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Screening of colored and green capsicum cultivars for economic traits and antioxidant potential grown at mid-hill climatic conditions of Uttarakhand

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Abstract

Six genotypes of bell pepper viz. Red Capsicum, Yellow Capsicum, Violet Capsicum (colored capsicums) and EC 579997, DARL 70, CW-51 (Green capsicums) were evaluated at Defence Institute of Bio-Energy Research, Field Station, Pithoragarh. Data were recorded for their yield and yield attributing traits, antioxidant constituents and antioxidant activity. The antioxidant activity of these genotypes has been estimated by ABTS and DPPH methods. Antioxidant constituents viz. ascorbic acid, total chlorophyll, tannin content, phenolic contents, total carotenoids, total flavonoids content were also estimated. The results showed significant differences for all the characters studied among these genotypes. Fruit length, fruit circumference, fruit weight, 1000 seed weight and seed yield were found the contributing characters for yield improvement. The antioxidant constituents and antioxidant activity in bell pepper were found in increasing order in colored fruits rather than green fruits. Red Capsicum was found the best towards quantitative traits and antioxidant potential followed by Yellow Capsicum and Violet Capsicum cultivars.

Keywords: Bell pepper, Cultivar, Antioxidant constituents, DPPH, ABTS

Introduction

Bell pepper (*Capsicum annuum* L var. *grossum* Sendt) is an annual herbaceous vegetable crop also known as sweet pepper, Shimla mirch, green pepper or capsicum. It is one of the most important and highly remunerative crops in mid-hills of Uttarakhand. Nowadays, health conscious people are much interested in colored vegetables and fruits because they are full of nutritional value, antioxidant constituents and antioxidant capacity. It is confirmed during epidemiological studies that there is positive relation between consumption of these food items and death rate of human beings due to life style diseases such as cardiovascular disease, cancer and other degenerative diseases. Among colored vegetables, capsicum is the important one. It comes in different colors viz. green, red, yellow, orange and violet. In the beginning all capsicums are green in color and during maturity they change their color. Green capsicums are immature and slightly bitter in taste while colored capsicums are fully matured and good in taste. As the fruit matures, it changes its color due to accumulation of carotenoids and becomes sweeter and more nutritional. Colored capsicums are the best sources of carotenoids like beta- capsanthin, capsorubin (mainly in red capsicums) cryptoxanthin, zeaxanthin (mainly in yellow capsicums), quercetin and leutin (mainly in orange capsicums). Violet color capsicum is having violet plants with violet leaves, flowers and fruits. Violet fruits contains anthocyanin pigments (Topuz and Ozdemir 2007; Wahyuni et al. 2011). The colored capsicums are more expensive than the green capsicums because harvesting in these capsicums is done at full maturity. The plants of these capsicums stay in fields for longer time and require more intercultural operations than the green capsicums.

In capsicum, yield is a quantitative character which is governed by yield attributing traits viz: fruit length, fruit width, fruit weight, fruit yield, seed weight and seed yield. Production of good quality vegetables is the primary factor of commercial vegetable cultivation for better economic returns. It is the chemical composition that mainly plays a crucial role for determining the quality of vegetables which makes them worth acceptable for consumption. The ascorbic acid or vitamin C has a great potential against heart diseases, cancer, blood pressure and high cholesterol (Antonious 2009; Byers and Perry 1992). Chlorophyll is another bio-molecule recognized

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as a health promoting phytochemical. The phenols, flavonoids and tannins are the secondary metabolites synthesized by the plants. These are potentially powerful antioxidants that can protect the human from free radicals damage (Velioglu et al. 1998; Chu et al. 2002; Kaur and Kapoor 2002; Materska and Perucka 2005; Nadeem et al. 2011). Antioxidant activity is an important parameter to establish the health functionality of a food product. The majority of the antioxidant activities of vegetables are due to ascorbate, phenols, flavonoids and tannins (Badami and Channa 2007).

Capsicum is a rich source of vitamin 'A' and 'C'. Occurrence of diverse antioxidants makes it an important food ingredient, known to protect against diseases (Kaur at al. 2007). Antioxidants are related with reduced cancer risks. Free radical is capable of inducing oxidative damage to human body. Antioxidants are the compounds which terminate the attack of reactive species and reduce the risks of diseases (Meena et al .2012). Diets rich in vegetables are thus associated with protection of different types of cancers. Due to economic and nutritional benefits of bell pepper, a study was conducted for screening of quantitative traits and antioxidant potential of red, yellow, violet and green capsicums.

Material and Methods

The experiment was carried out at Defence Institute of Bio-Energy Research, Field Station, Pithoragarh, Uttarakhand. The six genotypes of bell pepper viz. Red Capsicum, Yellow Capsicum, Violet Capsicum (colored capsicums) and EC 579997, DARL-70 & CW-51 (Green Capsicums) were grown in randomized block design with three replications under green house structures in two consecutive years i.e. 2018-19 and 2019-2020. Pithoragarh is situated at an altitude of 5500 feet above the sea level. This place is situated in western Himalayas, which extends from $29^0$29' N to $30^0$49' N latitude and $85^0$05' E to $81^0$31' E longitude. The annual rainfall is approximately 1250 mm, out of which 70-75% is received during the rainy season. The temperature of the place ranges from a maximum of 35^0C in summer to a minimum of -2^0C during winter. The variety/genotype were evaluated for quantitative traits viz. fruit length (cm), fruit width (cm), fruit weight (g), fruit yield/plant (kg), seed weight of 1000 seed (g) and seed yield/plant (g). Antioxidant constituents viz. ascorbic acid (mg/100g), total chlorophyll (mg/100g), tannin content (mg/g), phenolic contents (mg/g), total carotenoids (µg/ml), total flavonoids (mg/g) were also estimated. Antioxidant activity (IC_{50}mg/ml) in aqueous solution was estimated by ABTS and DPPH method. Such information will not only be helpful in identifying high yielding genotypes with better quality but also be helpful in further breeding programme for the development of new cultivars.

Yield and its yield attributing traits: Seedlings of all six genotypes were raised in nursery and transplanted in green house structures. Application of fertilizers, plant protection measures and other agro techniques were followed as per the recommendations. The crop was planted at 60 x 60 cm spacing and net plot area was kept 7.2 m^2. Data were recorded on yield and its yield attributing traits.

Antioxidants evaluation: Three replicates each comprising of a homogenous mass of capsicum fruits from 5 randomly selected plant were screened for antioxidant activity (IC_{50} mg/ml) through ABTS and DPPH method, ascorbic acid (mg/100g), total chlorophyll (mg/100g), tannin content (mg/g), phenolic contents (mg/g), total Carotenoids (µg/ml), total flavonoids (mg/g). The edible fruits were dehydrated in a hot air oven below 40^0C, powdered and stored for analysis. 1 g dried powder was extracted by cold maceration with 100 ml water and kept for 24 hrs by shaking occasionally. The aqueous filtrate was collected and stored.

Ascorbic acid assay: The chemical analysis of fresh fruits included determination of ascorbic acid (mg/100g) by 2, 6 di chlorophenol indol phenol titration method (AOAC, 1980). Ascorbic acid was estimated by volumetric method. 5 g of the fresh sample was extracted with 4% oxalic acid and volume made to 100 ml and centrifuged. 5 ml of this supernatant was pipette out, added with 10 ml of 4% oxalic acid and titration was done against the dye. Ascorbic acid reduces the 2, 6-dichlorophenol dye to a colorless leuco- base and gets oxidized to dehydro-ascorbic acid.

Chlorophyll assay: Chlorophyll content was estimated through the method developed by Rangana, S. (1976). 1 g finely cut and well mixed sample of leaf or fruit tissues was made to a fine pulp with the addition of 20 ml of 80% acetone. Centrifuged (5000 rpm for 5 min) and the supernatant were transferred to a 100 ml volumetric flask. The residue was grinded with 20 ml of 80% acetone, centrifuged and transferred the supernatant to the same volumetric flask. This process was repeated until the residue becomes colorless. The clear washings were collected in the volumetric flask and volume was made to 100 ml with 80% acetone. Thus chlorophyll was extracted in 80% acetone and absorption at 663 nm and 645 nm were read in a spectrophotometer. Using the absorption coefficients, the amount of chlorophyll was calculated.

Determination of total tannin content: Total tannin contents were determined by Folin- Denis method (Schanderi et al. 1970). 0.5 g of powder was boiled for 30 min with 75 ml of double distilled water. It was cooled, centrifuged at 2000 rpm for 20 min and supernatant was collected in 100 ml volumetric flask and the volume was made up with double distilled water. 1 ml of this solution was transferred to a 100 ml volumetric flask containing 75 ml water and 5 ml Folin-Denis reagent + 10 ml of sodium carbonate solution were added and diluted up to 100 ml with water. After shaking, the absorbance was measured at 700 nm after 30 min. Total tannin content of the sample was measured equivalent to tannic acid by using standard calibration curve of tannic acid.

Estimation of total phenolic contents: The total phenolic contents in the plant extract were determined by using Folin- Ciocalteu method (Malik et al. 1980). The plant extract was taken and volume make up to 3 ml with distilled water. Then 0.5 ml Folin-ciocalteau reagent and 2 ml of 20% Na_2CO_3 were added in each tube. A blue color was developed due to the complex redox reaction with phosphomolibdic acid in folin ciocalteau reagent. The test solutions were warm for 1 minute, cooled and absorbance was measured at 650 nm. The concentration of total phenol was measured equivalent to catechol (mg catechol equivalent of phenol/ g of sample) by using standard calibration curve of catechol.

Carotenoid assay: Total carotenoids concentration was quantified according to Rodriguez-Amaya and Kimura (2004). 1 g fresh sample was homogenized in 20 ml acetone and the supernatant decanted. This process was repeated until attaining complete removal of all pigments. The sample was filtered and washed with 30 ml acetone, the acetone evaporated and the dry sample dissolved in 60 ml petroleum ether. The resulting solution was filtered, transferred quantitatively to a 100 ml volumetric flask, and volume completed with petroleum ether. Of this solution, 2 ml were placed in a test tube with 8 ml petroleum ether. Absorbance was read at 475 nm (ECIL, Double Beam UV- VIS Spectrophotometer 5704SS) and concentration calculated with a â-carotene curve. The results were expressed as µg/ml.

Estimation of total flavonoids: The total flavonoid contents were determined by Aluminum chloride colorimetric method (Chang et al. 2002). The assay was performed using 0.5ml of plant extract. To each test tube 1.5ml methanol, 0.1ml aluminum chloride solution, 0.1 ml potassium acetate solution and 2.8 ml distilled water added and mixed well, then kept for 30 min. and measure the absorbance at 415 nm against the suitable blank (all reagents except aluminum chloride). The concentration of total flavonoid contents of the sample was measured equivalent to quercetin by using standard calibration curve of quercetin.

Anti-oxidant Assay

DPPH Method: The DPPH (2, 2-diphenyl-1-picrylhydrazyl) method (Hatano et al. 1989; Kedare and Singh 2011) was used for estimating free radical scavenging activity of the methanol extracts of samples. 2 ml of methanol extract (4 mg/ml) taken in test tube and final volume of 3 ml was made with methanol. The absorbance of the mixture was measured after 40 min at 517 nm against methanol as blank. Ascorbic acid was used as standard. The free radical scavenging activities (%) of tested samples were evaluated by comparing with a control (2 ml DPPH and 1 ml of methanol). Each sample was then measured in triplicate and averaged. The free radical scavenging activity (FRSA) was calculated using the formula: FRSA= [(Ac-At)/Ac-As] x 100, where Ac=Absorbance of control, As=Absorbance of standard and At = Absorbance of test. IC_{50} value = (conc. of test/ FRSA nearest to the 50%) × 50

ABTS Method: The Total Antioxidant Activity was determined according to the method given by (Re R et al. 1999). ABTS (2,2-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) reacts with potassium persulphate to produce ABTS Radical Cation ($ABTS^{\bullet+}$), a blue green chromogen with absorption maxima at 734 nm. the extent of decolorization is a significant indicator of antioxidant activity of the sample. the effects of antioxidants on $ABTS^{\bullet+}$ radical cation is due to its hydrogen donating availability which is observed by a change in color radical cation ($ABTS^{\bullet+}$) to colorless ABTS. The percentage inhibition of $ABTS^{\bullet+}$radicals at different concentrations were determined using the following formulae and further IC_{50} value was calculated:

FRSA(%) = [(Ac-At)/Ac] ×100, Where, Ac = Absorbance of Control, At = Absorbance of Test.

IC_{50} value = (conc. of test/ FRSA nearest to the 50%) × 50

The results were represented as Mean ± Standard Deviation (N=3). The results were interpreted using One-way analysis of variance (ANOVA) and Duncan's test at 0.05 probability levels by SPSS 16.0 Software.

Results and Discussion

Yield and its yield attributing parameters: The analysis of variance revealed that there were significant differences in all the parameters studied in this

experiment. Values are expressed as Mean ± SD (N=3) Different alphabets represents the significant differences among the capsicum genotypes (Table 1). Fruit length, fruit circumference, fruit weight, 1000 seeds weight and seed yield are the contributing characters for yield improvement. Fruit length was measured from the stem end to the blossom end of the fruit. Red Capsicum exhibited maximum fruit length (12.27 cm) followed by Yellow Capsicum (10.70 cm). Violet Capsicum showed minimum fruit length (5.03 cm). Fruit circumference was measured in centimeters at the broadest part of the fruit. Again Red Capsicum showed maximum fruit circumference (26.23 cm) followed by Yellow Capsicum (94.35 cm). For weight of fresh fruit, five fruits from each picking were randomly taken and weighed to calculate average fruit weight. Fruit weight ranged from 39.30 to 118.33g. Fruits of Red Capsicum weighed (118.33g) and minimum fruit weight (39.30g) was exhibited by genotype Violet Capsicum. Fruit yield per plant was calculated by the total weight of all the marketable fruits from all the pickings was divided by number of plants to get fruit yield per plant. Red Capsicum exhibited highest fruit yield per plant (2.190 kg) followed by Yellow Capsicum genotype (1.915 kg). Genotype EC 579997 showed minimum fruit yield/plant (1.220 kg). 1000 seed weight was measured by weighing in electronic balance. 1000 seed weight was found maximum (6.722 g) in Red Capsicum followed by Yellow Capsicum (6.073 g). Seed yield per plant was calculated by the total seed weight of matured fruits from the plants from the net plot area and divided it by number of plants in that plot area to get seed yield per plant. Seed yield per plant (g) ranged from 6.657-14.029 g. Red Capsicum gave highest seed yield per plant (14.029 g) and genotype DARL 70 exhibited 6.657g seed yield/plant. Evaluation study for yield attributing traits and quality parameters of colored sweet pepper germplasm was also carried out by Sultana et al (2020). The bell peppers have diversity in shape, size, yield, quality and other traits. So for the exploitation of their potential, region specific selection of good cultivars is the need of hour.

Antioxidant constituents: Values are expressed as Mean ± SD (N=3). Different alphabets represent the significant differences among the capsicum genotypes (Table 2). Ascorbic acid content (mg/100g) ranged from 45.27-180.86 mg/100g. Red Capsicum exhibited maximum ascorbic acid content (180.86 mg/100g) followed by Yellow Capsicum (128.50 mg/100g) and DARL 70 (102.62 mg/100g). Bell peppers are the excellent source of vitamin C. Studies conducted by other scientists also proved that the concentration of this vitamin increases with fruit ripeness. Howard et al. (1994), Simmone et al. (1997) and Navarro et al. (2006) studied that there was higher increase in vitamin C content from immature stage of fruits (green) to fully matured stage (red). Ascorbic acid determination in various colored peppers by titration method was also carried out by Nerdy and Nerdy (2016).

Chlorophyll is a natural dye extracted from green plants. It is the pigment which helps in the process of photosynthesis and also used in cosmetics and food industry (Humphery 2004). In our study, the range of total chlorophyll (mg/100g) was from 1.18-5.00 mg/100g. Genotype DARL 70 (Green Capsicum) exhibited maximum amount of chlorophyll (5.0 mg/100g) followed by EC 579997 (4.46 mg/100g) and California Wonder (2.84 mg/100g). Yellow capsicum exhibited minimum chlorophyll content (1.18 mg/100g). Chlorophyll determination in green peppers was also studied by Manolopoulou et al. (2016).

Tanin contents (mg/g) ranged from 16.67-29.09. Maximum tannin content was estimated in Red Capsicum (29.09 mg/g) followed by Yellow Capsicum (25.46 mg/g) followed by CW-51(21.85 mg/g). The range of phenolic content (mg/g) was from 5.03 to 8.18 mg/g. Red capsicum exhibited the maximum range (8.18 mg/g) and minimum ranged was exhibited by genotype DARL 70. Bell pepper is richest in polyphenolic compounds such as tannins and phenols with having strong antioxidant potential. Their level in the fruits increases with the stages of ripening (Nadeem et al. 2011; Hallmann and Rembialkowska 2012).

Table 1: Yield and related attributes of bell pepper cultivars

Capsicum germplasm	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	Fruit yield per plant (kg)	1000 Seed weight (g)	Seed yield per plant (g)
Red Capsicum	12.27±0.945a	26.23±0.862a	118.33±6.506a	2.190±0.274a	6.722±0.011a	14.029±0.195a
Yellow Capsicum	10.70±0.400b	24.43±0.665b	94.35±5.131b	1.915±0.102b	6.073±0.064b	11.271±0.006b
Violet Capsicum	05.03±0.351f	12.23±0.152f	39.30±5.131e	1.586±0.077c,d	5.449±0.013d	09.730±0.004c
EC 579997	07.23±0.208e	15.60±0.264e	67.65±7.505d	1.220±0.026e	5.695±0.006c	09.993±0.003c
DARL 70	08.47±0.208d	19.45±0.180c	81.00±3.605c	1.695±0.022b,c	4.842±0.025f	06.657±0.852e
CW 51	09.45±0.152c	17.50±0.200d	90.35±2.516bc	1.412±0.012de	5.343±0.020e	08.342±0.326d

Values are expressed as Mean±SD (N=3) Different alphabets represents the significant differences among the capsicum genotypes

Table 2: Antioxidant constituents in bell pepper cultivars

Capsicum germplasm	Ascorbic acid (mg/100g)	Total chlorophyll (mg/100g)	Tanin (mg/g)	Phenols (mg/g)	Total carotenoids (µg/ml)	Flavonoids (mg/ml)
Red Capsicum	180.86±0.432a	1.32±0.005e	29.09±0.421a	8.18±0.160a	265.83±0.020a	4.24±0.046a
Yellow Capsicum	128.50±0.739b	1.18±0.000f	25.46±0.398b	7.47±0.150b	170.85±0.020b	2.00±0.025b
Violet Capsicum	45.27±0.577d	2.36±0.005d	20.51±0.617d	6.30±0.045d	80.72±0.017c	1.67±0.017c
EC 579997	81.97±0.034c,d	4.46±0.000b	16.67±0.000f	5.20±0.015e	66.71±0.011f	1.31±0.000f
DARL 70	102.62±0.548b,c	5.00±0.005a	19.58±0.067e	5.03±0.057e	68.12±0.025e	1.13±0.115e
CW 51	97.99±0.753b,c	2.84±0.005c	21.85±0.438c	6.96±0.017c	70.33±0.028d	1.97±0.020d

Values are expressed as Mean ± SD (N=3) Different alphabets represents the significant differences among the capsicum genotypes Anti-oxidant activity using DPPH and ABTS method

Table 3: Antioxidant activity in bell pepper cultivars

Capsicum germplasm	Antioxidant activity (ABTS method)	Antioxidant activity (DPPH method)
Red Capsicum	0.566±0.004f	1.017±0.000f
Yellow Capsicum	0.799±0.007e	1.169±0.006e
Violet Capsicum	1.058±0.000c	1.952±0.011b
EC 579997	1.113±0.002d	2.085±0.022a
DARL 70	1.190±0.003a	1.615±0.003c
CW 51	1.127±0.001b	1.492±0.003d

Values are expressed as Mean ± SD (N=3) Different alphabets represents the significant differences among the capsicum genotypes

Capsicum is good source of carotenoids among vegetable crops. During the estimation of total carotenoids (µg/ml), Red capsicum showed maximum total carotenoids (265.83µg/ml) and minimum range was exhibited by EC 579997(66.71µg/ml). Our results are agreed with the study made by Simmone et al. (1997) in which ß carotene was highest in red fruits and lowest in green fruits. The flavonoid contents (mg/g) ranged from 1.13 to 4.24 mg/g. Red Capsicum exhibited the maximum range (4.24 mg/g) and DARL70 showed the minimum range (1.13 mg/g). Total flavonoid contents also showed ripening-dependent changes in study made by Howard et al. (2000) and Ghasemnezhad et al. (2011).

Data revealed (Table 3) that antioxidant activity i.e. inhibition concentration 50 (IC_{50}) ranged from 0.566 to 1.190 mg/ml through ABTS method in aqueous extract. Red capsicum exhibited lowest IC_{50} value (0.566 mg/ml) i.e. highest antioxidant activity was found followed by Yellow Capsicum (0.799 mg/ml) and Violet Capsicum (1.058 mg/ml). DARL-70 (Green Capsicum showed highest IC_{50} value (1.190 mg/ml) i.e. lowest antioxidant activity. With DPPH method in aqueous extract, the range of IC_{50} value (mg/ml) varied from 1.107-2.085 mg/ml. Red Capsicum exhibited lowest concentration (1.107 mg/ml) means highest antioxidant activity and EC579997 (Green Capsicum) exhibited highest concentration (2.085 mg/ml) i.e. lowest antioxidant activity. There is negative correlation between antioxidant constituents and IC_{50} values means higher the phytochemicals constituent lesser the IC_{50} value, therefore, higher the antioxidant activity. The antioxidant activity in bell pepper is also dependent on ripening. A well ripened fruit showed highest antioxidant activity due to accumulation of more ascorbic acid, polyphenols and carotenoids in matured stage. Our study is well confirmed by the study made by Howard et al. (1994); Guil-Guerrero et al. (2006); Deepa et al. (2007) and Bhandari et al. (2013).

From this study, it was concluded that colored bell peppers are richer in antioxidant constituents and antioxidant activity than green bell peppers. Red capsicums followed by Yellow capsicum were better in yield and its yield attributing traits. These lines can be used in our further breeding programme for the development of improved quality cultivars.

सारांश

शिमला मिर्च की चार रंग वाली किस्मों जैसे– लाल शिमला मिर्च, पीली शिमला मिर्च, बैंगनी शिमला मिर्च और हरी शिमला मिर्च की ईसी 57997, डी.ए.आर.एल.–70, सी.डब्ल्यू.–51 का मात्रात्मक एवं गुणात्मक मूल्यांकन रक्षा ऊर्जा अनुसंधान संस्थान, पिथौरागढ़ (उत्तराखण्ड) में किया गया। उपज एवं उपज विशेषता क्षमता, प्रति ऑक्सीकारक तत्त्वों एवं प्रति ऑक्सीकारक क्षमता के आंकड़ों को अभिलेखित किया गया। सभी आंकड़ों में लाल शिमला मिर्च उत्तम पायी गयी। प्रति ऑक्सीलाकरक तत्त्व, प्रति ऑक्सीकारक क्षमता रंगीन शिमला मिर्च में हरी शिमला मिर्चों की तुलना में अधिक पायी गयी। ये सभी प्रभेद अग्रिम प्रजनन कार्यक्रम में नयी प्रजातियों को विकसित करने में प्रयोग में लायी जा सकती हैं। रंगीन शिमला मिर्च जिनमें ज्यादा प्रति ऑक्सीकारक क्षमता विद्यमान है, औषधीय एवं पोषण मूल्यों की उपस्थिति का भी संकेत प्राप्त होता है। रंगीन शिमला मिर्च प्राकृतिक प्रति ऑक्सीकारक तत्वों के रूप में प्रयोग में लायी जा सकती है।

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