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Delineating bioactive properties of sweet pepper advanced breeding lines adapted to Indian mid-Himalayas: A Chemometric approach

Kumari Shubha[*,], N. K. Hedau, R. S. Pal, Anirban Mukherjee[**], Lakshmi Kant and A. Pattanayak**

ICAR-Vivekananda Institute of Hill Agricultural Research, Almora 263 601, Uttarakhand

ABSTRACT

Malnutrition, heart, respiratory and pulmonary diseases are becoming most critical in Mid Himalaya. Vegetables rich in micronutrients, antioxidants and bioactive compounds can help to solve the issue. Sweet pepper is one of the best choices but as it is a warm and humid loving crop and bioactive compounds are highly varied with attitude and temperature condition, the study was conducted to identify promising antioxidant rich sweet pepper lines adapted to hill condition. Thirty eight advanced breeding lines (ABLs) of sweet pepper were evaluated for variations in total polyphenol, total carotenoids, ascorbic acid and antioxidant activity in mid Himalayan region. Antioxidant activity was measured using two *in vitro* assays *viz*. 2, 2-diphenyl-1-icrylhydrazyl (DPPH) 2,2′-Azino-bis(3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS). Additionally colour parameter capsanthin, pungency compound capsaicin and fruit firmness were evaluated. Among ABLs, significant differences ($p \leq 0.05$) were obtained with respect to antioxidant composition and antioxidant activity. Ascorbic acid and total phenol varied from 39.42 - 85.12 mg/100 g and 13.01 - 71.24 mg GAE /100g fresh weight (fw) respectively, while ABTS % Inhibition and DPPH % Inhibition varies from 10.95-80.05 and 11.19-49.18 respectively. Chemometric tools like principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were applied to understand possible classification sweet pepper ABLs based on bioactive antioxidant compounds, antioxidant potentiality and fruit firmness. PCA revealed that the first two components represented 67.78% of the total variability in the total variation. AHC classified cultivars into four main groups on the basis of the measured parameters. Results suggested that VLCP-16-1, VLSM-3 and Mukteshwar for Vitamin C; VLCP-16-57, VLCP-16-54, Mukteshwar and VLCP-16-52 for polyphenols, VLCP-16-54, VLCP-16-57, VLCP-2016-52 and showed highest antioxidant activity. The result will help in advance breeding of sweet pepper for development of nutritionally rich varieties.

Key words: *Capsicum annuum*, antioxidant activity, principal component analysis, Agglomerative hierarchical clustering.

INTRODUCTION

Food and nutrition insecurity is a serious challenge in the Indian mid Himalaya. More than 30% of the population suffers from food insecurity and around 50% face some form of malnutrition, where women and children are suffering the most. Survey revealed that over 30% of the children less than 5 years in Indian Mid Himalaya suffers from under-nutrition (NFHS, 11). As per as disease is concern ischaemic heart disease, lower respiratory infections, chronic obstructive pulmonary disease are the major diseases of mid Himalaya causes severe loss of life (healthdata.org, 6). Vegetables plays important role in eliminating food and nutrition insecurity. It not only provides necessary nutrients but antioxidant bioactive compounds which helps in promoting health as well.

Sweet Pepper (*Capsicum annum* L.), is one of those vegetables which contain high levels nutrients and antioxidants. They are fast gaining popularity, not only for their attractive colours (red, green and yellow), unique taste and aroma, but also for their health-promoting properties. Sweet peppers exhibit large genetic diversity in terms of colour, shape, size and chemical composition besides these differ significantly in their antioxidant properties, vitamins and other bioactive compounds. Fresh sweet peppers have long been recognized as an excellent source of vitamin C. In addition, Sweet peppers are rich in polyphenols, particularly the flavonoids, quercetin and luteolin (Lee, Howard, & Villalon, 10). Carotenoids, a class of natural pigments responsible for the diverse colours in fruits and vegetables are also found abundance in peppers. Mainly the oxygenated carotenoids provitamin A (a- and b-carotene and b-cryptoxanthin) and xanthophylls are predominantly present in Sweet peppers. These fat-soluble bioactive compounds show potential action against certain cancers, prevent gastric ulcer, stimulate the immune system, prevent cardiovascular diseases and protect against age-related disease like macular degeneration and cataracts (Krinsky & Johnson, 8). It is clear that sweet pepper is an important crop for hilly region. Due to high cost of transportation and loss of nutrition

*Corresponding author's E-mail: shubhaveg@gmail.com

**ICAR-Research Complex for Eastern Region, Patna 800014, Bihar

during transport it is generally not imported from plains.

Realising the importance various government research institutes and private seed companies started focusing on sweet pepper breeding for high altitude worldwide. However, scanty information is available on level of antioxidant compounds and antioxidant potentiality of Indian cultivars particularly Indian mid-Himalayas. The antioxidants availability is highly subject to temperature and environment. Therefore, the present study was conducted to have detailed information on antioxidant properties of sweet pepper cultivars of Indian Mid Himalaya with desired genetic architecture rich in nutraceutical content, and serve as a reference material to develop nutraceutical rich sweet pepper. Generation of such information will benefit both breeders as well as consumers. In the present study, various bioactive compounds, colour properties, pungency properties; fruit firmness and antioxidant activity of ABLs which are well adapted to north western Himalayan region were investigated. Fruit firmness is a desirable character for ensuring long transportation of peppers in difficult terrains. The study was conducted with the aim to classify sweet pepper cultivars of Indian Mid-Himalaya based on levels of bioactive compounds, colour properties and antioxidant activity and to find out the relationship of bioactive compounds, colour properties, pungency property, fruit firmness with antioxidant potentiality.

MATERIAL AND METHODS

In the present study, thirty four ABLs along with two local and two exotic lines of bell pepper (Table 1) were grown in a field experiment at ICAR-VPKAS, Experimental farm, Hawalbagh, Almora, Uttarakhand, India. Hawalbagh situated at Indian mid-Himalayas range (Shivalik hills). The site was located at 29°36' N, 79°40' E and at an elevation of 1250 m above mean sea level. The plants raised in a semi-climate-controlled green house, where evaporative cooling carried out with a pad and fan system.

The temperature ranged from 20–25°C during the growing season. All sweet peppers ABLs received similar standard cultivation practices with regard to nutrition supply and irrigation during the entire growing season. Out of thirty eight lines, eleven lines are yellow and rests are red in colour. All the fruits analyzed were harvested at the same time when 50% of the fruit showed a transition from green to red or yellow. Three replicates were taken for each genotype. Immediately after harvest, the fruits were placed in polyethylene bags and transported under refrigerated at -20°C until analyzed. Quantitative analysis was carried out for total polyphenol, total carotenoids, capsanthin (ASTA 20), capsaicin, ascorbic acid firmness of fruits and antioxidant activities. The entire analysis was completed within fifteen days of sample collection.

Total polyphenols content: Total polyphenols content (TPC) was determined spectrophotometrically by the Folin-Ciocalteu method (Singleton & Rossi., 16). Extracts (150 µL) were mixed with 850 µL of double distilled water. A blank was prepared using double distilled water instead of a sample. Subsequently, 50 µL of Folin-Ciocalteu Reagent (1 N) were mixed with the sample or blank. The reaction mixture was allowed to stand at room temperature for 6 min. and then 2.5mL of 20% Na_2CO_3 solution were added to each mixture and allowed to stand at room temperature for 90 min. The absorbance was measured at 725 nm. A standard curve of gallic acid (purity ≥98%) was constructed (20-100 mg/L). Results are expressed as mg gallic acid equivalent (GAE) per g fresh weight.

Determination of scavenging effects on DPPH radicals**:** The DPPH assay was done by measuring the decrease in absorbance of methanolic DPPH solution at 515 nm. The stock solution was prepared by dissolving 24 mg DPPH with 100mL methanol and then stored at -20 0C until further use. The working solution was obtained by mixing 10mL stock solution with 45mL methanol to get an absorbance of 1.17 ± 0.02 units at 515 nm. Fruit extracts (150 µL) of different cultivar were allowed to react with 2850 µL of the DPPH solution for 24 h in the dark and absorbance was taken at 515 nm. The radical scavenging activity was calculated as a percentage of DPPH discoloration using the equation:

DPPH • radical scavenging (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

Where A_{sample} is the absorbance of the solution when the extract/reference has been added at a particular level, and $A_{control}$ is the absorbance of the DPPH solution without addition of extract.

Determination of scavenging effect on ABTS radicals: The ABTS assay was done by measuring the decrease in absorbance of methanolic ABTS solution at 745 nm in the presence of the extract (Arnao *et al*., 2). The stock solutions included 7.0 mM ABTS solution and 2.3 mM ammonium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was diluted by mixing 1.0 mL ABTS solution with 3.0 mL methanol to obtain an absorbance of 0.9 ± 0.02 units at 745. Fruit extracts (200 µL) of different cultivars were allowed to react with 2000 µL of the ABTS solution for 30 min in dark condition and absorbance was taken at 745 nm.

Table 1: List of sweet pepper advanced breeding lines under study, source of seed, anther colour and their fruit characters.

S. No.	Genotype	Source	Anther colour	Fruit colour	Number of locules per fruit	Fruit position	Fruit shape
1	VLCP-16-1	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
2	VLCP-16-3	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
3	VLCP-16-9	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
4	VLCP-16-10	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Cylindrical
5	VLCP-16-11	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
6	VLCP-16-14	ICAR-VPKAS, Almora	Pale Yellow	Red	4	Erect	Blocky
7	VLCP-16-15	ICAR-VPKAS, Almora	Yellow	Yellow	4	Pendant	Blocky
8	VLCP-16-16	ICAR-VPKAS, Almora	Yellow	Yellow	5	Pendant	Blocky
9	VLCP-16-5	ICAR-VPKAS, Almora	Yellow	Yellow	5	Pendant	Blocky
10	VLCP-16-6	ICAR-VPKAS, Almora	Blue	Red	4	Pendant	Cylindrical
11	VLCP-16-17	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Cylindrical
12	VLCP-16-19	ICAR-VPKAS, Almora	Yellow	Red	5	Pendant	Blocky
13	VLCP-16-21	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
14	VLCP-16-59	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
15	VLCP-16-2	ICAR-VPKAS, Almora	Yellow	Red	5	Pendant	Blocky
16	BLCPN.4	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Elongate
17	VLCP-16-60	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
18	VLCP-16-61	ICAR-VPKAS, Almora	Yellow	Red	5	Erect	Blocky
19	VLCP-16-62	ICAR-VPKAS, Almora	Yellow	Red	4	Erect	Blocky
20	VLCP-16-37	ICAR-VPKAS, Almora	Blue	Red	4	Pendant	Blocky
21	VLCP-16-38	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
22	VLCP-16-39	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
23	VLCP-16-40	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
24	VLCP-16-41	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
25	VLCP-16-42	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
26	VLCP-16-45	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
27	VLCP-16-47	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
28	VLCP-16-48	ICAR-VPKAS, Almora	Yellow	Red	4	Pendant	Blocky
29	VLCP-16-52	ICAR-VPKAS, Almora	Yellow	Yellow	4	Pendant	Blocky
30	VLCP-16-53	ICAR-VPKAS, Almora	Yellow	Yellow	4	Pendant	Blocky
31	VLCP-16-54	ICAR-VPKAS, Almora	Yellow	Yellow	4	Pendant	Blocky
32	VLCP-16-55	ICAR-VPKAS, Almora	Yellow	Yellow	4	Pendant	Blocky
33	VLCP-16-56	ICAR-VPKAS, Almora	Yellow	Yellow	4	Pendant	Blocky
34	VLCP-16-57	ICAR-VPKAS, Almora	Yellow	Yellow	4	Pendant	Blocky
35	VLCP-16-58	ICAR-VPKAS, Almora	Yellow	Yellow	4	Pendant	Blocky
36	VLSM-3	AVRDC Taiwan, line ISPN 2 # 2	Yellow	Red	4	Pendant	Blocky
37	Mukteshwar	ICAR-VPKAS, Almora	Yellow	Yellow	4	Pendant	Blocky
38	VLCP-2	AVRDC Taiwan, line ISPN 2 # 3	Yellow	Red	4	Pendant	Blocky

The percentage inhibition was calculated using the equation:

ABTS radical scavenging (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

Where A_{sample} is the absorbance of the solution when the extract/reference has been added at a particular level, and $A_{control}$ is the absorbance of the ABTS solution without extract.

Ascorbic acid content: Ascorbic acid content of the tuber was determined at final harvesting stage by the method of Ranganna (14). Sample of 10 ml was taken and made upto 100 ml volume with 3 % HPO3 and filtered. Standard ascorbic acid solution was prepared by taking 50 mg ascorbic acid and making up its volume upto 50 ml with 3 % HPO3 solution; an aliquot of 5 ml from this solution was made up to 50 ml with 3 % HPO_3 solution. 42 mg of $NaHCO_3$ was dissolved in 150 ml hot distilled water. 50 mg of the dye, 2, 6- dichlorophenol indophenol was added in it and the volume was made upto 200 ml with distilled water to prepare the Dye solution. 5 ml of standard ascorbic acid solution was taken and mixed with 5 ml of 3 % HPO_3 solution. Dye was filled in a pipette and titration was done till a pink color appears that persists for at least 15 seconds. 5 ml of sample was blended with 50 ml of 3 % HPO3 solution and filtered. 2 ml was taken from this solution and titrated against the dye. Dye Factor is 0.5/Titre. The calculations were done with the help of the following formula (Rangana, 14):

$$\text{Ascorbic acid} = \frac{\text{Titre} \times \text{Dye Factor} \times \text{Volume made up}}{\text{Aliquat taken} \times \text{weight of sample}} \times 100$$

Estimation of Capsanthin/ colouring matter (ASTA Units)[A.O.A.C, 1980]: 0.5 gm of fresh Sample was taken in 100ml of volumetric flask, diluted to volume with acetone and cock tightly. A clear portion of the extract was transferred to cell and exhibition was measured at 465nm using acetone as blank: ASTA colour value for Capsanthin = $[(A_{ext}$ at 465nm) × (16.4 $I_f)]$/g sample

Where, I_f (Correction factor) = Declared OD of NBS std. at 465 nm

Observed OD of NBS std. at 465nm

Standard of NBS (National Board of Spice) is 1M Ferrous ammonium sulphate and declared OD is 0.64. In the spectronic, declared OD is equal to observed OD, there was no need to multiply with I_f.

Capsaicin content estimated by spectrophotometric measurement of the blue coloured component formed as a result of reduction of phosphomolybdic acid to lower acids of molybdenum (Sadasivam & Manikkam, 15). Two grams of fresh sample was extracted with 10 ml of dry acetone using pestle and mortar and centrifuged at 10,000 rpm for 10 min and 1ml of supernatant was pipetted into a test tube and evaporated to dryness in a hot water-bath. The residue was then dissolved in 0.4 ml of NaOH solution and 3 ml of 3% phosphomolybdic acid. Absorbance was measured for the clear blue solution, thus obtained, at 650 nm using reagent blank (5 ml of 0.4% NaOH+3ml of 3% phosphomolybdic acid). Capsaicin content calculated from the standard curve was expressed as mg /100 g Fw.

The property firmness, i.e. the maximum force applied to puncture the pepper tissue, was measured as an indicator of texture. Firmness of samples was measured using a Texture Analyzer (Texture Technologies Corp., TA, XT2, Scardale, NY, USA). The puncture diameter was 2 mm, with compression of pre-test, test and post-test were 10, 10, 20 mm/sec respectively with a trigger force 0.0250 Kg. The maximum force was measured by making one puncture in each fresh pepper sample, using 4 slabs per treatment. The mean value of maximum firmness for each treatment was then calculated and the results were expressed as Kg.

Statistical analysis

Data represents the mean of three replicate analyses. Analysis of data was performed using the Statistical Analysis System software (SAS). With the objective to categorize the ABLs according to their bioactive properties the chemometric data was subjected to two approaches viz., principal component analysis (PCA) and agglomerative hierarchical cluster analysis (AHC) PCA and AHC was performed using The Unscrambler software package (Version 9.7; CAMO, Norway).

RESULTS AND DISCUSSION

The general statistical parameters for various characters are presented in Table 2.

Ascorbic Acid is water-soluble antioxidant by desirable quality of its high reducing power. It is a cofactor for enzymes involved in the biosynthesis of collagen, carnitine, and euro transmitters in vitro, and it can reduce a variety of reactive oxygen species and reactive nitrogen species. Ascorbic acid content was found to vary significantly (≤0.05) among sweet pepper ABLs. Ascorbic acid content in these ABLs ranged from 39.42 to 85.12 mg/100g. The results show about 1.15 fold variations in ascorbic acid content among ABLs. The highest content was recorded in Mukteshwar (85.13 mg/100g) followed by VLSM-3 (85.00 mg/100g) and VLCP-16-1 (84.67 mg/100g) and also fulfilled 100% RDA for vitamin C. Variation in ascorbic acid content can be attributed to multiple factors such as greenhouse temperature fluctuations, cultivar response and sampling variation (Deepa *et al.,* 4).

Table 2: Estimates of variance for the traits studied in sweet pepper advanced breeding lines.

	TCar	TCap	ASTA	Vit C	TPh	DPPH	ABTS	FIRM
Min.	0.20	6.67	0.13	39.42	13.01	11.19	10.95	0.41
Max.	9.46	39.55	3.32	85.13	71.24	49.18	80.05	1.17
Mean	4.53	16.58	0.77	58.19	29.81	23.35	39.64	0.80
Std dev.	2.37	7.60	0.64	13.27	12.91	9.94	16.85	0.19
Std error	1.37	4.39	0.37	7.66	7.46	5.74	9.73	0.11
C.V	52.41	45.81	83.11	22.80	44.78	42.58	42.51	23.44

Total carotenoids (TCar), total capsaicin (TCap),total polyphenol (TPh), , capsanthin (ASTA 20), ascorbic acid (VitC) firmness of fruits (FIRM) and antioxidant activities (DPPH and ABTS)

The focus of dietary antioxidant research in recent years has shifted from vitamin C, vitamin E and ß- carotene to natural phenolic photochemical. This may be due to its unique capacity to counteract reactive oxygen species, thus minimizing molecular damage (Koley et al., 7) *in vitro* and *in vivo* biological systems (Powels & Ness, 12) necessitates their quantification in foods. In the present study total phenolics were quantified in 38 ABLs in pepper. The total phenolic content ranged from 13.01 mg GAE /100g (VLCP-16-21) to 71.24 mg GAE /100g (VLCP-16-57) fresh weight with a mean value of 29.81±7.46 mg GAE/100 g. The other genotypes which showed elevated total phenolic content are VLCP-16-54 (64.32 mgGAE/100 g), Mukteshwar (56.82mg GAE/100 g) and VLCP-16-52 (55.43 mg GAE/100 g). These ABLs could be exploited in breeding programs for the development of varieties with enhanced health and nutritional benefits. Recent research results on anti-proliferation and apoptosis induction properties of phenolic compound to colon cancer cell line suggested that sweet pepper can be potential ingredient for the development of functional food for lower intestine health.

Capsaicin (8-methyl-n-vanillyl-6-non-enamide) is the dominant pungency principle unique to the genus, Capsicum, which imparts flavour and also has therapeutic uses for its anticarcinogenic properties (Surh & Lee, 17). Capsaicin content in these ABLs ranged from 6.67 to 39.55µg/100g. The highest content was recorded in Mukteshwar (39.55µg/100g) followed by VLCP-2016-52 (36.41 µg/100g). However, it was interesting to note that though there were drastic differences in capsaicin levels between ABLs, the magnitude of variation was 4.92 fold. The wide variation observed in different ABLs may be attributed to inherent variation in the levels of peroxidase enzymes in different sweet pepper cultivars. The sweet pepper ABLs analysed in the present study contains low content of capsaicin compared to pungent spice red pepper, which contributes to their characteristic flavour and also makes them suitable for culinary preparation. Carotenoids are lipophilic yellow-orange-red pigments found in plants and play a special role in protecting tissues from light and oxygen. Changes in the content and structure of carotenoids in plants can also be considered as markers of environmental damages (Arimboor *et al*, 1). The keto-carotenoids, capsanthin, capsorubin and cryptocapsin are unique *Capsicum* carotenoids. Capsanthin, the major carotenoid in ripe fruits, contributes up to 60% of the total carotenoids. In this study, total carotenoids content and capsanthin are analysed separately on fresh weight basis (fwb). The total carotenoids content among 38 ABLs ranged from 0.20 to 9.46 mg/100 g fresh weight depicting approximately 48.7 time variation. ABLs with higher amount of carotenoids are VLCP-2 (9.45mg /100 g FW), BLCPN.4 (9.06 mg/100 g FW) and VLCP-16-59 (25.88 mg/100 g FW). Similarly capsanthin content (expressed in ASTA value) ranged from 0.13 to 3.32 fwb depicting approximately 24.53 time variation. The high or low carotenoid content for a given ABLs depends upon on various factors: the level of expression of the genes governing carotenogenesis, morphological and physiological character inherent to the ABLs, and growth conditions. All these factors taken together may influence the performance of a cultivar with respect to phytochemical content. The total carotenoids and capsanthin content of the ABLs were found lower in level as compared to the reported level. This may be due to lack of optimum climate condition (warm and humid condition) in mid Himalaya region or the sampling of fruit which was done during transition from green to red or yellow stage (preferred by local consumers rather than fully ripen stage). Daood *et al*., (4) have reported there was increase in total carotenoids content in sweet peppers at the last stages of ripening. Both (genotype × environmental and genotype × maturity) interactions plays significant role in determining carotenoids content and capsanthin content which indicates that both environmental

condition and maturity are determinants of the content of antioxidant constituents in sweet peppers.

Antioxidants are compounds that reduce oxidative stress. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Free radicals and other reactive oxygen species generated in living organisms leads to many diseases including cancer, cardiovascular diseases, cataracts, asthma, hepatitis, liver injury and immunodeficiency diseases (Lee *et al.,*9). The use of synthetic antioxidants is an older practice and their safety could be questioned by the consumers. Therefore alternative natural phyto-compounds with efficient antioxidant activity have been paid increasing attention in recent days. In the present study, antioxidant activity was measured by two different methods, namely free radical (DPPH) and ABTS cation radical scavenging assay.

The DPPH is a stable free radical with a maximum absorbance at 515-517 nm. In the presence of an antioxidant, DPPH radical form a stable molecule (1,1-diphenyl-2-picrylhydrazine) by gaining one more electron or hydrogen atom from the antioxidant and consequential decreases UV absorbance indicates the scavenging potentials of the antioxidant extract. DPPH assay results indicated hydrophilic antioxidant activity ranged from 11.19 to 49.18 (% inhibition as compared to control) depicting approximately 3.39 time variation.

ABTS cation radical scavenging activity: ABTS assay also known as Trolox equivalent antioxidant capacity (TEAC) assay, the green–blue stable radical cationic chromophore, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$) is produced by oxidation, and has absorption maxima at 414, 645, 734, and 815 nm. In this cation radical scavenging method, the activity of tested sample extracts was expressed as micromolar equivalent of Trolox solution, having an antioxidant capacity equivalent to 1 g of the extarct under the experimental investigation. The present experiment ABTS radical ranged from 10.95 to 80.05 (% inhibition as compared to control). This wide variation observed in both DPPH and ABTS assay. It may be due to differences either in potency or in the concentration of reducing substances (mainly phenolics). Sweet peppers contain numerous phenolic compounds, and not all of the genotypes may contain a similar profile or relative proportions of compounds within the profile. Differences in these profiles may subsequently result in complex changes in antioxidant activity or other bioactivities (Pulido *et al*., 13). Various researchers have used different assay systems for determining antioxidant activity in Capsicum (Lee *et al*., 10). The variability in their assay systems does not allow us to make suitable comparisons. Based on both DPPH and ABTS free radical scavenging assays, VLCP-16-54, VLCP-16-57, VLCP-16-52 and Mukteshwar seem to be promising cultivars having high antioxidant activity. The high antioxidant activities in fruits are largely attributed to phenolic compounds as activity is generally considered to be dependent on their structure and content of it in sweet pepper. To further understand the relationship between antioxidant activity and total phenols, the correlation coefficient was calculated. The correlation analysis showed (Figs. 1 and 2) a strong relationship between total phenol and antioxidant activity measured by DPPH and ABTS with correlation coefficient, $r^2 = 0.8218$ DPPH, $r^2 = 0.8639$ ABTS (significant at $P \leq 0.05$).

These results suggest that the antioxidant activity of sweet pepper is mainly derived from the contribution of phenolics in sweet pepper. The literature reports concerning the relationship of phenols and antioxidant activity are accordant with our finding; Wang *et al* (18) found strong correlation between antioxidant activity and total phenolic content in blueberry.

Firmness is a significant textural attribute for sweet pepper fruit and directly influences their fruit quality and postharvest life. Currently, firmness is determined by the texture analyse firmness tester, which records maximum force for a steel probe of specific size and shape to penetrate fruit, for a pre-determined distance. In this study fruits of 38 ABLs were analysed separately and fruit firmness ranged from 0.19 to 1.17 Kg fresh weight depicting approximately 5.16 time variation. The highest fruit

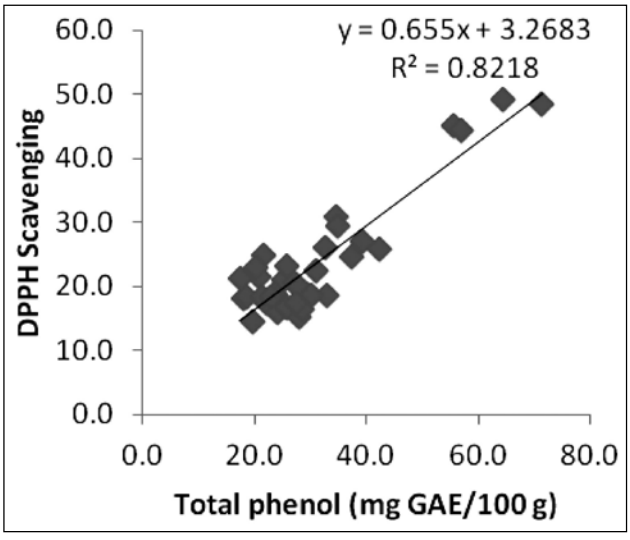

Fig. 1. DPPH radical scavenging activity of total phenol content.

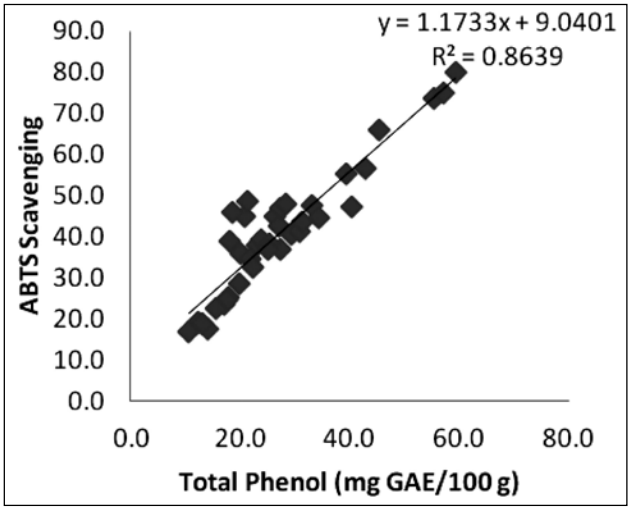

Fig. 2. ABTS radical scavenging activity of total phenol content.

firmness recorded in Mukteshwar (1.17) followed by VLCP-16-15 (1.15) and VLCP-16-61 (1.12). Fruit firmness is directly co-related to shelf-life and suitability to long term transportation. Fruit firmness is important criteria to select variety appropriate for mid Himalayan region due to lack of suitable systemic market in this area. Small scatter market required transition which causes physical injuries, loss of firmness and crispiness and ultimately results in the rejection of such fruit, with consequent commercial and monetary loss.

Chemometrics is a multivariate mathematical approaches which often permit a relatively simple representation of similarities between samples on the basis of more-or-less complex analytical data. Recently chemometrics become a very active research area and widely used to study the association of antioxidant component with antioxidant potentiality in vegetables with chemometrics techniques. (Koley *et al*.,7). In the present study PCA and AHC were performed as chemometric approaches to evaluate bioactive properties of sweet pepper. PCA is a mathematical tool which performs a reduction in data dimensionality and allows the more visualisation of underlying arrangement in experimental data and relationships between data and samples. Therefore, PCA was performed for thirty eight sweet pepper ABLs to observe any possible cluster within analysed samples. A new data set of 8 orthogonal variables (PCs) was generated by PCA (Table 3).

The first principal component had the highest eigen value of 4.287 and accounted for 53.593 % of the total variability of the data set. The second PC had the eigen value of 1.135 accounted for 14.18% respectively. The remaining six generated PCs (*i.e.*PC 3 to PC 8) yielded progressively smaller six values contributed variability in the dataset (32.23% total). Therefore, according to Keiser rule, only the first 2 PCs explaining about 67.77% of the total variability were selected and subjected to the varimax orthogonal rotation for a clear interpretation of the data. Total variance explained by principal component analysis was shown in Table 3

The first rotated factor (PC1) explained about 52.10% of the total variability and the second (PC2) presented 15.68% of the total variability respectively. Table 4 shows the most significant varimax rotated PCs generated from different variables as well as their statistical loadings in the present study. Factor loadings are squared correlation between original, measured variables and the factor/rotated factors derived from PCA and represent significant contribution to the overall variability. Based on the theoretical outlook of the PCA described by Hair *et al*. (5) the significant factor loading values higher than or equal to 0.7 were used to identify the significant variables and attributes

Table 3: Total variance explained by principal component analysis.

PC	Initial Eigen values			Extraction Sums of Squared Loadings			Rotation Sums of Squared		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.28	53.59	53.59	4.28	53.59	53.59	4.16	52.09	52.09
2	1.13	14.18	67.77	1.135	14.18	67.77	1.25	15.67	67.77
3	0.82	10.32	78.10						
4	0.67	8.49	86.59						
5	0.50	6.29	92.89						
6	0.24	3.09	95.98						
7	0.18	2.31	98.29						
8	0.13	1.70	100.00						

Table 4: Varimax rotated [a] factor loadings of the significant principal components (PCs).

Variables	Component	
	1	2
TCar	-0.63	-0.348
TCap	0.845	-0.198
ASTA	0.748	-0.039
Vit C	0.636	-0.011
TPh	0.89	0.175
DPPH	0.89	0.174
ABTS	0.713	0.421
FIRM	-0.026	0.924

Extraction Method: Principal Component Analysis, Rotation Method: Varimax with Kaiser Normalization
[a]Rotation converged in 3 iterations

in each eigen vector or principal components (PCs).

The PC 1 was highly positively contributed by Capsaicin content, ASTA units, ascorbic acid, polyphenol content, DPPH and ABTS antioxidant activity. However, carotenoides negatively attributed towards PC1. The PC2 was highly positively contributed by firmness of fruits. Fig 1a illustrates PCA scores plot for different sweet pepper advanced breeding lines. VLCP-16-52, VLCP-16-54 and VLCP-16-57 group close on lower side of left based on PCA score are recorded promising lines for total polyphenol (TPh), firmness of fruits (FIRM) and antioxidant activities (DPPH and ABTS). Fig. 3b illustrates the relationships between the parameters studied in the present work. Not surprisingly, polyphenol and ABTS antioxidant activity and are clustered together on the left hand side of the plot and were found to be significantly correlated as evidenced by their Pearson correlation coefficients. Similarly correlation coefficients showed total capsaicin (TCap), total polyphenol (TPh), Capsanthin (ASTA), ascorbic acid (Vit C) firmness of fruits (FIRM) and antioxidant activities (DPPH and ABTS) are found in opposition to Total carotenoids (TCar) and occupied a unique location at the very top of the figure.3b.

Hierarchical cluster analysis (HCA): In hierarchical cluster analysis, samples are grouped on the basis of similarities, without taking into account the information about the class membership. The results obtained following HCA shown as a dendrogram (Fig. 4) in which four well-defined clusters are visible in different colour. Samples will be grouped in clusters in terms of their nearness or similarity. Furthermore, Ward method classifies two major clusters which can be further divided into four sub-groups (Cluster I, II, III and IV). The results of our study were following the PCA.

The analysis revealed a distinct cluster of high, low and intermediate performing ABLs. It is interesting to see that the all high polyphenol, firmness and antioxidant activities containing ABLs were placed in same cluster I showing their unique genetic makeup for high polyphenol, firmness and antioxidant activities. Incidentally, most of the ascorbic acid (Vit C) rich ABLs *viz*.VLCP-16-1, VLCP-16-3, VLCP-16-56, VLCP-16-58, BLCPN.4, VLSM-3 and Mukteshwar were grouped together in Cluster II. Cluster III consisted of 11 ABLs as outlier probably due to its high carotenoids (TCar) and total capsaicin (TCap) and low phenolics content and ABTS and DPPH activity and cluster IV was again an outlier that consisted of the seventeen ABLs due to its overall lower performance in total phenolics, total carotenoids, antioxidant potentiality and other variables.

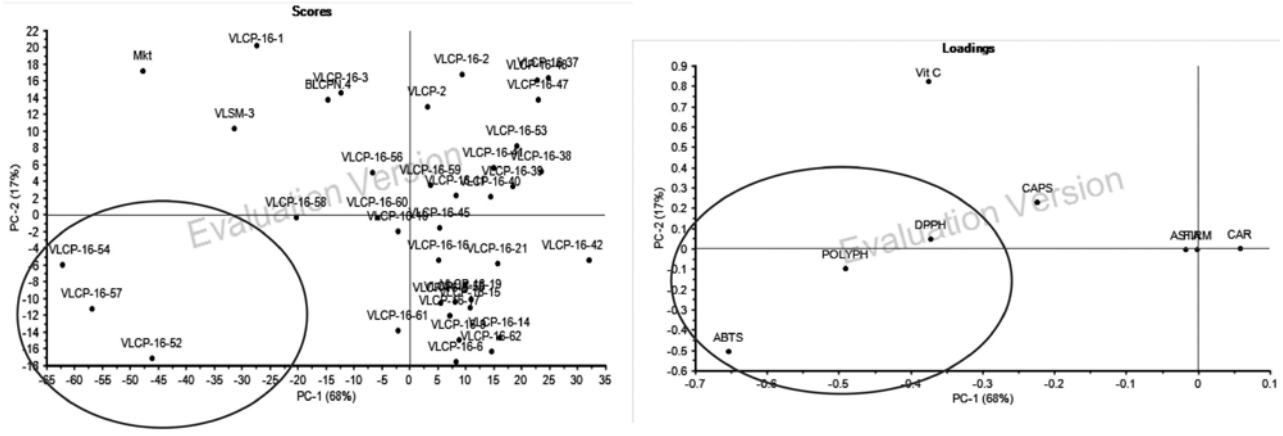

Fig. 3. Principal component analysis (PCA) plots. (a) PCA scores plot for different sweet pepper advanced breeding lines and (b) Loading plots for different bioactive compounds on PC1 and PC2.

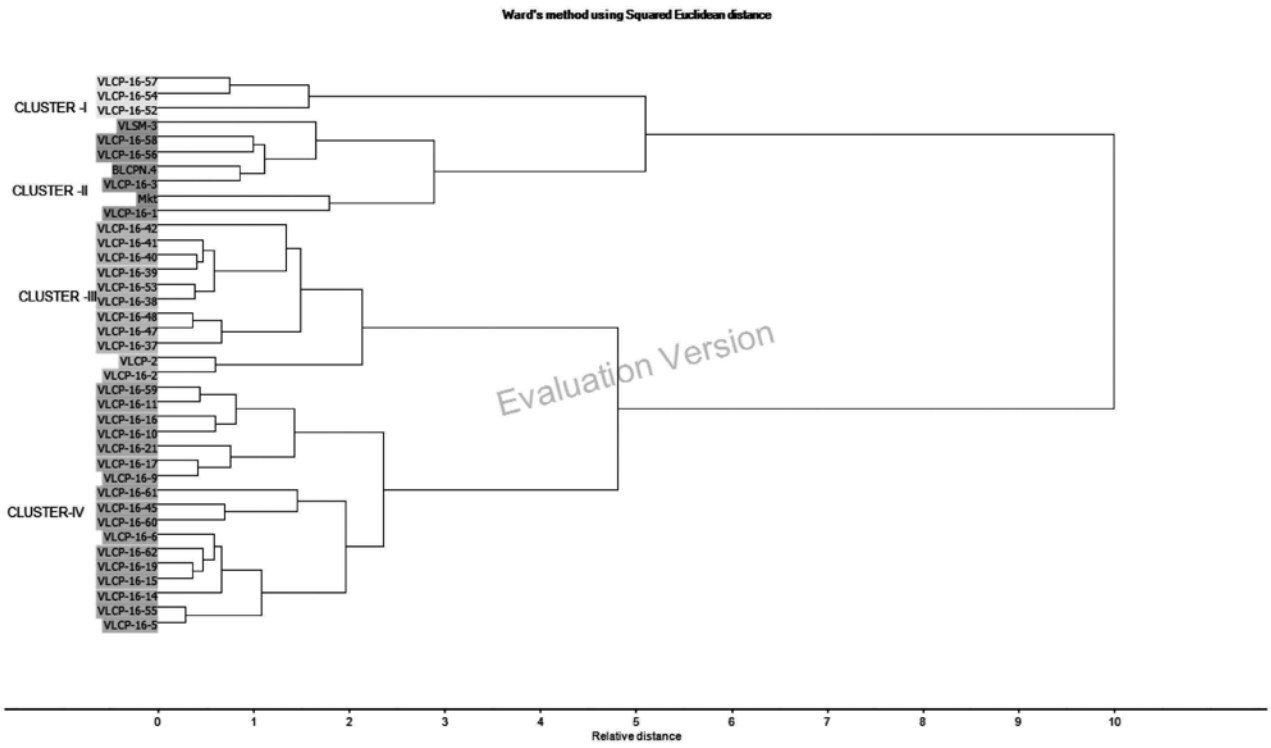

Fig. 4. Agglomerative hierarchical cluster analysis (AHC) of bioactive compounds and *in vitro* antioxidant activities from sweet pepper ABLs.

Collectively our data emphasize the existence of wide variations in antioxidant content (ascorbic acid, carotenoid, capsaicin and total polyphenol content), ASTA value, and antioxidant activity among sweet pepper cultivars. Results suggested that VLCP-16-1, VLSM-3 and Mukteshwar for Vitamin C; VLCP-16-57, VLCP-16-54, Mukteshwar and VLCP-16-52 for polyphenols, VLCP-16-54, VLCP-16-57, VLCP-2016-52 and showed highest antioxidant activity (DPPH and ABTS) and VLCP-2, BLCPN.4 and VLCP-16-59 for total carotenoids. These genotypes have a rich dietary composition and very suitable to grow under mid Himalaya region and to be released as new nutritionally rich cultivars for on-farm production. Texture of VLCP-16-15, VLCP-16-61 and Mukteshwar are highly firm and very suitable for long transport. Alternatively, these lines could also be of instant significance for further use as a donor in the future breeding which will further provide an option for cultivation of such vegetables in the nutritionally insecure and remote mountain areas.

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