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Determination of phenols, ascorbic acid, capsaicin and colour values in chilli (*capsicum annuum* L.)

Rahul Sonaniya, SK Singh, RS Shukla and VP Bagde

Abstract
The genus *Capsicum* generally recognized as chilli pepper is a major spice crop rich in ascorbic acid, capsaicin content and carotenoids. An investigation was started on 48 diverse chilli genotypes to analyses the biochemical components (total phenols, ascorbic acid, capsaicin content and color values) to identify the promising lines dominated with nutritive and resistance traits. Fully matured and dried chilli fruits of each genotype were utilized for analysis of different quality components. It was noted that among the 48 genotypes capsaicin content was ranged from 0.19% - 0.40%, ascorbic acid was from 92.38 mg/100g - 175.85 mg/100g, phenol content was from 9.47 mg/g - 18.78 mg/g and colour value were ranged from 33.82 - 53.55 for L*, 11.28 - 28.87 for a* and 13.36 - 38.13 for b*. Genotype MPJCC5 showed highest ascorbic acid content (175.85 mg/100g) whereas maximum phenol and capsaicin content was recorded in MPJCC12 (18.78 mg/g) and Pusa jwala (0.40%). Maximum color value for L* a* b* were noted for MPJCC15 (53.55), MPRJJCC1 (28.87) and MPJCC15 (38.13) respectively. The identified promising genotypes for different quality components will be of great importance in chilli hybrid breeding program and a these lines will be utilized as donor for the development of high quality chilli lines/hybrids coupled with high economic importance.

Keywords: *Capsicum annuum* L., ascorbic acid, phenol, color value (L* a* b*), hunter color, capsaicin content

Introduction

Chilli (*Capsicum annuum* L.) is a major spice cum vegetable crop used all over the world in diverse form. India is the single major producer and exporter of chilies, meeting approximately half of the world's intake demand (Sajjan *et al*., 2020) [25].

In 2020-21, total area of chilli in India was 7, 32,213 ha, with the production of 19, 88,304 lakh tons with vast majority of production occurring in Andhra Pradesh (42%) followed by Telangana (20.48%) and then by Madhya Pradesh (14.71%). Total area and production of spices in Madhya Pradesh is 6, 99,994 ha and 32, 37,655 tons respectively. Chilli covers the area of 1, 13,366 ha (16.19%) and production of 2, 62,616 lakh tons (8.1%) (Spice board, India 2020).

Chilli has some important marketable qualities. Some varieties are well-known for red colour because of the pigment capsanthin plays a major role for the different color values as economic importance in different food and cosmetic industries. Chilli price is basically based on its shape size and colour values. Color scales are a powerful tool used in agriculture for estimating maturity of fruits. A variety of color scales have been used to define color. Those frequently used in the food-processing industries are the Hunter color L, a, b system and CIE LAB color scales (Giese, 2000) [10]. During study of color of red chilli, Ramkrishnan and Francis (1973) observed that both color values a and L decreased during heating and they advocated the use of L*a as the color quality parameter while Nagle *et al*. (1979) [20] and Carnavale *et al*. (1980) found that there were varying levels of correlation of total pigments and tristimulus L, a, b color values. There was poor correlation between total pigments and a value, while b value and a/b showed better correlation with total pigment.

The compounds liable for the pungency of the chilli peppers fit to group of secondary metabolites known as capsaicinoids. Two chief capsaicinoids found in chillies are capsaicin and dihydrocapsaicin. Capsaicin and dihydrocapsaicin characterize about 77-98% of total capsaicinoids content in peppers. Capsaicin is a very steady alkaloid, which is stable under exposure to heat and cold, has no flavour, colour, aroma and is insoluble in water, but effortlessly soluble in fat or some organic solvents (DeWitt and Bosland 2009) [9]. Capsaicin is acknowledged for his pharmacological, neurological and dietetic effectiveness.

It has also significant antibiotic activity and the ability to reduce the cholesterol level in blood (Gurnani *et al*. 2016) [11]. Capsaicin is also capable to kill some types of cancer cells (Anandakumar *et al*. 2013, Dawan *et al*. 2017, Prasad *et al*. 2006) [3, 8, 22] and provide 861 November 8–9, 2017, Brno, Czech Republic 24 years relief in arthritis and respiratory ailments (Prasad *et al*. 2006) [22], it is also antioxidant and has anticancer, antiarthritic and analgesic properties (Dawan *et al*. 2017, Prasad *et al*. 2006) [8, 22].
Plant phenolics are secondary metabolites that constitute one of the most common and widespread groups of substances in plants. Phenolics biogenetically arise from the shikimate-phenyl-propanoids flavonoids pathways (Lattanzio *et al*., 2006) [17]. Plants need phenolic compounds for pigmentation, growth, resistance to pathogens and for many other functions. Therefore, they represent adaptive characters that have been subjected to natural selection during evolution.
Chili pepper is also known for its richness in ascorbic acid, a very essential antioxidant for human nutrition and proper functioning of body (Igwemmar *et al*., 2013; Mohammed *et al*., 2013) [13, 19]. Fresh green chili pepper contains more vitamin C than citrus fruits and fresh red chili pepper has more vitamin A than carrots (Chigoziri and Ekefan, 2013) [6]. Chili pepper is also suitable for the diets of the obese and is useful in the control of cancer of the stomach and colon (TayebRezvani *et al*., 2013; Dang *et al*., 2014) [27, 7].

Material and Methods

Forty eight chili genotypes were collected form 12 states were grown at breeder seed production unit (soybean), Department of Plant Breeding and Genetics, College of Agriculture, JNKVV, Jabalpur (Table. 1). All the genotypes were grown in RBD (Randomized block design) with 3 replications during the *Rabi*/summer 2020-21, *Kharif* 2021 and *Rabi/*summer 2021-22. Matured fruits were isolated and dried for the estimation of quality components. All the biochemical estimation was completed at Department of Food Science and Technology, JNKVV, Jabalpur. For the quality analysis, all the genotypes were analyzed for assessment of capsaicin, phenol, vitamin C content and color values.

Table 1: List of genotypes with their collection centers and year of collection

S.no	Name of genotype	Collection from	Year of collection
1	MPJCC 1	Bedia, M.P.	2014
2	MPJCC 2	Bedia, M.P.	2014
3	MPJCC 3	Bedia, M.P.	2014
4	yellow chilli	Khargone, M.P.	2014
5	MPJCC 4	Khargone, M.P.	2014
6	MPJCC 5	Khargone, M.P.	2014
7	MPJCC 6	Khargone, M.P.	2014
8	MPJCC 7	Badwani, M.P.	2014
9	MPJCC15	Badwani, M.P.	2014
10	MPJCC16	Badwani, M.P.	2014
11	MPJCC17	Shajapur M.P.	2014
12	MPJKJCC1	Baramullaka, Kashmir	2014
13	MPBJCC2	Patna, Bihar	2014
14	MPJCC14	Khargone, M.P.	2014
15	MPJCC12	Khargone, M.P.	2014
16	MPJCC21	Bedia, M.P.	2014
17	MPJCC18	Bedia, M.P.	2014
18	MPJCC 13	Bedia, M.P.	2014
19	MPJCC19	Khandwa, M.P.	2015
20	MPDJCC 1	Delhi	2015
21	MPRJCC 1	Jodhpur, Rajasthan	2015
22	MPKJCC 1	Banglore, Karnataka	2015
23	pusa jwala	Jodhpur, Rajasthan	2015
24	MPRJCC 2	Jodhpur, Rajasthan	2015
25	MPOJCC 1	Bhubaneswar, Odisha	2015
26	MPJCC20	Sagar, M.P.	2015
27	MPJCC24	Alipur, M.P.	2015
28	MPRJCC 3	Sikar, Rajasthan	2015
29	MPRJCC4	Sikar, Rajasthan	2015
30	MPRJCC5	Sikar, Rajasthan	2015
31	MPMJCC 1	Amravati, Maharashtra	2015
32	MPUPJCC 1	Prayagraj, U.P.	2015
33	MPUPJCC 2	Lucknow, U.P.	2015
34	MPHRJCC1	Faridabad, Haryana	2016
35	MPOJCC2	Bhubaneswar, Odisha	2016
36	MPDJCC2	Delhi	2016
37	MPBJCC 1	Patna, Bihar	2016
38	MPJCC 8	Khargone, M.P.	2016
39	MPJCC22	Jhabua, M.P.	2016
40	MPJCC8	Jhabua, M.P.	2016
41	MPJCC9	Khargone, M.P.	2016
42	MPJCC10	Khargone, M.P.	2016

43	MPMGJCC 1	Shillong, Meghalaya	2016
44	MPMGJCC 2	Shillong, Meghalaya	2016
45	MPUPJCC3	Banaras, U.P.	2016
46	Kashi Anmol	IVRI, U.P.	2016
47	MPJCC23	Raipur, Chattisgarh	2016
48	MPUPJCC4	Prayagraj, U.P.	2016

Quality Traits

Phenol content

Total phenolic compounds were determined by mixing 2.0 mL extract solution, 1.0 mL of 10% Folin-Ciocalteau reagent, 2.0 mL sodium carbonate (20%), and 2.5 mL distilled water. The reaction mixture was incubated at room temperature for 30 min in the dark. The absorbance of the solution was measured at 765 nm. Total phenol content was subsequently calculated and expressed as µg gallic acid equivalents (GAE) per gram of (µg GAE/g dw).

Ascorbic acid content (mg/100g)

Ascorbic acid was estimated by the method as described by Rangana (1986) using 2, 6-dichlorophenol indophenol dye. Dye factor was calculated by titrating 5 ml standard ascorbic acid plus 5 ml (3%) meta-phosphoric acid against 2, 6-dichlorophenol indophenol till pink colour appeared and volume used was noted.

$$\text{Dye factor} = \frac{0.5}{\text{Titre value}}$$

Ascorbic acid was estimated by taking 5g of sample, volume made upto 100 ml with (3%) meta-phosphoric acid and filtered. Then aliquot of 10 ml was taken in a titration flask and titrated against 2, 6-dichlorophenol indophenol till light pink colour appeared (which persisted for 15 seconds). Vitamin C was calculated using the following formula:

$$\text{Ascorbic acid}\left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{Titre value x dye factor x volume made up}}{\text{ml of filtrate taken for x weight of sample estimation}} \times 100$$

Color values (L*, a*, b*)

The L*, a*, b* color values were observed by Hunter colorimeter (Model CR-2000, Minolta, Osaka, Japan), equipped with an 8-mm measuring head and a c illumination (6774 K). The meter was calibrated using the manufacturer's standard white plate. Color changes were quantified in the L*, a*, b* color space. L *, refers to lightness of the color of the sample fruit and ranges from black = 0 to white = 100. A negative value of a* indicates a green color where the positive value indicates red-purple color. A positive value of b* indicates a yellow colour and the negative value a blue colour (McGuire, 1992).

Capsaicin content

Capsaicin is extracted from red pepper (chillies) with ethyl acetate and made to react with ethyl acetate solution of vanadium oxychloride. Then it is read at 720nm. This method is sensitive and is useful to measure small quantities (less than 0.05%).

Sample was grinded well to pass through No. 40 sieve. Two gram sample was taken in 100mL volumetric flask and made it stand for 24h to extract (otherwise reflux the contents for 2.5h) and then make up to volume. Diluted 1mL of extract to 5mL with ethyl acetate and then 0.5mL vanadium oxychloride solution was added and shaked. Reading was taken at 720nm in a spectrophotometer and absorbance value was substracted.

Calculation

$$\%\ \text{Capsaisin} = \frac{\mu g\ capsaisin}{1000x1000} X \frac{100}{1} X \frac{100}{2} = \frac{\mu g\ Capsaicin}{200}$$

Results and Discussion

Forty eight individual plants were screened based on their phenotypic performance and evaluated for quality parameters *viz*. phenol content (mg/g), ascorbic acid (mg/100g), colour value (L*,a*,b*), capsaicin content (%). The mean performances of these characters are presented in Table 2, quality character's value is presented in Table 3 and color value is described in Table 4.

Phenol content (mg/g)

Phenol content was ranged from 9.47 mg/g to 18.78 mg/g with a mean performance of 13.25 mg/g. This finding is supported by the work of Ayob *et al*. (2021), Hamed *et al*. (2019) and Latha & Hunumanthraya (2018) [4, 12, 16]. The minimum phenol content was recorded by MPJKJCC1 (9.47 mg/g) while the maximum phenol content was recorded in MPJCC12 (18.78 mg/g). Genotypic and phenotypic coefficients of variation were 18.76 and 19.25 per cent, respectively. This trait exhibited high heritability (95.02%) coupled with low expected genetic advance (4.99).

Ascorbic acid (mg/100g)

Ascorbic acid content was ranged from 92.38 to 175.85 mg/100g with a mean performance of 142.94 mg/100g. The similar Kopta *et al*. (2020), Kennao *et al*. (2020) and Hamed *et al*. (2019) [15, 14, 12] shows similar results in their studies. The minimum ascorbic acid content was recorded by MPDJCC1 (92.38 mg/100g) and the maximum ascorbic acid content was recorded in MPJCC 5 (175.85 mg/100g). Genotypic and phenotypic coefficients of variation were 13.67 and 14.12 per cent, respectively. This trait exhibited high heritability (93.85%) coupled with high genetic advance (39.00).

Capsaicin content (%)

The capsaicin content for selected genotypes ranged from 0.19% to 0.40% with mean performance of 0.31%. Similar results were noted by Popelka *et al*. (2017), Tilahun *et al*. (2013), Toontom *et al*. (2012) and Sanatombi *et al*. (2008) [21, 28, 29, 26]. The minimum capsaicin content was recorded by MPJCC14 (0.19%). The maximum capsaicin content was recorded with Pusa jwala (0.40%). Genotypic and phenotypic coefficients of variation were 15.56 and 18.67 per cent, respectively. This trait exhibited moderate heritability (69.47%) coupled with low expected genetic advance (8.25).

Table 2: Estimates of genetic parameters for quality parameters of chill

Character	Range		Mean	GCV (%)	PCV (%)	Heritability (%) broad sense	GA
	Max	Min					
X1	18.78	9.47	13.25	18.76	19.25	95.02	4.99
X2	175.85	92.38	142.94	13.67	14.12	93.85	39.00
X3	0.40	0.19	0.31	15.56	18.67	69.47	8.25

X1 = Phenol content (mg/g)
X2 = Ascorbic acid (mg/100g)
X3 = Capsaicin content (%)

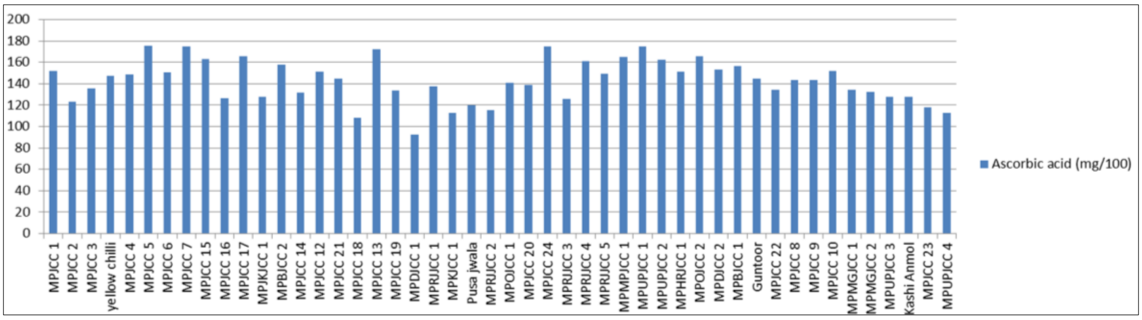


Fig 1: Ascorbic acid (mg/100)

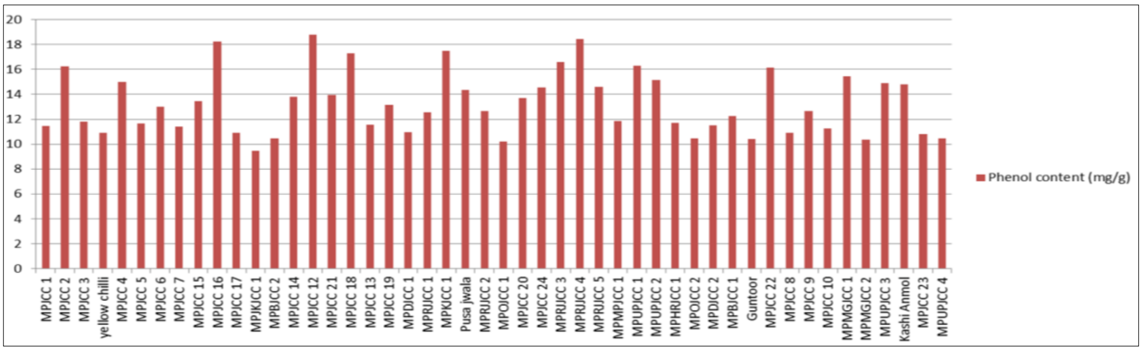
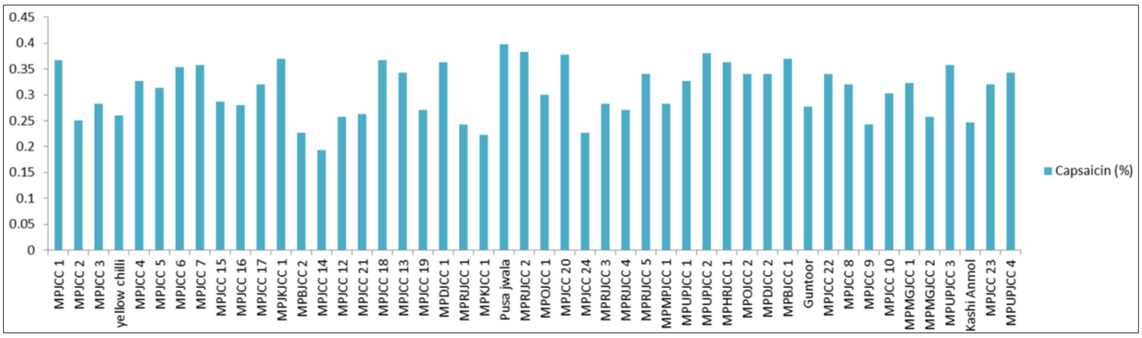


Fig 2: Phenol content (mg/g)



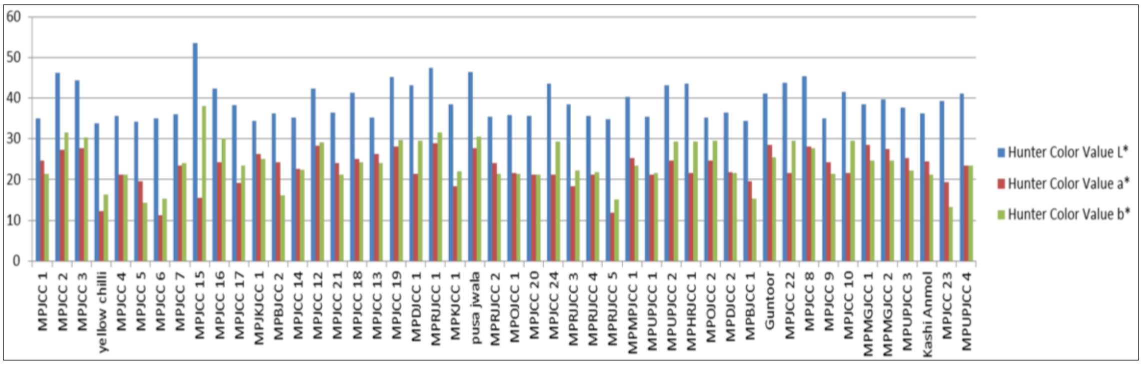


Fig 3: Capsaicin (%)

Table 3: Performance of chilli genotypes for quality parameter

S.no	Genotypes	Phenol content (mg/g)	Ascorbic acid (mg)	Capsaicin (%)
1	MPJCC 1	11.48	151.99	0.367
2	MPJCC 2	16.22	123.18	0.250
3	MPJCC 3	11.82	135.67	0.283
4	yellow chilli	10.90	147.36	0.260
5	MPJCC 4	15.01	148.54	0.327
6	MPJCC 5	11.64	175.85	0.313
7	MPJCC 6	13.00	150.47	0.353
8	MPJCC 7	11.40	174.74	0.357
9	MPJCC 15	13.46	163.23	0.287
10	MPJCC 16	18.26	126.71	0.280
11	MPJCC 17	10.92	165.94	0.320
12	MPJKJCC 1	9.47	127.83	0.370
13	MPBJCC 2	10.47	157.86	0.227
14	MPJCC 14	13.80	131.62	0.193
15	MPJCC 12	18.78	151.34	0.257
16	MPJCC 21	13.93	144.57	0.263
17	MPJCC 18	17.30	108.38	0.367
18	MPJCC 13	11.54	172.13	0.343
19	MPJCC 19	13.16	133.43	0.270
20	MPDJCC 1	10.96	92.38	0.363
21	MPRJJCC 1	12.54	137.73	0.243
22	MPKJCC 1	17.51	112.73	0.223
23	Pusa jwala	14.34	119.77	0.397
24	MPRJJCC 2	12.67	115.30	0.383
25	MPOJCC 1	10.23	141.13	0.300
26	MPJCC 20	13.72	139.23	0.377
27	MPJCC 24	14.55	174.83	0.227
28	MPRJJCC 3	16.59	125.73	0.283
29	MPRJJCC 4	18.42	160.90	0.270
30	MPRJJCC 5	14.59	149.67	0.340
31	MPMPJCC 1	11.86	164.93	0.283
32	MPUPJCC 1	16.29	174.97	0.327
33	MPUPJCC 2	15.15	162.70	0.380
34	MPHRJCC 1	11.71	151.47	0.363
35	MPOJCC 2	10.48	165.89	0.340
36	MPDJCC 2	11.51	153.14	0.340
37	MPBJCC 1	12.26	156.87	0.370
38	Guntoor	10.39	144.51	0.277
39	MPJCC 22	16.16	134.20	0.340
40	MPJCC 8	10.92	143.40	0.320
41	MPJCC 9	12.63	143.31	0.243
42	MPJCC 10	11.26	152.13	0.303
43	MPMGJCC 1	15.43	134.36	0.323
44	MPMGJCC 2	10.34	132.16	0.257
45	MPUPJCC 3	14.88	128.00	0.357
46	Kashi Anmol	14.82	127.87	0.247
47	MPJCC 23	10.81	118.24	0.320
48	MPUPJCC 4	10.44	112.57	0.343
Mean		13.25	142.94	0.31
CV		4.30	3.50	7.32
CD@5%		0.92	8.11	0.05
CD@1%		1.22	10.74	0.07

Colour values (L*, a*, b*) in chilli

Colour in chilli is the principle criterion for assessing its quality value. The major coloring pigments in chilli are Capsanthin and Capsorubin comprising 60% of the total carotenoids. Other pigments are Beta-carotene, Zeaxanthin, Violaxanthin, Neoxanthin and Lutein. Effect of colour values (L*, a*, b*) of chillies powder samples is indicated in Table 4.

L* (Darkness)

The different L* color values of chillies obtained and described in the table 1.4. Range of L* value falls 33.82 - 53.55 with a mean of 39.24. Sajjan *et al*. (2020) [25] found the nearby results. The maximum values were recorded in MPJCC15 (53.55), followed by MPRJJCC1, (47.34), Pusa jwala (46.37), MPJCC2 (46.3), MPJCC8 (45.45) and so on while the minimum values were found in MPRJJCC5 (34.89) followed by MPJKJCC1 (34.45), MPBJCC1 (34.34), MPJCC 5 (34.2), yellow chilli (33.82).

a* (Green)

The different a* color values of chillies obtained and described in the table 4. Range of a* value falls 28.87 to 11.28 with a mean of 22.92. The maximum values were recorded in MPRJJCC1 (28.87) followed by Guntoor (28.63), MPMGJCC1 (28.62), MPJCC12 (28.33), MPJCC8 (28.21) and so on while the minimum values were found in MPKJCC1 followed by (18.39), MPJCC15 (15.43), yellow chilli (12.33), MPRJJCC5 (11.82), MPJCC6 (11.28). Similarity was seen in the report developed by Aleksandra and Biserka (2004).

b* (Yellow)

The different b* color values of chillies obtained and described in the table 4. Range of b* value falls 38.13 to 13.36 with a mean of 24.12. Similar result was seen by Ahmed *et al.* (2002) [1]. The maximum values were recorded in MPJCC15 (38.13) followed by MPRJJCC1 (31.68), PJCC2 (31.64), Pusa jwala (30.66), MPJCC3 (30.34) and so on while the minimum values were found in MPBJCC1 (15.42) followed by MPJCC6 (15.4), MPRJJCC5 (15.04), MPJCC5 (14.35), MPJCC23 (13.36).

Table 4: Value of chilli based on Hunter color

S.no.	Genotypes	Hunter Color Value		
		L*	a*	b*
1	MPJCC 1	35.05	24.63	21.4
2	MPJCC 2	46.3	27.38	31.64
3	MPJCC 3	44.42	27.82	30.34
4	yellow chilli	33.82	12.33	16.25
5	MPJCC 4	35.74	21.32	21.17
6	MPJCC 5	34.2	19.56	14.35
7	MPJCC 6	34.98	11.28	15.4
8	MPJCC 7	35.96	23.37	23.97
9	MPJCC 15	53.55	15.43	38.13
10	MPJCC 16	42.36	24.25	30.12
11	MPJCC 17	38.23	19.25	23.41
12	MPJKJCC 1	34.45	26.35	25.12
13	MPBJCC 2	36.25	24.23	16.24
14	MPJCC 14	35.21	22.68	22.35
15	MPJCC 12	42.38	28.33	29.15
16	MPJCC 21	36.38	24.15	21.25
17	MPJCC 18	41.26	25.12	24.23
18	MPJCC 13	35.33	26.22	24.13
19	MPJCC 19	45.14	28.05	29.73
20	MPDJCC 1	43.26	21.51	29.62
21	MPRJJCC 1	47.34	28.87	31.68
22	MPKJCC 1	38.41	18.39	22.13
23	pusa jwala	46.37	27.81	30.66
24	MPRJJCC 2	35.51	24.05	21.33
25	MPOJCC 1	35.91	21.63	21.42
26	MPJCC 20	35.74	21.32	21.17
27	MPJCC 24	43.51	21.26	29.26
28	MPRJJCC 3	38.39	18.41	22.31
29	MPRJJCC 4	35.69	21.19	21.84
30	MPRJJCC 5	34.89	11.82	15.04
31	MPMPJCC 1	40.36	25.32	23.45
32	MPUPJCC 1	35.46	21.32	21.71
33	MPUPJCC 2	43.14	24.62	29.25
34	MPHRJCC 1	43.52	21.55	29.41
35	MPOJCC 2	35.21	24.62	29.52
36	MPDJCC 2	36.38	21.92	21.55
37	MPBJCC 1	34.34	19.67	15.42
38	Guntoor	41.22	28.63	25.42
39	MPJCC 22	43.68	21.53	29.45
40	MPJCC 8	45.45	28.21	27.62
41	MPJCC 9	35.05	24.23	21.4
42	MPJCC 10	41.62	21.66	29.51
43	MPMGJCC 1	38.45	28.62	24.63
44	MPMGJCC 2	39.65	27.61	24.68
45	MPUPJCC 3	37.62	25.23	22.14
46	Kashi Anmol	36.25	24.56	21.23
47	MPJCC 23	39.23	19.41	13.36
48	MPUPJCC 4	41.12	23.45	23.42

Conclusion

The study revealed that biochemical composition is the great parameter to judge the genetic components of the particular genotypes. These traits are importance for the nutrient value and marketable traits that results increase economic status and fulfil the essential nutri-components for the proper growth and development of body. The genotypes reported promising for total carotenoids will be of very good components for the utilization in consumption as good source of antioxidants to the prevention of the development of chronic disease such as diabetes and cancer. Genotype MPJCC5 showed highest ascorbic acid content (175.85 mg/100g) among the forty eight genotypes studied. These genotypes will be of great parental line for the utilization as donor in the breeding program to the development of high ascorbic content genotypes. Maximum phenol content was recorded in MPJCC12 (18.78 mg/g) and this genotype was reported as resistant to chilli leaf curl virus resistance. Capsaicin content was reported maximum in Pusa jwala (0.40%). As Pusa Jwala is commercially known for its export category in dried form and in this investigation the finding supported the importance of this genotype as a quality component especially for capsaicin content to the various industries and food preparations.

The genotype Pusa Jwala is also a very good combiner and maximum breeders preferred this to include as a parental line for the development of high yielding with pungent chilli hybrids/varieties. Colour value is also a very good trait in chilli breeding to enhance the quality component resulting high marketable food and cosmetic industries. Genotype MPJCC15 (53.55), MPRJJCC1 (28.87) and MPJCC15 (38.13) showed maximum color value for L*, a* and b* respectively. This component also be of preferred by the industries to the natural food colorant and also to attract the food products for the consumers. These identified promising genotypes for different biochemical components will be of great importance in future chilli breeding program.

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Conflict of interest
None.

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