/g) [15].

These BC_2F_4-based introgressed inbreds (BC_1F_4 for VQL2-based progeny) were evaluated for various morphological characteristics along with the irrespective recurrent and donor parents. These inbreds were grown under randomised complete block design (RCBD) with two replications at IARI Experimental Farm, New Delhi during *kharif* 2013. Morphological characters *viz*., (i) leaf angle (ii) leaf attitude (iii) brace root pigmentation (iv) glume base pigmentation (v) glume pigmentation (vi) anther pigmentation (vii) angle of tassel branch (viii) attitude of tassel branch and (ix) silk pigmentation were recorded as per DUS guidelines [16]. Besides, quantitative characters such as (i) plant height (ii) cob height (iii) days to 50% anthesis (iv) days to 50% silking (v) cob length (vi) cob girth (vii) number of kernel rows (viii) test weight (100 kernels) and (ix) grain yield were also recorded. Similarity for each of the introgressed inbreds was calculated as ratio of 'number of characters resembling respective recurrent parent' to the total number of characters, and expressed in percentage.

Results and discussion

Introgressed inbreds possessed similar characteristics of their respective recurrent parents for majority of characters *viz*., brace root, glume and anther pigmentation, angle of tassel branch, days to 50% silking, number of kernel rows, test weight and grain yield. VQL1- and HKI161-based inbreds showed 88.9-100% similarity with their respective recurrent parents (Table 1). Phenotypic similarity 94.4% was observed for VQL2-based MAS-derived inbred, while 100% similarity was seen in case of V335-based inbred. V345-based inbreds showed 88.9% similarity, whereas β-carotene rich versions of HKI323 exhibited 83.3-94.4% phenotypic similarity. MAS-derived versions of HKI1105 had 77.8-83.3% phenotypic resemblance to its recurrent parent (Table 1). This high degree of phenotypic similarity among these introgressed inbreds (with their respective recurrent parents) is attributed to the high recovery (83.1-93.7%) of recurrent parent genome (RPG) achieved through genome-based SSR markers used in the background selection [15, 17].

However, introgressed inbreds also differed from their respective recurrent parent for few characters, and resembled the characteristics of donor parent. For example, HKI1105 had pigmentation in glume base, but both the introgressed inbreds *viz*., HKI1105-PV-A and HKI1105-PV-B did not show any pigmentation in the base of the glume (Table 1). In case of silk pigmentation, both these introgressed inbreds lacked pigmentation in silk when compared to their recurrent parent, HKI1105 which had purple coloured silk. The introgressed inbred, VQL2-PV-A also lacked pigmentation in silk in contrast to the recurrent parent, VQL2 which possessed pigmentation in the silk. Similarly, HKI323-PV-B had straight leaf attitude, contrast to droopy leaf attitude of HKI323 (Table 1). Further, reduction in plant height was observed in the introgressed inbreds of HKI323, *viz*., HKI323-PV-A and HKI323-PV-B as compared to their original HKI323. Trend for increase in plant height was observed in one of the introgressed inbreds *viz*., V345-PV-A and HKI1105-PV-B, as compared to original inbreds. Cob height increased in case of VQL1-PV-B and VQL1-PV-C as compared to VQL1. Similar observation was recorded in case of V345-PV-A, while the same decreased in HKI161-PV-B, as compared to their respective recurrent parent. One of the introgressed inbreds of HKI1105 also showed difference for cob length. One each of the introgressed inbreds of VQL1, HKI323 and HKI1105 showed reduced cob girth as compared to original inbreds. However, another version of HKI1105 showed increase in cob girth (Table 1). Characteristics that showed difference between respective recurrent parent and introgressed inbreds are highly useful for registration of genotypes [18]. Besides, they also act as morphological marker to unambiguously differentiate the *crtRB1*-derived introgressed inbreds from the original inbreds in the field, especially during

Table 1. Morphological characteristics of β-carotene rich introgressed inbreds

S.No.	Genotypes	Generation	β-carotene (ìg/g)#	Contrasting characteristics*		% Phenotypic similarity*
				DUS-characteristics	Yield-related traits	
1.	VQL1-PV-A	BC_2F_4	17.5	-	-	100.0
2.	VQL1-PV-B	BC_2F_4	17.1	-	cob height	94.4
3.	VQL1-PV-C	BC_2F_4	16.4	-	cob height, cob girth	88.9
4.	VQL2-PV-A	BC_1F_4	16.3	silk pigmentation	-	94.4
5.	V335-PV-A	BC_2F_4	16.4	-	-	100.0
6.	V345-PV-A	BC_2F_4	13.4	-	plant height, cob height	88.9
7.	V345-PV-B	BC_2F_4	8.6	attitude of tassel branch	day to 50% anthesis	88.9
8.	HKI323-PV-A	BC_2F_4	9.2	-	plant height	94.4
9.	HKI323-PV-B	BC_2F_4	10.1	leaf attitude	plant height, cob girth	83.3
10.	HKI161-PV-A	BC_2F_4	16.0	-	-	100.0
11.	HKI161-PV-B	BC_2F_4	15.1	leaf angle	cob height	88.9
12.	HKI1105-PV-A	BC_2F_4	13.3	glume base pigmentation, silk pigmentation	cob length, cob girth	83.3
13.	HKI1105-PV-B	BC_2F_4	14.1	glume base pigmentation, silk pigmentation	plant height, cob girth	77.8

#=based on Muthusamy et al. [15]; *=Comparison with respective recurrent parents; -=represents no contrast.

large scale seed production and certification. The contrasting features are possibly due to the effects of minor proportion of donor genome (6.3-16.9%) present in the introgressed progenies, and/or its interaction with the RPG [15, 17]. Though multiple SSR markers per linkage groups were used in the background selection, some segment were not adequately covered leading to existence of minor proportion of donor parent genome [19].

Besides, some of the introgressed inbreds showed intermediate phenotype to their recurrent and donor parents. The introgressed inbred, V345-PV-B had curved attitude of tassel branch, whereas the recurrent parent, V345 had strongly curved tassel branch. Similarly, HKI161-PV-B showed wide leaf angle as compared to small leaf angle in HKI161. The appearance of new types not present in either of the parents could be due to segregation and fixation of new combination of genes/alleles (in introgressed progenies) contributed by both the recurrent and donor parents.

These introgressed inbreds are rich in kernel β-carotene (8.6-17.5 µg/g) as compared to their recurrent parents (1.3-1.5 µg/g) [15]. Analysis of variability for kernel carotenoids among large set of diverse maize inbreds (especially of Indian origin) revealed that mean kernel β-carotene is quite low (<1 µg/g) [8-10]. Thus newly developed β-carotene rich maize inbreds possessing rare genetic variation in *crtRB1* gene hold enormous potential to be utilized in the pro-vitamin A enrichment programme. Comprehensive characterization of these introgressed inbreds would help in their effective utilization in the breeding programme. Hybrids generated using these inbreds would help in alleviating vitamin A deficiency in humans as observed in earlier studies [15, 20].

The present study thus led to the comprehensive morphological characterization of *crtRB1*-based inbreds in the genetic background of VQL1, VQL2, V335, V345, HKI161, HKI323 and HKI1105. Introgressed inbreds showed high degree of resemblance with their respective recurrent parents for majority of morphological characters due to higher recovery of RPG undertaken during the MABB programme [15]. Some versions of introgressed inbreds showing contrast (from their original parent) for some of the morphological characters would be useful in registration and unambiguous identification in the field. These newly developed β-carotene enriched maize inbreds thus assumes great significance in alleviating vitamin A deficiency in humans.

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