Analysis of Genetic Variability for Nutritional Quality Traits in Hot Pepper (*Capsicum annuum* L.)

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Variability and association analysis were studied among 42 genotypes of hot pepper (*Capsicum annuum* L.) comprising exotic (Taiwan), landrace and open-pollinated varieties for some important nutritional quality traits. The phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits under study and difference between GCV and PCV was not high, indicating that these quality traits were not much influenced by the environmental factors. Heritability ranged from 38.8% (manganese μg g^{-1}) to 99.9% (capsaicin %). High heritability and high genetic advance were observed for ascorbic acid, total phenols, iron (Fe) and beta carotene content in fruits indicating involvement of additive gene action. Association analysis exhibited both positive and negative correlations among the traits under study.

Key Words: Hot pepper, Variability, Correlation, Nutritional quality

Introduction

Hot pepper (*Capsicum annuum* L.) is an important vegetable as well as spice crop, not only because of its economic importance, but also for the nutritional value of its fruits, mainly due to the fact that they are an excellent source of natural red pigment capsanthin and antioxidant compounds (Howard *et al.*, 2000). It is an excellent source of vital micronutrients such as vitamin C and vitamin E (Minguez-Mosquera & Hornero-Mendez, 1994, Daood *et al.*, 1996; Gnayfeed *et al.*, 2001). The important states growing hot pepper are Andhra Pradesh, Orissa, Maharastra, West Bengal, Karnataka, Rajasthan and Tamil Nadu. Total world production of hot pepper has been estimated to be 14–15 million t per year (Weiss, 2002). Capsaicinoids (heat principle), have aroused great interest lately owing to their biological and therapeutic importance (Surh and Lee, 1996; Szolcsanyi, 2004). Capsaicnoids are alkaloids specific for *Capsicum* sp. A wide spectrum of antioxidant compounds are present in pepper fruits. It includes antioxidant vitamins (Vitamin E, C and β-carotene), phenolic compounds, carotenoides and xanthophylls. One of the important antioxidant is phenolic compounds, which occurs in peppers in connection with sugars (Materska *et al.*,2003 a, b). The level of antioxidants differs between accessions, generally hot peppers are a better source of them than the sweet ones (Daood *et al.*, 1996; Gnayfeed *et al.*, 2001). Phenolic compounds retard or inhibit lipid autoxidation by acting as radical scavengers (Namiki, 1990) and consequently, are essential antioxidants that protect against propagation of the oxidative chain. Another important antioxidant-vitamin C, an important compound of pepper fruits, chelates heavy metal ions (Namiki, 1990), reacts with singlet oxygen and other free radicals, and suppresses per oxidation, reducing the risk of arteriosclerosis, cardiovascular diseases, and some forms of cancer (Harris, 1996). Carotenoid pigment, vitamin E, vitamin C are located in the pericarp of pepper fruit, whereas, capsaicinoids are distributed in different parts. Genetic variability, especially for quality in given sets of germplasm has not been studied much. Study related to pepper as functional food or as a source of micronutrient are not meager, but, systematic screening study for the quality evaluation of hot pepper and use of the information for the development of better varieties is lacking. Hence, the present was undertaken to analyze the extent of variability in respect of nutritional quality present in available germplasm of hot pepper to breed better quality hot pepper varieties.

Materials and Methods

The present experiment was conducted in VPKAS, Almora at experimental farm, Hawalbagh (29°36' N, 79°40' E and 1250 m asl). Forty two lines of hot pepper (exotic, local and open pollinated popular varieties) were grown in the field under completely randomized block design with three replications in *kharif* season (June-September), 2007. The crop was raised as per the recommended package of practices. Peppers were harvested at green mature stage. Three replicates of 42 genotypes were analyzed for different functional (β-carotene, ascorbic acid, total phenolics and capsaicin) and nutritional attributes (phosphorous, potassium, zinc,

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Copper, iron and manganese). The detailed information regarding source of chilli lines is mentioned in Table 1.

Chemical Analysis

Determination of ascorbic acid content

L-ascorbic acid (LAA) was estimated extracted and quantified by HPLC as described by Abdulnabi *et al.* (1997) with minor modifications. The sample (10 g) was homogenized with solution (10 ml) containing meta-phosphoric acid (0.3 M) and acetic acid (1.4 M) for 15 minutes at room temperature. The mixture was filtered through Whatman No. 4 to obtain clear extract. All samples were extracted in triplicate. Mobile phase was Acetonitrile: Methanol: Tetrahyrofuran (45:50:5; v/v/v) at a flow rate of 1.0 ml min^{-1} and detected at 254 nm.

Determination of β-carotene content

β-Carotene in the pepper samples was according to 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). Pepper samples (10g) were extracted with 40 ml ethanol (99.8%) and 10 ml 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 washing with distilled water and then passed through sodium sulfate. Hexane was removed under reduced pressure at 45^0C using rotary evaporator. The standards and pepper isolates were dissolved in 10 mL of hexane prior to HPLC analysis. A mobile phase ran at 0.8 ml min^{-1} and consisted of water containing 0.01% formic acid: Acetonitrile (95:5 v/v). β-Carotene was detected at 450 nm using a UV-Vis detector. The column was equilibrated to the original mobile phase concentration prior to the next sample injection.

Determination of total phenolics content

Total phenolics content of the MeOH extracts was determined by the Folin-Ciocalteu assay and catechol was used as standard (Singleton and Rossi, 1965). Sample (500 mg) was weighed into 50 ml plastic extraction tubes and vortexed with 25 ml extraction solvent (80% ethanol). The sample with the extraction solvent was than heated at 60^0C (water bath) for 1h, allowed to cool to room temperature, and homogenized for 30s with a sonicator. Two hundred fifty microlitres (three replicates) were introduced into screw cap test tubes; 1.0 ml of Folin-Ciocalteu's reagent and 1.0 ml of sodium carbonate (7.5%) were added. The tubes were vortexed and heated for 15 minutes at 45^0C. The absorption at 765 nm was measured (Model U 2001, Hitachi UV/Vis spectrophotometer) and the total phenolic content was expressed as catechol equivalents in mg per 100 g dry material.

Determination of capsaicin content

Capsaicin content was determined using

Table 1. List of chilli lines analyzed for genetic variability

Accession No.	Code of lines	No. of lines	Source
ICPN 10 # 1	VLC-16-I-1, VLC-16-I-2, VLC-16-II-1,VLC-16-II-3	4	AVRDC through NBPGR
ICPN 10 # 3	VLC-18-I-1, VLC-18-I-2	2	AVRDC through NBPGR
ICPN 10 # 5	VLC-20-I-2, VLC-20-II-1	2	AVRDC through NBPGR
ICPN 10 # 6	VLC-21-II-3-1, VLC-21-II-3-2	2	AVRDC through NBPGR
ICPN 10 # 7	VLC-22-I, VLC-22-I-2-1, VLC-22-I-2-2, VLC-22-IV-1, VLC-22-V-1-1, VLC-22-V-1-2, VLC-22-VII-1,	7	AVRDC through NBPGR
ICPN 10 # 8	VLC-23-I-1-1, VLC-23-II-2-1, VLC-23-II-2-2	3	AVRDC through NBPGR
ICPN 10 # 9	VLC-24-I-1	1	AVRDC through NBPGR
ICPN 10 # 10	VLC-25-1	1	AVRDC through NBPGR
ICPN 10 # 13	VLC-28-II-1, VLC-28-II-2, VLC-28-III-1	3	AVRDC through NBPGR
ICPN 10 # 14	VLC-29-1, VLC-29-II-1-1, VLC-29-II-1-2	3	AVRDC through NBPGR
ICPN 10 # 15	VLC-30-I-1, VLC-30-II-1,VLC-II-2	3	AVRDC through NBPGR
ICPN 10 # 16	VLC-31-I-1	1	AVRDC through NBPGR
ICPN 10 # 17	VLC-32-1, VLC-32-3, VLC-32-4	3	AVRDC through NBPGR
ICPN 10 # 19	VLC-34-2	1	AVRDC through NBPGR
Lakhori Mirch	-	1	Salt, Dist. Almora, Uttarakhand
Janjeera Mirch	-	1	Salt, Dist. Almora, Uttarakhand
Berry Mirch	-	1	Salt, Dist. Almora, Uttarakhand
Lal Mirch	-	1	Salt, Dist. Almora, Uttarakhand
Pusa Sadabahar	-	1	Released variety from IARI
Pusa Jwala	-	1	Released variety from IARI

spectrophotometer. Sample (500 mg) was weighed into 50 ml plastic extraction tubes and extracted with 10 ml extraction solvent (acetone) for 3 h. Then, the sample with the extraction solvent was centrifuged and 1.0 ml extract was taken in a beaker and evaporated. The residue was dissolved in 0.4% sodium hydroxide solution (5 ml) and 3 ml 3% phosphomolibdic acid was added. The mixture was kept for 1.0 h and absorbance was taken at 650 nm after centrifugation. The capsaicin content was expressed as per cent dry material.

Determination of nutritional attributes

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

Statistical analysis

The genotypic and phenotypic coefficient of variation, heritability (broad sense) were calculated by standard statistical procedure given by Burton and De Vane 1953; Johnson *et al.* (1955). The genotypic and phenotypic correlation coefficient was calculated by a method described by Singh and Choudhary (1979).

Results and Discussion

Variation among 42 genotypes of hot pepper was found highly significant for the characters under study (Table 2). The estimates depicting the genetic variability including genotype range, mean genotypic and phenotypic variation, genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), heritability (broadsense)and genetic advance are presented inTable 3.

Wide variation (0.20-0.36 mg $100g^{-1}$) was observed in β-carotene content in evaluated pepper lines, suggesting considerable levels of genetic diversity. The result was not consistent with the report by Gnayfeed *et al.* (2001), where it was reported that paprika red pepper contained 171-250 mg g^{-1} β-carotene, but similar to the report by Howard *et al.* (2000). The latter reported 337-800 mg $100g^{-1}$ β-carotene in *Capsicum annuum* fresh fruit. Ascorbic acid content in pepper accessions ranged from 25-217 mg $100g^{-1}$, consistent with the report by Howard *et al.*, 2000 and Gnayfeed *et al.*, 2001. Total phenolics ranged between 38.4-188.1 mg $100g^{-1}$ catechol equivalent. Capsaicin content ranged between 0.08-0.67%, which is far below than the report by Contreras-Padilla and Yahla, 1998. It was reported that paprika accession contained 383-1075 mg g^{-1} (Gnayfeed *et al.*, 2001) and 997-1240 mg g^{-1} (Ayuso *et al.*, 2008) dry matter capsaicinoid. Phosphorous and potassium content in pepper ranged between 0.33-0.59 and 2.9-6.7% (dry matter basis). It was reported that physiologically mature peppers were rich in mineral content than green one (Jadczak and Grzeszczuk, 2004). Iron and zinc content ranged from 146-317 mg g^{-1} and 11.4-32.6 mg g^{-1}, respectively (Table 3). The phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits under study and difference between GCV and PCV is not much except manganese contents(mg g^{-1}) in fruits indicating that these quality traits were not much influenced by the environmental factors. Similar findings were reported by Shirshat *et al.* (2007), that difference between PCV and GCV was less in case of ascorbic acid content in fruits (Table 3). The high heritability coupled with high genetic advance was recorded for ascorbic acid, total phenols, iron (Fe) and β- carotene contents in fruits indicating the

Table 2. Analysis of variance for quality nutritional attributes in forty-two genotyps of hot pepper

Traits	Mean Sum of Squares (d.f.)			CD (5%)
	Replication (02)	Genotypes (41)	Error (82)	
K	0.00055	1.7815**	0.0071	0.137
P	0.000003	0.011456**	0.000047	0.011
Zn	0.2729	51.937**	0.549	1.198
Cu	0.0213**	6.954**	0.0037	0.100
Fe	8.822	4429.450**	46.51	11.078
Mn	0.492	14.312**	4.925	3.604
â-carotene	0.0007	0.347**	0.0034	0.096
Ascorbic acid	52.66	6643.68**	33.88	9.455
Total Phenolics	279.50*	4532.57**	68.67	13.460
Capsaicin	0.0244**	0.045**	0.000019	0.007

** Significant at 1 per cent level * Significant at 5 per cent level

Table 3. Range, mean, variance, coefficient of variation, heritability and genetic advance for different quality nutritional attributes of hot pepper

Traits	Units	Range	Mean	Variance		Coefficient of Variation (%)		Heritability in broad sense (%)	Genetic advance 5%
				Genotypic	Phenotypic	Genotypic	Phenotypic		
K	%	2.88-6.69	4.596	0.591	0.599	16.732	16.833	98.8	1.58
P		0.326-0.585	0.463	0.004	0.004	13.327	13.409	98.8	0.13
Zn	mg g^{-1}	11.4-32.60	19.827	17.131	17.675	20.875	21.204	96.9	8.39
Cu		3.93-9.067	7.214	2.317	2.321	21.101	21.118	99.8	3.13
Fe		146.10-316.8	193.94	1460.98	1507.49	19.709	20.020	96.9	77.52
Mn		11.80-22.90	17.63	3.129	8.054	10.033	16.098	38.8	2.27
β-carotene	mg $100g^{-1}$	0.20-0.36	0.275	0.115	0.118	12.316	12.501	97.0	0.69
Ascorbic acid		25.00-217.00	67.79	2203.27	2237.15	69.246	69.777	98.5	95.96
Total phenolics		38.41-188.10	94.63	1487.97	1556.64	40.761	41.691	95.6	77.69
Capsaicin	%	0.08-0.670	0.267	0.015	0.015	46.240	46.268	99.9	0.25

Table 4. Genotypic (r_g) phenotypic (r_p) correlation coefficient among different quality nutritional attributes of hot pepper

Traits		K	P	Zn	Cu	Fe	Mn	â-carotene	Ascorbic acid	Tot. Phenolics	Capsaicin
K	r_g	1.0	0.6573	0.2048	-0.1852	-0.0163	0.6126	-0.1165	0.0449	0.0208	-0.0591
	r_p	1.0	0.6506	0.1965	-0.1849	-0.0116	0.3834	-0.1132	0.0438	0.0209	-0.0583
P	r_g		1.0	0.0585	-0.0953	0.0748	0.5806	-0.0982	0.0920	0.0480	0.0248
	r_p		1.0	0.0635	-0.0944	0.0839	0.3199	-0.0890	0.0913	0.0442	0.0246
Zn	r_g			1.0	0.2141	0.2547	0.4551	0.0237	-0.0213	-0.0430	0.0053
	r_p			1.0	0.2092	0.2611	0.2999	0.0210	-0.0210	-0.0389	0.0050
Cu	r_g				1.0	0.2402	0.0767	0.2277	-0.3681	0.0793	0.2286
	r_p				1.0	0.2353	0.0377	0.2256	-0.3646	0.0788	0.2280
Fe	r_g					1.0	0.4285	0.1754	-0.1198	0.3304	0.1169
	r_p					1.0	0.2364	0.1799	-0.1163	0.3170	0.1155
Mn	r_g						1.0	-0.1355	-0.1374	-0.0415	0.1884
	r_p						1.0	-0.1320	-0.0830	-0.0011	0.1164
â-carotene	r_g							1.0	-0.3311	-0.1105	0.0541
	r_p							1.0	-0.3230	-0.1052	0.0531
Ascorbic acid	r_g								1.0	0.2316	-0.0685
	r_p								1.0	0.2243	-0.0679
Totat phenolics	r_g									1.0	0.1365
	r_p									1.0	0.1323
Capsaicin	r_g										1.0
	r_p										1.0

involvement of additive gene action for expression of these traits. Johnson *et al.* (1955) suggested that traits with high heritability coupled with high genetic advance would respond to select better than those with high heritability and low genetic advance. Heritability is not an exact parameter because it could be high even when genetic advance is very low (Table 3). However, expected genetic gain can be high only if the genetic variance is high (Allard, 1960). Burton and Devane (1953) suggested that GCV along with heritable estimates would give a better picture of the amount of progress expressed by phenotypic selection.

The assessment of genetic potentiality of important quality traits and their association is of paramount importance to carryout the effective selection of genotype with desired quality traits. Correlation coefficient of important quality traits were estimated at genotypic and phenotypic levels. In present study, the genotypic correlation coefficients were higher in magnitude than their respective phenotypic ones and positive correlation was observed (β carotene with P, Zn, Fe, Mn, β carotene and total phenols; total phenols with P, Fe, and ascorbic acid; ascorbic acid with K and P; β carotene with Zn and Cu, ascorbic acid and lycopene) indicating that there is an inherent association among these traits (Table 4).

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