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Biochemical characterization of elite maize (*Zea mays*) germplasm for carotenoids composition

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ABSTRACT

A set of 100 inbred lines comprising of 50 normal and 50 quality protein maize (QPM) were analyzed for carotenoids composition such as total carotenoids, β-carotene, β-cryptoxanthin and zeaxanthin. Seven QPM {HKI-3-4-8-6, HKI 34(1+2)-1, HKI 164-4(1-3), NP-06-07R-76-8, NP-06-07R-80-6, LQPM-42 and LQPM-40} and 9 normal lines {DML-288, DML-2, DML-112, DML-309, DML-45, BAJIM-08-27, BAJIM-13-1, HKI 1105 and HKI 1155} were found to possess significantly higher carotenoids as compared to the check (DMRQPM 103). Kernel colour intensity and total carotenoid contents showed highly significant positive correlation ($r = 0.491^{**}$), whereas no significant correlation was observed between kernel colour and β-carotene ($r = 0.014$). Based on the carotenoids studied, a set of 16 lines {HKI-3-4-8-6, HKI 34(1+2)-1, HKI 164-4(1-3), NP-06-07R-76-8, NP-06-07R-80-6, LQPM-42, LQPM-40, DML-288, DML-2, DML-112, DML-309, DML-45, BAJIM-08-27, BAJIM-13-1, HKI 1105 and HKI 1155} was identified as promising lines which can effectively be utilized in the future breeding programmes towards the development of nutritionally improved maize (*Zea mays* L.).

Key words: β-carotene, β-cryptoxanthin, Carotenoids, Maize, QPM, Zeaxanthin

Micronutrient malnutrition is a major concern globally, but most prevalent in the developing countries where majority of the population relies on staple foods like wheat, rice and maize (*Zea mays* L.) which are deficient in major micronutrients (Harrison 2010). Micronutrient deficiencies could be addressed through supplementation and food fortification, but biofortification (enriching the nutrition composition of staple crops through plant breeding) is a viable option. It is a cheap and sustainable approach which can easily reach the rural population. By producing staple foods whose edible portions are denser in bioavailable minerals and vitamins, farmers can produce crop varieties that naturally reduce anemia, cognitive impairment, and other nutritionally related health problems such as vitamin A deficiency (VAD) without compromising agronomic productivity (Nestel *et al.* 2006). It is seen as an upcoming strategy for dealing with deficiencies of micronutrients in the populations whose diet is heavily based on the staple grains (Ortiz-Monasterio *et al.* 2007) as compared to earlier approaches such as food fortification and supplementation (Nestel *et al.* 2006, Bouis and Welch 2010).

Maize occupies third place after wheat and rice and is a staple food for a large segment of population worldwide particularly in the Asian as well as African countries. Maize alone is responsible for providing 15% of the total protein and 20% of the total calories in the human diet (Sofi *et al.* 2009). In addition to its nutritional importance, maize represents one of the most important sources of carotenoids (Vallabhaneni *et al.* 2009, Kuhnen *et al.* 2011). The yellow maize kernel exhibits wide natural variation for carotenoids that may be exploited through plant breeding (Buckner *et al.* 1990). Improving the micronutrient balance of maize through biofortification is therefore an economically and socially sound way to address micronutrient malnutrition, including VAD, on global scale (Tanumihardio *et al.* 2008). Quality protein maize (QPM), a nutritionally improved form of maize, contains 2-3 times higher concentration of essential amino acids such as tryptophan and lysine than normal maize. However, limited information is available on the carotenoids composition of QPM and normal maize elite germplasm from India. Thus, a clear need emerges to assess the genetic variability for micronutrients particularly carotenoids composition such as total carotenoids, β-carotene, β-cryptoxanthin and zeaxanthin in order to use this information in developing cultivars that are nutritionally superior. The present study was therefore conducted to evaluate carotenoids composition in elite lines and identify

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potential donors for developing nutritionally superior maize cultivars.

MATERIALS AND METHODS

A diverse panel of 100 inbred lines (50 QPM and 50 normal) developed at the Indian Institute of Maize Research (IIMR), New Delhi; Hill Agricultural Research and Extension Centre (HAREC), Bajaura (HPKV, Palampur) and CIMMYT was used in this study. The lines were selected with diverse genetic background, showing better agronomic performance, resistance to major diseases and insect-pests of maize prevalent in various maize growing ecologies. The lines differed for their phonological and agronomic characteristics and its attributes, viz. flowering, maturity, plant height, seed colour, cob length, cob girth etc. The QPM lines showing higher lysine and tryptophan were included for study. The material represents seed kernel color from white to dark orange. The inbred DMRQPM 103, a line registered with the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India was used as check. Full panel of inbred lines along with check was grown at Delhi and Bajuara (25 QPM and 25 normal at each center). Self pollinated ears from each entry (three replications) were harvested at maturity stage; seeds were shelled under shade and stored in dark at 4°C to prevent carotenoids degradation by high temperature and light. The different lines were visually sorted with respect to their kernel color and classified as dark orange, orange, yellow and pale yellow, and color intensity was correlated with the total carotenoid as well as β-carotene content. The QPM samples were confirmed for protein quality (lysine, tryptohan and protein content) and subjected to biochemical analysis for carotenoids composition. Individual samples were ground into fine powder using a Cyclotech Mill (Model 1093, FOSS, Sweden).

Carotenoids extraction was carried out in powdered samples under dim light to prevent the photo-oxidation as per the method of Rodriguez-Amaya and Kimura (2004). Total carotenoids were measured at 450 nm absorbance and calculated using the formula given by Rodriguez-Amaya and Kimura (2004). Carotenoids composition was estimated using ultra performance liquid chromatography (UPLC) system (Waters). 5 µl injection volume was used for the UPLC-PDA analysis carried out using ACQUITY Ultra performance LC^{TM} system linked to a photodiode array (PDA) 2996 detector (Waters, Milford, MA,USA). Empower version 2 software was used for data acquisition and processing. UPLC chromatographic separation was performed on a reverse-phase column ACQUITY UPLC^{R} BEH 130A^{0} C18, 1.7 µm, 2.1 × 100 mm (Waters) with the mobile phase consisting of solvent A {ACN:MeOH (7:3 v/v)} and solvent B; HPLC grade water (100%). Sample and column temperatures were set at 25°C and 32°C, respectively. Samples concentration of various carotenoids was calculated based on the performance of standards (β-carotene, β-cryptoxanthin, zeaxanthin) procured from SIGMA Chemical, USA.

Data was subjected to descriptive statistics and analysis of variance (ANOVA) was conducted using SAS 9.2 Software. Pearson's simple correlation coefficient between different carotenoid components and kernel colour were computed using mean values.

RESULTS AND DISCUSSION

Variability for different traits in normal and quality protein maize

Significant variation was observed in carotenoids of QPM germplasm (Table 1). Total carotenoids in QPM germplasm varied from 2.66 (CML 153) to 75.96 (LQPM-24) µg/g. Most of the QPM lines accumulate between 10-40 µg/g of total carotenoids, whereas 10 lines exhibited between 40-50 µg/g, and the same number of lines was found to possess more than 50 µg/g total carotenoids. β-carotene, the primary pro-vitamin A component, varied significantly from 0 to 4.58 [NP-06-07R-76-8] µg/g, whereas, β-cryptoxanthin, another important vitamin A precursor, also varied widely from 0 to 4.58 µg/g (Table 1) with the highest value being exhibited by NP-06-07R-76-8. The inbred NP-06-07R-76-8 was found to be the best QPM line having highest concentrations of β-carotene and

Table 1 Kernel composition of total carotenoids, β-carotene, β- cryptoxanthin and zeaxanthin content of normal and QPM lines

Maize type	Total carotenoids (µg/g)		β-carotene (µg/g)		β- Cryptoxanthin (µg/g)		Zeaxanthin (µg/g)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
QPM	35.02	2.66-75.96	2.12	0-4.58	1.93	0-3.38	10.22	0-18.08
Normal	37.57	1.67-64.06	2.14	0-4.54	1.90	0-3.31	10.15	0-18.55
Promising QPM lines	HKI 161, HKI-3-4-8-6, LQPM-24, LQPM-43, CML169		HKI 161, HKI 34(1+2)-1, HKI 164-4(1-3)-1, HKI-3-4-8-6, LQPM-43, LQPM-40, NP-06-07R-76-8, NP-06-07R-80-6		HKI-3-4-8-6, LQPM-15-1, NP-06-07R-76-8, LQPM-42, LQPM-33, LQPM-13		DMRQPM 102, HKI 162-2-3, NP-06-07R-76-8, LQPM-42, LQPM-33, LQPM-13	
Promising normal lines	DML-268, DML-310, DML-288, BAJIM-13-8, BAJIM-08-26		LM-5, DML-2, DML-288, HKI 1105, HKI 1155		DML-310, DML-308, DML-39		DML-308, DML-309, BAJIM-13-5, BAJIM-13-1, BAJIM-13-3, DML-39	

β-cryptoxanthin. It also accumulated high concentration of zeaxanthin (16.90 µg/g), which is an important carotenoid having health benefits ranging from maintaining normal vision to reducing oxidative stress. Seven QPM lines (HKI-3-4-8-6, HKI 34(1+2)-1, HKI 164-4(1-3), NP-06-07R-76-8, NP-06-07R-80-6, LQPM-42 and LQPM-40) were found to be promising for carotenoids as compared to the check DMRQPM 103. QPM has almost double the quantity of two essential amino acids, lysine and tryptophan than normal maize. The development of QPM was considered a significant breakthrough as it helps in alleviating protein energy malnutrition in populations eating maize as staple food. Further characterization of QPM for carotenoids and the identification of carotenoids rich QPM germplasm will complement maize breeding for enhanced nutritive value.

Normal maize germplasm also showed wide variability for carotenoids (Table 1). Total carotenoids ranges from 1.67 (CML 121) to 64.06 (DML-268) µg/g. β-carotene, β-cryptoxanthin and zeaxanthin concentrations ranged from 0 to 4.54 (LM-5), 0 to 3.31 (DML-308) and 0 to 18.55 (DML-39) µg/g, respectively (Table 1) implying that an almost 4 times natural variation exists for pro-vitamin A carotenoids in the studied germplasm. DML-288 was found to be the best line followed by DML-2, HKI 1105, HKI 1155, DML-112, DML-309, BAJIM-13-1, DML-45 and BAJIM-08-27 in terms of carotenoids composition. ANOVA revealed significant variation for total carotenoids, β-carotene, β-cryptoxanthin and zeaxanthin among all the 100 inbred lines, indicating the possibility of genetic improvement for the target trait in the breeding programme. The normal lines of Delhi showed higher total carotenoids as compared to QPM lines (Fig 1). Amongst different subgroups highest variability was observed within normal germplasm from Delhi center for β-carotene (Fig 2) and β-cryptoxanthin as well as zeaxanthin. Similar genetic variability for total carotenoid content in maize has also been reported earlier with a range of 9.46 to 42.84 µg/g (Cardoso *et al.* 2009) and 9.9 to 40.0 µg/g (Hulshof *et al.* 2007). It has been reported that predominant carotenoids in maize kernels, in decreasing order of concentration are zeaxanthin, β-carotene and β-cryptoxanthin (Safawo *et al.* 2010). Almost similar trend was observed in our study as zeaxanthin is found to be the major carotenoid followed by β-carotene and β-cryptoxanthin. β-carotene contains two provitamin A structures (two nonhydroxylate β-ionine rings) and β-cryptoxanthin contains one in single nonhydroxylated β-ionone ring which emphasizes on increasing β-carotene through maize biofortification (Safawo *et al.* 2010).

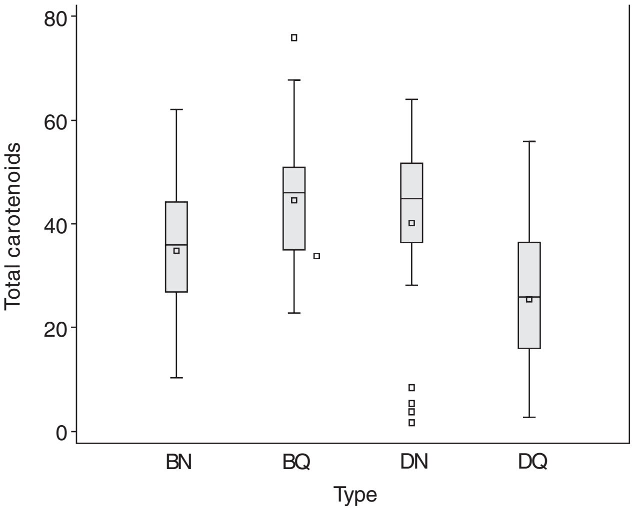

Fig 1 Variability for total carotenoids (µg/g) in the experimental germplasm (BN: Bajaura normal, BQ: Bajaura QPM, DN: Delhi normal, DQ: Delhi QPM)

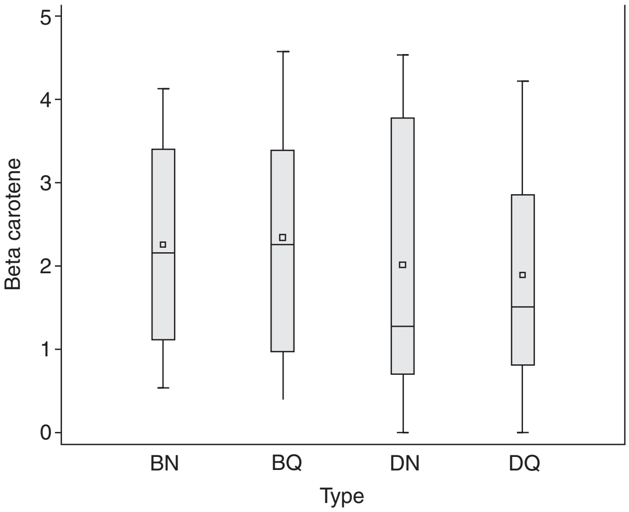

Fig 2 Variability for β-carotene (µg/g) in the experimental germplasm (BN: Bajaura normal, BQ: Bajaura QPM, DN: Delhi normal, DQ: Delhi QPM)

Based on the carotenoids composition including total carotenoids, β-carotene, β-cryptoxanthin and zeaxanthin, some most promising genotypes were identified which can be used as potential source of carotenoids for the development of nutritionally enriched maize hybrids. HKI-3-4-8-6, HKI 34(1+2)-1, HKI 164-4(1-3), NP-06-07R-76-8, NP-06-07R-80-6, LQPM-42, LQPM-40, DML-288, DML-2, DML-112, DML-309, DML-45, BAJIM-08-27, BAJIM-13-1, HKI 1105 and HKI 1155 were identified as most promising, micronutrients enriched inbreds.

Character association

There was a significant and positive correlation between total carotenoids and β-carotene in both QPM (r=0.481; P<0.01) and normal (r=0.384; P<0.01) maize accessions, whereas a highly positive correlation between total carotenoids and β-cryptoxanthin (r=0.494; P<0.01) and total carotenoids and zeaxanthin (r=0.469; P<0.01) only in case of normal maize accessions (Table 2). The present investigation did not observe any co-linearity between total carotenoids and β-carotene and therefore highlighted that selecting inbreds for provitamin A based on total carotenoids content may not always be effective. Both in case of QPM and normal maize accessions, β-cryptoxanthin and zeaxanthin showed highly significant positive correlation (r values 0.923 and 0.952, respectively

Table 2 Correlation among different carotenoids contents in maize

Maize type	Total carotenoid vs β-carotene	β-carotene vs β-cryptoxanthin	β-cryptoxanthin vs zeaxanthin	Total carotenoid vs β-cryptoxanthin	Total carotenoid vs zeaxanthin	β-carotene vs zeaxanthin
QPM	0.481^{**}	0.045	0.923^{**}	0.121	0.104	0.012
Normal	0.384^{**}	0.233	0.952^{**}	0.494^{**}	0.469^{**}	0.212

*, **Significant at 5% and 1% , respectively.

at $P<0.01$). Apart from β-carotene and β-cryptoxanthin, which are the major provitamin A carotenoids, maize is also a good source of zeaxanthin which plays beneficial role in human health (Krinsky *et al.* 2003). A positive correlation between these two components implies that breeding for both β-cryptoxanthin and zeaxanthin could be strategized simultaneously. Increased dietary intake of zeaxanthin has been associated with lowering the risk of cataracts, age-related macular degeneration and other degenerative diseases (Mares 2013, Abdel-Aal *et al.* 2013). The whole germplasm studied was categorized into five classes based on colour as white, light yellow, yellow, dark yellow and orange. In present study, orange coloured kernels had the highest total carotenoid concentration followed by dark yellow, yellow, light yellow and white kernels. Sivaranjani *et al.* (2013) also reported similar relationship between kernel colour and carotenoids concentration. Pearson correlation coefficient between kernel colour intensity and carotenoids was computed to access the relationship between kernel colour and total carotenoids content which showed highly significant positive correlation ($r = 0.491^{**}$), whereas no significant correlation was observed between kernel colour and beta-carotene content ($r = 0.014$). Maize contains a number of carotenoids such as beta carotene, beta cryptoxanthin, zeaxanthin and lutein. The contribution of beta carotene to the total carotenoids in maize is very little which might be reason for the absence of any correlation between kernel colour and beta carotene. The orange color of endosperm was found positively correlated with total carotenoids, but with a very weak positive correlation with provitamin A carotenoids (Harjes *et al.* 2008). Other findings also related the yellow color of maize kernels to the non provitamin A carotenoids indicating that markers assisted selection may prove much more efficient than selection based on colour for higher provitamin A maize (Quackenbush *et al.* 1961, Weber 1987). It also implied that visual selection for β-carotene cannot be undertaken in breeding for high β-carotene maize. Recently, Chandler *et al.* (2013) identified a QTL in B73 × T × 303 in the vicinity of the *crtRB1* gene on chromosome 10. Earlier, it has been shown that *crtRB1* alleles associated with higher β-carotene concomitantly decreased total carotenoid content in the kernel, indicating that selection for *crtRB1* alleles that increase β-carotene will result in a decrease of orange color (Yan *et al.* 2010). In the present study also, highest total carotenoids were found present in orange coloured kernels, whereas a low range for β-carotene was observed.

Prospects of micronutrient enriched maize

The final, permanent solution to micronutrient malnutrition in the developing countries is to make available staple foods that are dense in minerals and vitamins to provide a low-cost, sustainable strategy. Maize has successfully been targeted for biofortification for quality protein maize (Prasanna *et al.* 2001, Vasal 2001, Babu *et al.* 2005, Atlin *et al.* 2011, Gupta *et al.* 2013). The primary pre-requisite towards the development of biofortified maize is the availability of natural variation in the germplasm stock. A very low level of natural variation (0.02-1.75 μg/g) has earlier been reported for maize kernel β-carotene (Muthusamy 2014, Chandler *et al.* 2013). Chander *et al.* (2008) also observed very low level of carotenoids in a set of 87 Chinese inbreds. However, the variability in the range of 0 to 4.58 for β-carotene and a similar variation in β-cryptoxanthin, another provitamin A component, observed in the present germplasm may effectively be exploited for the development of micronutrient enriched maize.

From the above discussion it can be concluded that large variability existed in the carotenoid composition among QPM as well as normal maize germplasm. A number of QPM lines with high pro-vitamin A components have been identified, which can be used as a potential source germplasm for the development of biofortified maize. Based on the carotenoids studied, a set of 16 lines { HKI-3-4-8-6, HKI 34(1+2)-1, HKI 164-4(1-3), NP-06-07R-76-8, NP-06-07R-80-6, LQPM-42, LQPM-40, DML-288, DML-2, DML-112, DML-309, DML-45, BAJIM-08-27, BAJIM-13-1, HKI 1105 and HKI 1155} was identified as promising donors which can be utilized in the breeding programes towards the development of nutritionally improved maize.

REFERENCES

Abdel-Aal E M, Akhtar H, Zaheer K and Ali R. 2013. Dietary sources of lutein and zeaxanthin carotenoids and their role in eye health. *Nutrients* **5**: 1169–85.

Atlin G N, Palacios N, Babu R, Das B, Twumas-Afriyie S, Friesen D K, Groote H D, Vivek B and Pixley K V. 2011. Quality protein maize: Progress and prospects. *Plant Breeding Reviews* **34**: 83–130.

Babu R, Nair S K, Kumar A, Venkatesh S, Sekhar J C, Singh N N, Srinivasan G and Gupta H S. 2005. Two-generation marker-aided backcrossing for rapid conversion of normal maize lines to quality protein maize (QPM). *Theoretical and Applied Genetics* **111**: 888–97.

Bouis H E and Welch R M. 2010. Biofortification- a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Science* **50**: S20-S32.

Buckner B, Kelson T L and Robertson D S. 1990. Cloning of the y1 locus of maize, a gene involved in the biosynthesis of carotenoids. *Plant Cell* **2**: 867–76.

Cardoso W S, Peas M C D, Galvao J C C, Rios S D, Guimaraes P E D, Schaffert R E and Borem A. 2009. Variability of maize genotypes for grain carotenoid composition. *Pesquisa Agropecuária Brasileira* **44**: 164–72.

Chander S, Meng Y, Zhang Y, Yan J and Li J. 2008. Comparison of nutritional traits variability in selected eighty-seven inbreds from Chinese maize (*Zea mays* L.) germplasm. *Journal of Agriculture and Food Chemistry* **56**: 6506–11.

Chandler K, Lipka A E, Owens B F, Li H, Buckler E S, Rocheford T and Gore M A. 2013. Genetic analysis of visually scored orange kernel color in maize. *Crop Science* **53** :189–200.

Gupta H S, Babu R, Agrawal P K, Mahajan V, Hossain F and Nepolen T. 2013. Accelerated development of quality protein maize hybrid through marker-assisted introgression of *opaque-2* allele. *Plant Breeding* **132**: 77–82.

Harjes C E, Rocheford T R, Bai L, Brutnell T P, Kandianis C B, Sowinski S G, Stapleton A E, Vallabhaneni R, Williams M, Wurtzel E T, Yan J and Buckler E S. 2008. Natural genetic variation in *Lycopene Epsilon Cyclase* tapped for maize biofortification. *Science* **319**: 330–3.

Harrison G G. 2010. Public health interventions to combat micronutrient deficiencies. *Public Health Reviews* **32**: 256–66.

Hulshof P J M, Schuil T K, West C E and Hollman P C H. 2007. Quick screening of maize kernels for provitamin A content. *Journal of Food Composition Analysis* **20**: 655–61.

Krinsky N I, Landrum J T and Bone R A. 2003. Biological mechanism of the protective role of lutein and zeaxanthin in the eye. *Annual Review Nutrition* **23**: 171–201.

Kuhnen S, Lemos P M, Campestrini L H, Ogliari J B, Dias P F and Maraschin M. 2011. Carotenoid and anthocyanin contents of grains of Brazilian maize landraces. *Journal of the Science of Food and Agriculture* **91**: 1 548–53.

Mares J A. 2013. Relationships of lutein and zeaxanthin to age-related macular degeneration: Epidemiological evidence. (*In*) *Carotenoids and Retinal Disease*. Landrum J and Nolan J (Eds). Francis and Taylor, CRC Press, New York.

Muthusamy V, Hossain F, Nepolean T, Choudhary M, Saha S, Bhat J S, Prsanna B M and Gupta H S. 2014. Enrichment of kernel β-carotene in maize hybrids using marker-assisted backcross breeding strategy. (*In*) *Proceedings of 2nd Asian Maize Conference and Expert Consultation on Maize for Food, Feed, Nutrition, and Environmental Security,* Bangkok, Thailand, pp 207–13.

Nestel P, Bouis H E, Meenakshi J V and Pfeiffer W. 2006. Biofortification of staple food crops. *Journal of Nutrition* **136**: 1 064–7.

Ortiz-Monasterio I, Palacious-Rojas N, Meng E, Pixley K V, Trethowan R M and Pena-Bautista R J. 2007. Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *Journal of Cereal Science* **46**: 293–307.

Prasanna B M, Vasal S K, Kassahun B and Singh N N. 2001. Quality protein maize. *Current Science* **81**: 1 308–19.

Quackenbush F W, Firch J G, Rabourn W J, McQuistan M, Petzold W N and Kargl T E. 1961. Composition of corn, analysis of carotenoids in corn grain. *Journal of Agriculture and Food Chemistry* **9**: 132–5.

Reddy B V S, Ramesh S and Longvah T. 2005. Prospects of breeding for micronutrients and β-carotene-dense sorghums. *International Sorghum Millet Newsletter* **46**: 10–4.

Rodriguez-Amaya D B and Kimura M. 2004. HarvestPlus handbook for carotenoid analysis. Washington DC.

Safawo T, Senthil N, Raveendran M, Vellaikumar S, Ganesan K N, Nallathambi G, Saranya S, Shobhana V G, Abirami B and Gowri E V. 2010. Exploitation of natural variability in maize for β - carotene content using HPLC and gene specific markers. *Electronic Journal of Plant Breeding* **1**: 548–55.

Sivaranjani R, Prasanna B M, Hossain F and Santha I M. 2013. Genetic variability for total carotenoid concentration in selected tropical maize (*Zea mays*) inbred lines. *Indian Journal of Agricultural Sciences* **83**: 431–6.

Sofi P A, Wani S A, Rather A G and Wani S. 2009. Review article: Quality protein maize (QPM): Genetic manipulation for the nutritional fortification of maize. *Journal Plant Breeding and Crop Science* **1**: 244–53.

Tanumihardio S A, Bouis H, Hotz C, Meenakshi J V and McClafferty B. 2008. Biofortification of staple crops: an emerging strategy to combat hidden hunger. *Comprehensive Reviews on Food Science and Food Safety* **7**: 329–34.

Vallabhaneni R, Gallagher C E, Licciardello N, Cuttriss A J, Quinlan R F and Wurtzel E T. 2009. Metabolite sorting of a germplasm collection reveals the Hydroxylase3 locus as a new target for maize provitamin A biofortification. *Plant Physiology* **151**: 1635–45.

Vasal S K. 2001. Quality protein maize development: An exciting experience. (*In*) *Proceeding of Seventh Eastern and South Africa Regional Maize Conference,* Kenya, pp 3–6.

Weber E. 1987. Carotenoids and tocols of corn grain determined by HPLC. *Journal of American Oil Chemist Society* **64**: 1 129–34.

World Health Organization. 2009. Global prevalence of vitamin A deficiency in population at risk 1995-2005. (*In*) WHO global database on vitamin a deficiency, Geneva, pp 1–55.

Yan J B, Kandianis C B, Harjes C E, Bai L, Kim E H, Yang X H, Skinner D J, Fu Z Y, Mitchell S, Li Q, Fernandez M G S, Zaharieva M, Babu R, Fu Y, Palacios N, Li J S, DellaPenna D, Brutnell T P, Buckler E S, Warburton M L and Rocheford T. 2010. Rare genetic variation at *Zea mays crtRB*1 increases b-carotene in maize grain. *Nature Genetics* **42**: 322–7.