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Variability for functional and nutritional quality traits in sweet pepper (*Capsicum annuum* L.)

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ABSTRACT

Natural biodiversity for functional and nutritional quality traits is of prime importance in breeding programmes for developing nutritionally rich genotypes. The present investigation was carried out to identify lines of sweet pepper with high ascorbic acid content and important mineral nutrients like potassium, phosphorus, zinc, copper, iron and manganese. Forty accessions of sweet pepper (*Capsicum annuum* L.) were analyzed for their functional and nutritional composition. Wide variation was observed in functional quality traits like ascorbic acid content (22-129mg $100g^{-1}$), and β-Carotene (0.39-1.0mg $100g^{-1}$) suggesting a considerable level of genetic diversity. Wide variability was also noticed for nutritional composition (K, P, Zn, Cu, Fe & Mn) in the tested lines. Across accessions, concentration of ascorbic acid was negatively correlated with copper content ($r = -0.293$, $p < 0.05$) being significantly greater in two accessions, VHC 34 and VHC 37 (129 and 118.0 mg$100g^{-1}$, respectively) compared to other accessions. β-Carotene concentration was higher (0.85 to 0.99mg $100g^{-1}$) in six accessions, and lower (0.39 to 0.54mg $100g^{-1}$) in twenty four accessions. Greater variability present for quality traits holds an immense potential to help develop *Capsicum* lines with traits of high functional and nutritional quality. Therefore, this information is potentially useful in sweet pepper breeding programmes in the future.

Key words: Sweet pepper, variability, correlation, nutritional quality

INTRODUCTION

Exploring natural biodiversity as a source of novel alleles to improve productivity, adaptation, quality and nutritional value of crops is of prime importance in 21st Century breeding programmes (Fernie *et al*, 2006). Efforts are on to improve the quality of not only grains but also vegetable crops (Romer *et al*, 2000). Sweet pepper (*Capsicum annuum* L.) is an important vegetable not only because of its economic importance, but also for its nutritional value, mainly as an excellent source of natural red colour owing to the pigment capsanthin, and antioxidant compounds (Lee *et al*, 1995; Howard *et al*, 2000). Capsicum is an excellent source of vital micronutrients and vitamins such as Vitamin C and Vitamin E (Minguez-Mosquera & Hornero-Mendez, 1994; Daood *et al*, 1996; Gnayfeed *et al*, 2001). A wide spectrum of antioxidant compounds is present in fruits of pepper viz., Vitamin 'E', 'C' and β-carotene; phenolic compounds, carotenoides and xanthophylls. Levels of antioxidants vary among accessions. Generally, hot peppers are a better source of these than sweet ones (Daood *et al*, 1996; Gnayfeed *et al*, 2001). Antioxidant vitamin 'C', an important compound in pepper fruits, chelates heavy metal ions (Namiki, 1990), reacts with singlet oxygen and other free radicals, and suppresses peroxidation thereby reducing the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer (Harris, 1996). Carotenoids, vitamin 'E' and vitamin 'C' are located in the pericarp of pepper fruit, whereas, capsaicinoids are distributed in different parts of the fruit. Studies on evaluation of variability, especially for quality in given sets of germplasm, is lacking in sweet pepper. Therefore, the present study was undertaken to analyze extent of variability in nutritional quality present in the available germplasm of sweet pepper, to breed for varieties and hybrids with high nutritional quality.

MATERIAL AND METHODS

Plant material

Forty lines of capsicum (*Capsicum annuum* L.) including exotic types, landraces and cultivars were grown

in the field during *kharif* season (April–August) of 2008 and 2009 at the experimental farm, Hawalbagh (29°36' N, 79°40' E, 1250m above msl). Fruits were harvested at the mature green stage. Three replicates of 40 lines were analyzed for various functional and nutritional attributes: VHC 10, VL Shimla Mirch 2, VHC 15, VHC 13, VHC 45, California Wonder, VHC 19, VHC 21, VHC 23-1, VHC 23-2, VHC 24, VHC 25, VHC 42, VHC 41-1, VHC 40-1, VHC 26, VHC 27, VHC 43, VHC 41-2, VHC 20, VHC 28, VHC 29, VHC 30, VHC 46, VHC 31, VHC 32, VHC 33, VHC 34, VHC 36, VHC 37, VHC 38, VHC 39, VHC 40-2, VHC 44, VHC 4, VHC 6-1, VHC 6-2, VHC 3, VHC 2 and VHC 22.

Chemicals and reagents

All chemicals and reagents were procured from Merck India Ltd. Double-distilled water was used throughout the analysis.

Chromatographic condition

For estimation of β-carotene and ascorbic acid content in mature green fruits, HPLC system (Shimadzu, Japan) was operated equipped with a Hitachi pump (L-7100) UV-VIS detector (L-7400) controlled by Win Chrom chromatographic software. The HPLC column used was Purospher®, RP- C_{18} (4.6 × 250mm I.D.; 5μ). Samples were injected in 20μl volumes at ambient temperature. Quantification of β-carotene /ascorbic acid in the samples was achieved by comparing each peak retention time and area with the Standard.

Chemical analysis

Determination of ascorbic acid content

L-Ascorbic acid (LAA) was extracted and quantified by HPLC as per Abdulnabi *et al* (1997), with minor modifications. The sample (10g) was homogenized with a solution (10ml) containing meta-phosphoric acid (0.3M) and acetic acid (1.4M) for 15 minutes at room temperature. The mixture was filtered through Whatman No. 4 filter paper to obtain a clear extract. All the samples were extracted in triplicate. The mobile phase was acetonitrile:methanol: tetrahydrofuran (45:50:5 v/v/v) at a flow rate of 1.0ml min^{-1}, and detection was done at 254nm.

Determination of β-carotene content

β-carotene in pepper samples was extracted as per Ismail and Fun (2003), with minor modifications. The β-carotene Standard ($E_{1cm}^{1\%}$ = 2560 in hexane) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Pepper samples (10g) were extracted with 40ml ethanol (99.8%) and 10ml 100% (w/v) potassium hydroxide, and homogenized for three minutes. The mixture was saponified by heating for 30 minutes. Then, the mixture was partitioned thrice in n-hexane, followed by a wash with distilled water and passed through sodium sulfate. Hexane was removed under reduced pressure at 45°C using a rotary evaporator. The standards and pepper isolates were dissolved in 10ml hexane prior to HPLC analysis. A mobile phase was run at 0.8ml min^{-1} and consisted of water containing 0.01% formic acid:acetonitrile (95:5 v/v). β-carotene was detected at 450nm using a UV-VIS detector. The column was equilibriated to the original mobile-phase concentration prior to injection of the next sample.

Determination of nutritional attributes

Peppers were analyzed for nutrient parameters after di-acid digestion (HNO_3:$HClO_4$ 10:4 v/v). Potassium (K) content was determined by flame photometry, while Fe, Zn, Cu and Mn contents were analyzed using an atomic absorption spectrophotometer. Phosphorus (P) was estimated photometrically by development of phospho-molybdate complex (Taussky and Shorr, 1953).

Statistical analysis

Forty different lines of Capsicum were grown in the field under Completely Randomized Block Design, with three replications. Data represent the mean of three replicate samples for each *capsicum* type. Genotypic mean value of each parameter was used for statistical analysis, using SPSS programme (SPSS Inc., Version 10, Chicago, Illinois, USA). Correlation analysis (Brereton, 2003) and cluster analysis were performed using SPSS.

RESULTS AND DISCUSSION

HPLC methodology

Prior HPLC-analysis saponification has been recommended to remove chlorophyll and to hydrolyze carotenol (Scott, 1922). In our study, preliminary work determined 40°C to be the optimal saponification temperature which maximized retention of both xanthophylls and non- oxygenated carotenoids. A chromatogram of β-carotene Standard and best fruits extracts of the genotype is shown in part A of Figure 1. Polar or lipophobic extract of *Capsicum* fruits contains ascorbic acid, the major precursor of vitamin 'C' and capsaicinoids, the pungency principle. A chromatogram of L-Ascorbic acid Standard and fruit extract of the best genotype is shown in part B of Figure 1.

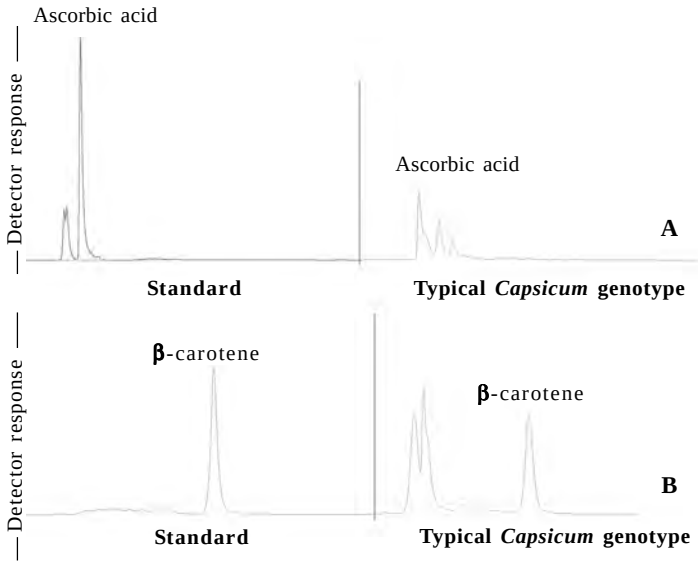

Fig 1. HPLC Chromatogram for L- ascorbic acid and β-carotene standard and in fruit extract of typical *Capsicum* genotype

Frequency Distribution of sweet pepper accessions

Forty accessions of sweet pepper were used for studying eight traits including two functional and six nutritional traits. Among the accessions, frequency distribution of ascorbic acid and β-carotene was classified into four groups. In both types of traits, the first group contributed maximum number of accessions. Frequency distribution for ascorbic acid is presented in Figure 2A. Group 1 contributed 17 out of 40 accessions tested; second, third and fourth group comprised of 12, 9 and 2 accessions respectively. Frequency distribution for β-carotene was classified into four groups with 24, 6, 4 and 6 accessions in each group, respectively (Fig. 2A). In the case of ascorbic acid, the frequency group showing high values (Group 4) contributed only 2 accessions, whereas, in the case of β-carotene, 6 accessions showing high values were observed in Group 4.

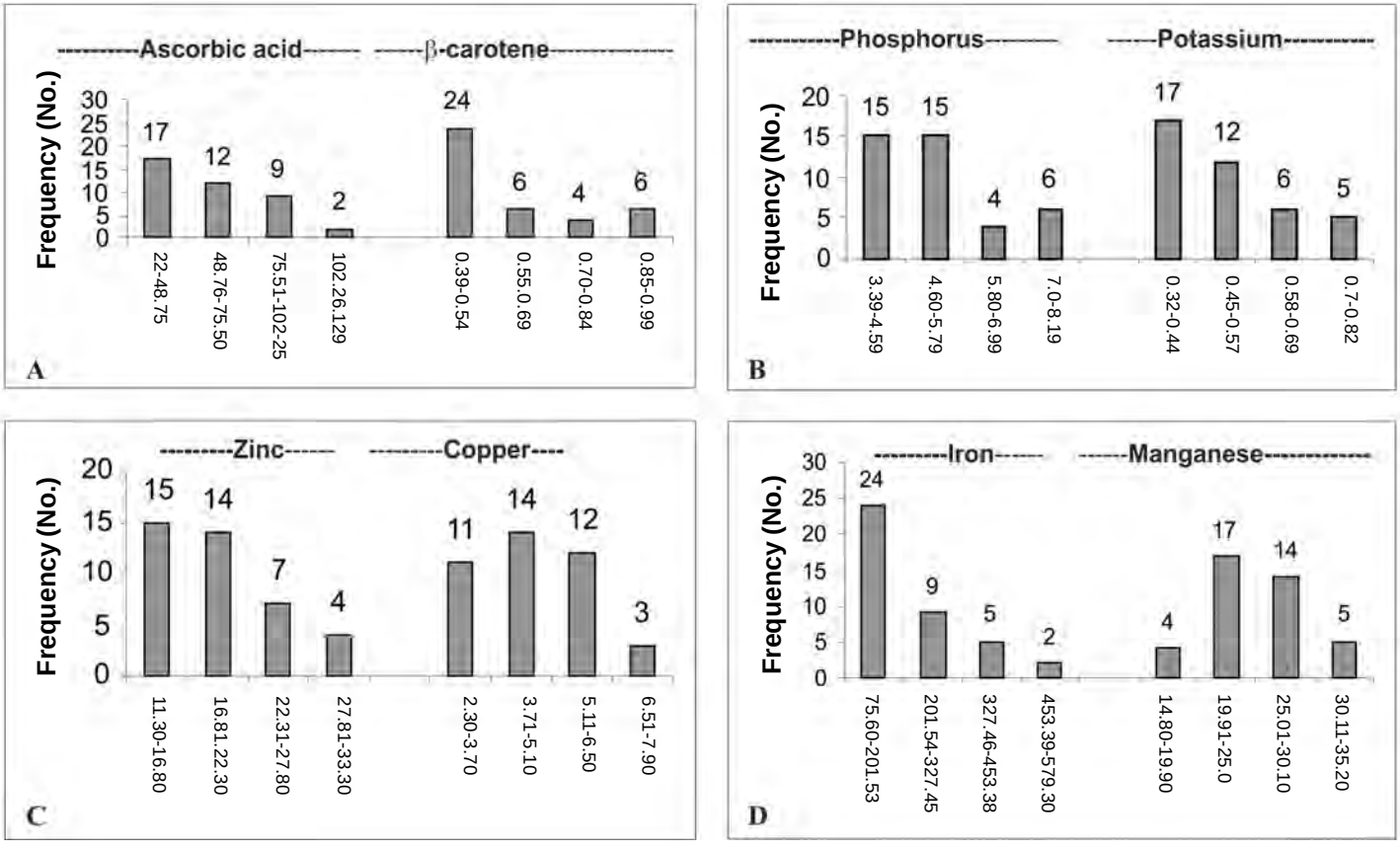

Fig 2. Frequency distribution of β-carotene and ascorbic acid (A); phosphorus and potassium (B); zinc and copper (C); Iron and manganese (D) content in the 40 *Capsicum* lines used

Frequency distribution for phosphorus and potassium content among the accessions also segregated into four groups (Fig. 2B). The first two groups of phosphorus and potassium contributed maximum number of accessions. Similarly, micronutrients also got grouped into four classes. In this case, for zinc and copper, the first two groups contributed maximum number of accessions. Frequency distribution for zinc and copper is presented in Fig. 2C. In the case of iron, maximum number of accessions (24) were observed in the first group, whereas, Group 2 of manganese contributed 17 accessions (Fig. 2D). The frequency group showing high values (Group 4) contributed 6, 5, 4, 3, 2 and 5 for phosphorus, potassium, zinc, copper, iron and manganese, respectively.

Variation in sweet pepper properties

Wide variations occurred in all the attributes tested (Table 1). This is due to the wide genetic base of the sweet pepper genotypes tested. Ascorbic acid content in these pepper accessions ranged from 22 to 129mg $100g^{-1}$ (fresh weight at physiological maturity), consistent with the report of Howard *et al* (2000), Gnayfeed *et al* (2001) and Saha *et al* (2010). L-Ascorbic acid content in *Capsicum annuum* fruit was reported to be 102-202mg $100g^{-1}$ fresh fruit (Howard *et al*, 2000). Wide variation (0.39-0.996mg $100g^{-1}$) was observed in β-carotene content in the accessions evaluated, suggesting a considerable level of genetic diversity. This is inconsistent with the report of Gnayfeed *et al* (2001) who found paprika red pepper to contain 171-250mg g^{-1} β-carotene. However, our result is in accordance with Howard *et al* (2000). The latter reported 337-800µg $100g^{-1}$ β-carotene in Capsicum *annuum* fresh fruit. Phosphorus and potassium content in pepper ranged between 0.32 – 0.82% and 3.39 – 8.19% , respectively, on dry matter basis, whereas, iron and zinc ranged between 75.6 – 579.3 and 11.3 – 33.3µg g^{-1}; manganese and copper varied from 14.8 to 35.2 and 2.3 to 7.9µg g^{-1}, respectively. Jadczak and Grzesczuk (2004) reported physiologically mature peppers to be richer in mineral content than green ones.

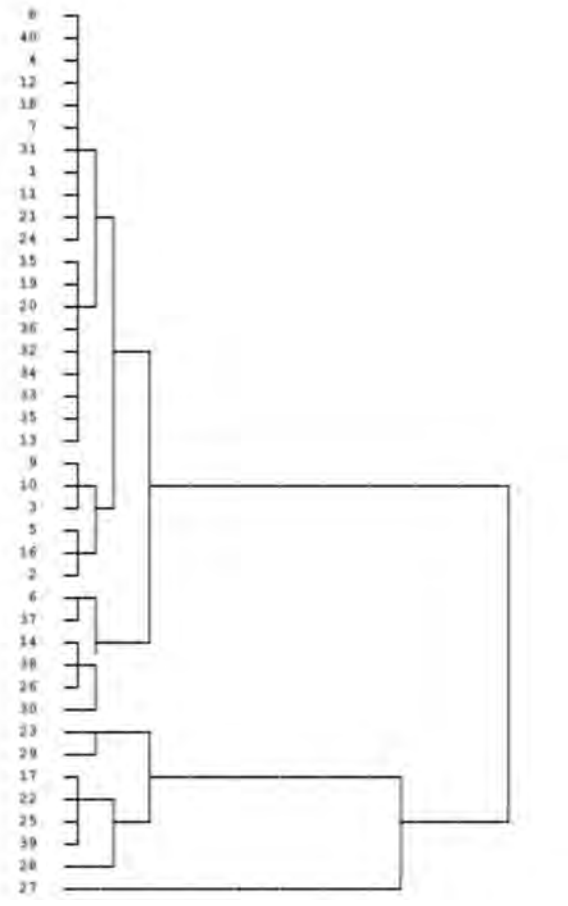

Fig 3. Dendrogram showing relationship among 40 lines used based on eight quality attributes.

Table 1. Basic statistical studies for functional and nutritional attributes in 40 *Capsicum* lines

Attribute	Unit	Min.	Max.	Mean	SD	Variance
			Functional properties			
Ascorbic acid	mg per 100g	22.00	129.00	59.73	25.66	658.70
β-carotene	mg per 100g (fresh weight at physiological maturity)	0.39	1.00	0.58	0.19	0.03
			Nutritional properties			
Phosphorus (P)	%	0.32	0.82	0.52	0.12	0.02
Potassium (K)	%	3.39	8.19	5.12	1.41	1.98
Iron (Fe)	µg g^{-1}	75.60	579.30	218.21	109.29	11944.70
Zinc(Zn)	µg g^{-1}	11.30	33.30	19.48	5.93	35.17
Manganese (Mn)	µg g^{-1}	14.80	35.20	24.76	4.73	22.39
Copper (Cu)	µg g^{-1}	2.30	7.90	4.68	1.42	2.01

Table 2. Correlation coefficient of functional and nutritional attributes

	Ascorbic acid Carotene	K	P	Zn	Cu	Fe	Mn
Ascorbic acid	-0.215	-0.056	-0.152	-0.073	-0.293*	0.241	-0.011
â-carotene		-0.014	-0.063	-0.118	0.131	-0.125	-0.153
Potassium (K)			0.764**	0.541**	0.350*	0.115	0.230
Phosphorus (P)				0.735**	0.618**	0.202	0.416**
Zinc(Zn)					0.610**	0.377**	0.481**
Copper (Cu)						-0.126	0.096
Iron (Fe)							0.648**

*, ** represent $P < 0.05$, 0.01, respectively

Correlation between functional and nutritional attributes

Few significant-correlation-coefficients among traits, from –0.293 (copper *versus* ascorbic acid content) to 0.764 (phosphorous *versus* potassium content), were observed but most values were low (Table 2). Potassium, phosphorus and zinc content correlated to three other nutritional attributes. Zinc was, besides, highly positively-correlated with all the nutritional traits, viz., potassium, phosphorus, copper, iron and manganese content (r = 0.541, 0.735, 0.61, 0.377 and 0.481, $p < 0.01$, respectively). Phosphorus content was also found to be highly correlated with potassium, zinc, copper and manganese content (r = 0.764, 0.735, 0.618 and 0.416, $p < 0.01$, respectively). Similarly, potassium content was correlated with phosphorous, zinc and copper content (r = 0.764, 0.541, $p < 0.01$ and 0.35, $p < 0.05$, respectively). Interestingly, ascorbic acid content was negatively correlated with copper content (r = -293 $p < 0.05$) and β-carotene content was not correlated with any of the traits under study.

Statistical procedure for classification

To visualize the pattern of clustering among sweet pepper accessions, hierarchical cluster analysis was used. The data matrix included as objects each of the eight attributes analyzed for the 40 accessions. Variables were attributes described in the experimental section. Pearson correlation was used as criterion for similarity, and furthest neighbour as the clustering method. Using the similarity level, these 40 sweet pepper accessions were classified into three main groups (Fig. 3). Differences existing between accessions studied for the variables selected were adequate to classify the accessions correctly.

Dendrogram of the 40 sweet pepper accessions showed three groups (Fig. 3). Cluster 1 consisted of 32 accessions (starting from the top, 8th to 30th accessions). Second cluster comprised of seven accessions (VHC 30, VHC 36, VHC 27, VHC 29, VHC 31, VHC 2 and VHC 34,) and the third cluster consisted of a single genotype (VHC 33) that is highly distinct from other accessions falling to the two clusters.

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