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Capsaicinoid content, pungency and antioxidant potential of Himalayan hot pepper

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ABSTRACT

Hot pepper (*Capsicum annuum* L.) is one of the most important vegetable crops grown worldwide due to its culinary values and potential pharmaceutical applications. Capsaicinoids in hot pepper are the secondary metabolites responsible for its pungency. Since capsaicinoid content depends on the environment, better-adapted genotypes with higher capsaicin content are always required. In the present study, we performed a biochemical screening of twenty-two North-Western Himalayan adapted hot pepper breeding lines. We determined their capsaicin content, dihydrocapsaicin content, total phenolic content, pungency, and antioxidant activity. The HPLC analysis quantified 1000 to 4010 µg/g capsaicin and 489 to 1863 µg/g of dihydrocapsaicin, translating to pungency of 16000 to 64160 SHU. The germplasm expressed 28.44% to 78.61% DPPH free radical scavenging activity, 121.29 to 394.294 µM Fe^{2+}/g dry weight (DW) ferric reducing antioxidant power (FRAP) and 4.31 to 8.56 mg/g GAE of total phenolics. Our results identified CITH-HP-91-13 and CITH-HP-92-13 as the breeding lines richest in capsaicinoids and antioxidant activity. The germplasm has been grouped into five clusters based on standardized squared Euclidean distance using Ward's hierarchical clustering and separation based on PCA biplot. Our study revealed significant differences among breeding lines for tested parameters, which indicate the possibility of exploiting them for quality improvement programme of Kashmiri pepper.

Keywords: *Capsicum annuum* L., Hot pepper, Capsaicinoids, Pungency, Antioxidant potential, Phenolics

INTRODUCTION

Hot pepper or chilli pepper (*Capsicum annuum* L.) is extensively used as flavouring and colouring spice across the world. Besides culinary value, hot pepper has pharmaceutical application due to its analgesic, neurological, gastroprotective, anti-obesity, anti-diabetic and dietetic properties (Basith *et al.,* 2). In modern times, chilli pepper is used in the pharmaceutical industry and as a traditional ethnomedicine (Basith *et al.,* 2). The North-Western Himalayan region is popular for Kashmiri chilli cultivation. Kashmiri chilli is a group of signature cultivars of Kashmiri origin with low pungency and high oleoresins. It imparts a bright red hue to local cuisines without adding too much piquancy. Nonetheless, highly pungent genotypes are also available in the region.

Culinary, disease therapeutic qualities of hot pepper are functions of capsaicinoids whose stable specific levels in a cultivar are imperative for its acceptance by food and pharmaceutical industries. However, environmental conditions significantly influence capsaicinoid content (Loizzo *et al.,* 9). This was also confirmed by our earlier study wherein North-Eastern chilli cultivar '*Bhut Jolokia*' accessions known to possess one of the highest capsaicin contents *i.e.,* ~1 million SHUs (Bosland and Baral, 3), expressed only about one-tenth of Scoville Heat Units (SHU) under North-Western Himalayan climate of Kashmir valley (Indrabi, 7). Hence, selecting highly adapted genotypes stable for capsaicinoid content in a particular geographical area is imperative. These highly adapted hot pepper genotypes with stable capsaicinoids can serve as sources of bioactive compounds and pungency and as breeding material for elite varieties development.

Although there are ample research reports on *Capsicum annuum* L. for capsaicinoid content and antioxidant potential but genotypes from North-Western Himalayas have not yet been evaluated (Dubey *et al.,* 4). Several hot pepper breeding lines of North-Western Himalayan adaptation are being maintained at ICAR-Central Institute of Temperate Horticulture, Srinagar. This scenario of chilli germplasm availability calls for its biochemical evaluation to authenticate the variation in pungency levels and phytochemical efficacy. The present study was formulated to quantify the variation for biochemical properties such as capsaicin, dihydrocapsaicin content, capsaicinoid-derived antioxidant activity and pungency among North-western Himalayan hot pepper breeding lines and

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identification of breeding lines with high pungency in the Kashmir region.

MATERIALS AND METHODS

Twenty-two hot pepper breeding lines developed from collections made from various Kashmir Valley regions were evaluated. These lines were morphologically uniform and stable for economic characteristics like yield and fruit traits. Fresh fruit samples were collected from the plants and dried in the shade. During experimentation and chemical analyses, analytical-grade reagents were used. Extraction was done in a Soxhlet extractor using HPLC-grade methanol as solvent, and a final concentration of 100 mg/ml was prepared.

HPLC analysis for capsaicinoids was conducted in Shimadzu HPLC (Kyoto, Japan). The solution was filtered through 0.2 µm syringe filters. HPLC separation was carried out with an injection volume of 20 µL, a flow rate of 1 ml min^{-1} with 35-40 minutes of run time. The analyses were done in three replications for each test sample. Both capsaicin and dihydrocapsaicin were detected at 210 nm. Chromatographic separations were performed on C 18 (250 mm × 4.6 mm), 5-µm column using a solvent system of 70% acetonitrile and 30% methanol in an isocratic mode. Prior analysis, the mobile phase was filtered through a 0.45 µm membrane filter (Millipore, Bedford, MA, USA). Class WP v6.1 (Shimadzu, Japan) software package was used for data acquisition and processing. The concentrations were measured with the help of respective peak areas of standards at particular retention time against the concentration and expressed as mg/g of dry sample. Under optimal isocratic conditions, capsaicin and dihydrocapsaicin were separated within 1.74 minutes and 2.3 minutes, respectively. The Scoville Heat Units (SHU) parameter indicates the pungency level. We categorized the pungency of the analysed samples as per Scoville (17).

Capsaicinoid-derived antioxidant potential by means of DPPH and FRAP assays was determined by methods described by Shotorbani *et al.* (18). Fruit samples were shade dried and powdered vigorously in a mortar and pestle. Three grams of fine powder were dissolved in measured levels of methanol. The Whatman filter paper was used to filter the extract. To avoid deterioration of antioxidant activity in the samples, extracts were processed immediately.

The 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay was done using the procedure described by Xu *et al.* (20) with slight modifications. The 15 µg/ml working solutions of each sample extract were prepared in methanol. To prepare the standard curve, ascorbic acid at 1, 2, 4, 6, 8, 10 and 15 µg/ml concentrations were used. The 1 ml of each sample extract was combined with 1 ml of 0.002% DPPH solution. The solution was mixed well and incubated in the dark for 30 minutes. The absorbance was recorded at 517 nm wavelength. The analyses were done in three replications. Any observed reduction in the absorbance of DPPH caused by the addition of test samples compared with control became the basis for calculating antioxidant activity in terms of percentage inhibition of DPPH radical (% IP). Per cent DPPH inhibition activity was estimated as , where is the absorbance of the control and is the absorbance of the sample. Methanol and DPPH in the ratio of 1:1 mixture was used as blank and standard (ascorbic acid) and DPPH mixture in the ratio of 1:1 was treated as control. The concentration of test sample and standard solution was 15 µg/ml.

The antioxidant capacity of samples was measured in terms of ferric ion reducing antioxidant power (FRAP) also. The FRAP reagent was prepared by mixing sodium acetate buffer (300 mmol/L at pH 3.6), 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mmol/L HCl (10 mmol/L) and $FeCl_3$ solution (20 mmol/L) in the ratio of 10:1:1 (v/v), respectively. The FRAP reagent was pre-warmed at 37 °C, and 1.8 ml was used for 200 µl of sample extracts. After 40 minutes, the absorbance of the solution was taken at 593 nm. The calibration curve was prepared based on $FeSO_4$ solutions at 0, 40, 80, 160, 320, and 640 µmol/L concentrations. The observations were determined with subsequent formulae determined on calibration curve: 0.998, where stands for absorbance and is µM Fe^{2+}/g fresh weight. Antioxidant activities of test sample extracts were finally depicted as µmol Fe^{2+}/g DW of the sample.

The potential of phenolic compounds as antioxidants were estimated by the modified Folin-Ciocalteau method (Omoruyi *et al.*, 11). Five ml of Folin-Ciocalteau reagent (previously diluted with distilled water 1:10 (v/v), and 4 ml (75% w/v) of sodium carbonate (Na_2CO_3) were combined with1 ml of test extract (1 mg/ml) and vortexed for 30 minutes for colour development. The absorbance of the extracts was recorded at 765 nm. A standard curve was made using gallic acid at 0 to 400 mg/L concentrations. Finally, concentrations were expressed as milligram gallic acid equivalent (mg GAE/g DW of sample extract). The formula used for the purpose was 0.997, where is absorbance and is gallic acid equivalent in mg/g.

The existence of significant differences among extracts for capsaicinoids, pungency, antioxidant potential and total phenolics were analysed. For the purpose, one-way analysis of variance (ANOVA) using Duncan's mean range test (DMRT) was carried

out. All statistical tests were done using 'SAS® Enterprise Guide 4.2' software at 5% significance level. Correlation study was performed using Pearson's test. Principal component analysis (PCA) and cluster analysis were performed using 'SAS® Enterprise Guide 4.2 software.

RESULTS AND DISCUSSION

Hot pepper is rich in capsaicin and dihydrocapsaicin, which are natural antioxidants (Ghasemnezhad *et al.,* 5). These capsaicinoids are also determining factors in establishing the commercial quality of hot pepper. In this study, there was significant variability in capsaicin and dihydrocapsaicin contents among tested lines. The capsaicin content ranged from 1000 µg/g (in SEL-1052-11) to 4010 µg/g (in CITH-HP-92-13) with an average of 2434.23 µg/g, while dihydrocapsaicin varied from 489 to 1863 µg/g with an average of 1143.82 µg/g. The highest capsaicin, as well as dihydrocapsaicin contents, was found in line CITH-HP-92-13. Based on capsaicinoid content and SHU were estimated to determine the pungency of germplasm. The highest pungency of 64160 SHU was recorded in CITH-HP-92-13 and the lowest pungency of 16000 SHU was recorded in SEL-1052-11. The average pungency of the germplasm was 38947.64 SHU. According to the Scoville Organoleptic Scale (Scoville, 17), out of the 22 lines, 19 came out to be highly pungent (> 25000 SHU) and 4 moderately pungent (3000–25000 SHU) (Table 1). It means that more than 86% of our tested germplasm is highly pungent, thus a promising genetic stock usable in pungent chilli breeding.

Our study has revealed considerably higher capsaicinoid content and pungency in NW Himalayan adapted chilli germplasm compared to many other germplasms studied worldwide. For example, Othman *et al.* (13) studied commercial pepper varieties of Riyadh city (KSA), and the mean capsaicin and dihydrocapsaicin contents were 1320 µg/g and 830 µg/g, respectively. Landraces of the Republic of Benin expressed capsaicin content range of 765 to 3070 µg/g (Orobiyi *et al.,* 12). In another study, the local commercial chilli powder products of Visakhapatnam, AP, had capsaicin content of 800 to 1300 µg/g. However, our tested germplasm lines have capsaicin content ranging from 1000 to 4010 µg/g with average of 2434.23 µg/g and dihydrocapsaicin content ranging from 489 to 1863 µg/g with an average of 1143.82 µg/g, which are much higher than found in all above studies.

Our germplasm, however, has generally lower capsaicin content than *Capsicum chinense* cv. Habanero, one of the hottest chillies in the world, having up to 200000 SHU (Johnson *et al.,* 8), as suggested by a study by Palma-Orozco *et al.* (14). The average capsaicin content of Habanero fruits dried at 40 to 70 ºC temperature was 9630 µg/g. In another study from Czechoslovakia, various Habanero varieties were found to have 1128 to 8215 µg/g of capsaicin, averaging 3900 µg/g (Popelka *et al.,* 16). However, in one study on Australian habanero cultivars, capsaicin contents ranged from 1474 to 3916 µg/g (Johnson *et al.,* 8). Comparing 1000 µg/g to 4010 µg/g capsaicin content of our germplasm, it is suggestive that NW Himalayan chilli may be comparable to or more pungent than Australian Habaneros. Hence, extensive studies involving larger sample sizes are needed. Moreover, agroecological conditions may also have a role to play.

Following capsaicinoid concentrations and pungency estimates, we compared the antioxidant

Table 1. Capsaicin and dihydrocapsaicin contents and pungency of hot pepper breeding lines.

S. No.	Line	Capsaicin (µg/g)	Dihydrocapsaicin (µg/g)	Pungency (SHU)
1)	CITH-HP-92-13	4010^{A}	1863^{A}	64160 ^{A}
2)	CITH-HP-91-13	3573^{B}	1522^{B}	57168 ^{B}
3)	SEL-1065-E	3300^{C}	1450 ^{C}	52800 ^{C}
4)	CITH-HP-1154	3000^{D}	1322^{D}	48000 ^{D}
5)	SEL-836-1-2	2937 ^{D}	1311 ^{D}	46992 ^{D}
6)	CITH-HP-85-13	2700^{E}	1311 ^{D}	43200 ^{E}
7)	CITH-HP-111-1	2673 ^{E}	1298 ^{D}	42768 ^{E}
8)	CITH-HP-1154-2-1	2623^{EF}	1260DE	41968 ^{EF}
9)	SEL-1050	2601^{EF}	1290 ^{D}	41616 ^{EF}
10)	CITH-HP-1154-3-1	2570 ^{EFG}	1243 ^{DE}	41120 ^{EFG}
11)	SEL-136-1-2	2501^{FGH}	1200 ^{EF}	40016 ^{FGH}
12)	CITH-HP-56-13	2493^{FGH}	1190 ^{EF}	39888 ^{FGH}
13)	CITH-HP-111	2483^{FGHI}	1185^{EF}	39728 ^{FGHI}
14)	SEL-1011-2 (E)	2423^{GHI}	1123^{FG}	38768 ^{GHI}
15)	SEL-1055-11	2390^{IH}	1150 ^{FG}	38240 ^{IH}
16)	CITH-HP-1154-1	2323^{IJ}	1101^{G}	37168 ^{IJ}
17)	CITH-HP-114-13	2200^{J}	1023^{H}	35200 ^{J}
18)	SEL-89	1800^{K}	847^{I}	28800 ^{K}
19)	KA2-SEL-1	1500^{L}	723^{J}	24000 ^{L}
20)	CITH-HP-42-13	1250 ^{M}	640^{K}	20000^{M}
21)	AL-4	1203 ^{M}	623 ^{K}	19248 ^{M}
22)	SEL-1052-11	1000 ^{N}	489^{L}	16000 ^{N}
	Mean	2434.23	1143.82	38947.64

(Note: Different letters indicate significant differences between means in the column as per Duncan's test)

potential of the germplasm *via* DPPH and FRAP antioxidant assays. DPPH is a very stable free radical and is extensively exploited to estimate the antioxidant potential of biological extracts. In the present study, the free radical scavenging potential of investigated lines estimated with DPPH assay revealed significant differences. The per cent inhibition of DPPH ranged from 28.44% in CITH-HP-91-13 to 78.61% in Sel-836-1-2 (Table 2). Fifteen lines comprising 68% of the tested germplasm elicited more than 50 per cent inhibition. The FRAP of tested germplasm ranged from 121.29 to 394.29 µM Fe^{2+}/g DW. Based on the highest FRAP value obtained, SEL-1050 is superior among all lines. It is worth noting that DPPH per cent inhibition and FRAP values did not correspond for the same germplasm. Although DPPH per cent inhibition was highest in SEL-836-1-2 (78.61), the FRAP was highest in SEL-1050 (394.29).

The total phenolic content of twenty-two extracts ranged from 4.31 to 8.56 mg GA/g DW (Table 2). SEL-1065-E expressed the highest total phenolics (8.56mg GA/g DW). The phenolic contents of chilli lines obtained in this study are similar to those observed in many other studies involving various species of *Capsicum* (Alvarez-Parrilla *et al.,* 1; Sora *et al.,* 19; Hamed *et al.,* 6). The absorbance obtained in test samples over a concentration range of 20-90 mg/L was linear (with 0.997.

To establish the relative competence of different assays and methods of biochemical estimations in chilli, we constructed a correlation matrix for DPPH and FRAP assays and total phenolic, capsaicin and dihydrocapsaicin contents obtained from HPLC (Table 3). Correlation coefficient is a statistic developed for determining the degree of association among parameters. Our results revealed a significant positive correlation among antioxidant assays, total phenolic, and capsaicinoid contents. This observation has strengthened the authenticity of these methods. The positive correlation ($P<0.05$) among TP-DPPH ($r = 0.3098$) was significant, whereas it was highly

Table 2. Antioxidant potential and total phenol content of twenty-two hot pepper breeding lines.

S. No.	Line	DPPH	Percent Inhibition	FRAP (µM fe^{2+}/g DW)	Total phenols (mg/g GAE)
1)	CITH-HP-92-13	11.51^{F}	64.28	122.00^{L}	4.90^{JK}
2)	CITH-HP-91-13	1.35^{K}	28.44	302.24^{FG}	6.08^{FGH}
3)	SEL-1065-E	12.67^{E}	68.37	274.94^{H}	8.56^{A}
4)	CITH-HP-1154	11.89^{F}	65.64	317.88^{EF}	6.48^{EF}
5)	SEL-836-1-2	15.57^{A}	78.61	332.24^{DE}	7.38^{C}
6)	CITH-HP-85-13	9.51^{G}	57.22	300.65^{FG}	6.12^{FGH}
7)	CITH-HP-111-1	3.28^{J}	35.27	341.88^{CD}	5.93^{GHI}
8)	CITH-HP-1154-2-1	11.54^{F}	64.39	315.53^{EFG}	4.97^{J}
9)	SEL-1050	14.96^{AB}	76.45	394.294^{A}	6.88^{D}
10)	CITH-HP-1154-3-1	3.60^{J}	36.41	363.588^{B}	8.06^{B}
11)	SEL-136-1-2	8.70^{H}	54.38	121.29^{L}	4.92^{JK}
12)	CITH-HP-56-13	13.41^{D}	70.99	356.65^{BC}	6.58^{DE}
13)	CITH-HP-111	4.86^{I}	40.84	314.00^{FG}	6.56^{DE}
14)	SEL-1011-2 (E)	3.25^{J}	35.15	170.00^{G}	5.16^{J}
15)	SEL-1055-11	15.44^{A}	78.16	298.59^{G}	7.53^{C}
16)	CITH-HP-1154-1	14.09^{C}	73.38	357.706^{BC}	6.19^{EFGH}
17)	CITH-HP-114-13	14.73^{B}	75.65	346.94^{BCD}	5.68^{I}
18)	SEL-89	3.18^{J}	34.93	160.76^{JK}	4.55^{L}
19)	KA2-SEL-1	4.67^{I}	40.16	148.35^{K}	4.31^{L}
20)	CITH-HP-42-13	11.28^{F}	63.48	229.41^{I}	4.78^{JK}
21)	AL-4	9.38^{G}	56.77	121.88^{L}	5.89^{HI}
22)	SEL-1052-11	9.02^{GH}	55.52	223.06^{I}	4.36^{L}
	Mean	8.93	57.02	268.81	5.99

(Note: Different letters indicate significant differences between means in the column as per Duncan's test)

Table 3. Correlation matrix for five biochemical parameters analysed.

	Total phenols	FRAP	Capsaicin	Dihydro-capsaicin
DPPH	0.3098*	0.3924	0.0782	0.0994
Total phenols		0.5464**	0.4450	0.4420
FRAP			0.2790**	0.2940**
Capsaicin				0.9897

(* Significant at p< 0.05, ** Significant at p< 0.01)

significant (P <0.01) among TP-FRAP (r = 0.5464), capsaicin–FRAP (r = 0.2790) and dihydrocapsaicin-FRAP (r = 0.2940). Observations on the same line have been made in studies on diverse *Capsicum annuum* L., germplasms (Nunez-Ramirez *et al.,* 10).

Principal component analysis (PCA) is done to partition variation and highlight strong patterns in a dataset. We performed PCA using two antioxidant assays (DPPH and FRAP) and three antioxidants (capsaicin, dihydrocapsaicin and total phenolics) which showed that the first two components (PC1 and PC2) explained 78.31% of the total variation. First principal component (PC1) accounted for 52.66% of the total variation and the variables responsible for separation along the PC1 included DPPH (0.22), TP (0.47), capsaicin (0.52) and FRAP (0.40) and DiCAP (0.52). The second principal component (PC2) explained 25.65% of the total variation. The accessions were separated by DPPH (0.58) (Fig. 1). Hierarchical cluster analysis grouped 22 genotypes into two main clusters with cluster-I represented by five genotypes (SEL-89, KA2-Sel-1, SEL-1052-11, AL-4 and CITH-HP-42-13) and cluster-II represented by sixteen genotypes (Fig. 2). Genotype CITH-HP-92-13 does not form the part of any cluster being most superior genotype with respect to capsaicin and dihydrocapsaicin contents. The contribution of individual genotypes to the antioxidant grouping and the relationship between the clusters was assessed by plotting PC1, PC2 and PC3. Expectedly, both PCA and cluster analysis were found equally effective in grouping the breeding lines based on their antioxidant contents (Patras *et al.,* 15).

In conclusion, it was observed that chilli, *Capsicum annuum* L., and breeding lines developed from collections made from North-Western Himalayas have high capsaicin and dihydrocapsaicin contents and antioxidant potential. Two lines *i.e.,* CITH-HP-92-13 and CITH-91-13, expressed the highest values for capsaicin content, dihydrocapsaicin content and FRAP activity. Clustering and PCA for these traits revealed significant differences among lines, which indicates the possibility of exploiting

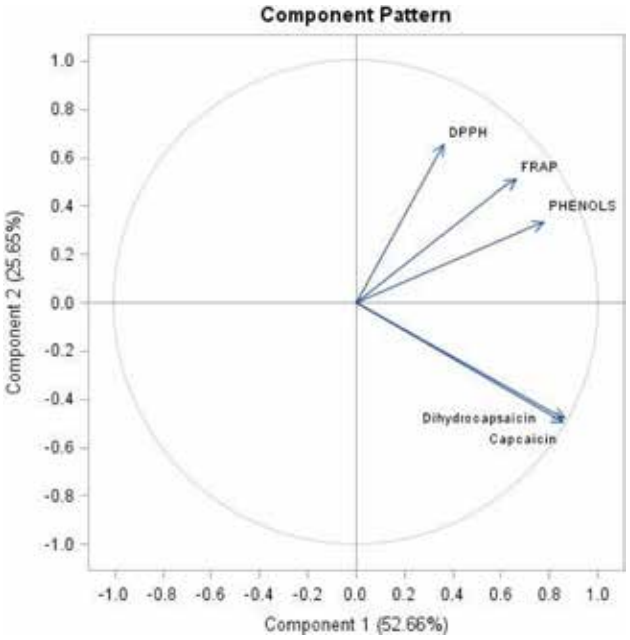

Fig. 1. Grouping of twenty-two hot pepper breeding lines based on standardized squared Euclidean distance using Ward's hierarchical clustering method and separation in PCA biplot.

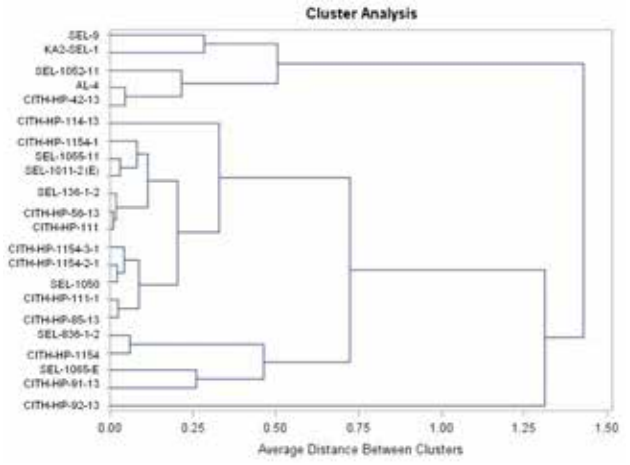

Fig. 2. Dendrogram based on capsaicin, dihydrocapsaicin, total phenols, FRAP and DPPH scavenging activity values obtained for twenty-two hot pepper breeding lines.

them for quality breeding for pharmaceutical and ethnomedical applications. Lines exhibiting high pungency can be used to breed for cuisine-specific targets like high pungency-high oleoresin, for example, combining the trait of high oleoresin (but low pungency) content of Kashmiri mirch with these high pungency breeding lines. These lines can also be used to decipher molecular mechanism and enzyme kinetics of rate limiting factor in capsaicinoids

biosynthesis. Most of the genotypes evaluated in this study were highly pungent because of high capsaicin and dihydrocapsaicin content and high content is strongly correlated with significant FRAP activity. Higher FRAP activity of chilli genotypes is due to scavenging potential of capsaicin and dihydrocapsaicin.

AUTHORS' CONTRIBUTION

Conceptualization of research (JIM, DBS, GM, OCS); Designing of the experiments (JIM, DBS); Contribution of experimental materials (DBS, GM); Execution of field/lab experiments and data collection (MAS, AS, RAS); Analysis of data and interpretation (JIM, GM, VD, SY); Preparation of the manuscript (JIM, GM, VD).

DECLARATION

The authors declare that there is no conflict of interest.

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REFERENCES

1. Alvarez-Parrilla, E., De la Rosa, L.A., Amarowicz, R. and Shahidi, F. 2011. Antioxidant activity of fresh and processed Jalapen˜oJalapeño and Serrano Peppers. *J. Agric. Food Chem.* **59**: 163–73.

2. Basith, S., Cui, M., Hong, S. and Choi, S. 2016. Harnessing the therapeutic potential of capsaicin and its analogues in pain and other diseases. *Molecules* **21**: 966.

3. Bosland, P.W. and Baral, J.B. 2007. 'Bhut Jolokia'—The world's hottest known chile pepper is a putative naturally occurring interspecific hybrid. *HortScience*. **42:** 222-24.

4. Dubey, R.K., Singh, V., Upadhyay, G., Pandey, A.K. and Prakash, D. 2015. Assessment of phytochemical composition and antioxidant potential in some indigenous chilli genotypes from North East India. *Food Chem.* **188:** 119-25.

5. Ghasemnezhad, M., Sherafati, M. and Payvast, G.A. 2011. Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annum*) fruits at two different harvest times. *J. Func. Food.* **3**: 44-49.

6. Hamed, M., Kalita, D., Bartolo, M.E. and Jayanty, S.S. (2019). Capsaicinoids, polyphenols and antioxidant activities of *Capsicum annuum*: Comparative study of the effect of ripening stage and cooking methods. *Antioxidants*. **8**: 364.

7. Indrabi, S.A. 2020. Molecular and biochemical characterization in chilli (*Capsicum annum L.*). *M.Sc. Thesis,* Division of Vegetable Science, Sher-e-Kashmir University of Agriculture Sciences and Technology, Kashmir.

8. Johnson, J.B., Mani, J.S. and Naiker, M. 2021. Correlations between capsaicin, dihydrocapsaicin and phenolic content in habanero chillies. *Biol. Life Sci. Forum.* 6: 30.

9. Loizzo, M.R., Pugliese, A., Bonesi, M., Menichini, F. and Tundis, R. 2015. Evaluation of chemical profile and antioxidant activity of twenty cultivars from *Capsicum annuum, Capsicum baccatum, Capsicum chacoense* and *Capsicum chinense*: A comparison between fresh and processed peppers. *LWT-Food Sci. Technol.* **64**: 623-31.

10. Nunez-Ramirez, F., González-Mendoza, D., Grimaldo-Juárez, O. and Díaz, L.C. 2011. Nitrogen fertilization effect on antioxidants compounds in fruits of habanero chili pepper (*Capsicum chinense*). *Int. J. Agric. Biol.* **13**: 827-30.

11. Omoruyi, B.E., Bradley, G. and Afolayan, A.J. 2012. Antioxidant and phytochemical properties of *Carpobrotus edulis* L. bolus leaf used for the management of common infections in HIV/AIDS patients in Eastern Cape Province. *BMC Complement. Altern.* **1**: 215.

12. Orobiyi, A., Ahissou, H., Gbaguidi, F., Sanoussi, F., Houngbeme, A., Dansi, A. and Sanni, A. (2015). Capsaicin and ascorbic acid content in the high yielding chili pepper (*Capsicum annuum* L.) landraces of Northern Benin. *Int. J. Curr. Microbiol. App. Sci.* **4**: 394-403.

13. Othman, Z.A.A., Ahmed, Y.B.H., Habila, M.A. and Ghafar, A.A. 2011. Determination of capsaicin and dihydrocapsaicin in Capsicum fruit samples using high performance liquid chromatography. *Molecules*. **16**: 8919-29.

14. Palma-Orozco, G., Orozco-Álvarez, C., Chávez-Villeda, A. A., Mixtega-Martínez, A. and Castro-Muñoz, R. 2021. Capsaicin content in red

habanero chilli (*Capsicum chinense* Jacq.) and its preservation after drying process. *Future Foods*. **4**: 100070.

15. Patras, A., Brunton, N.P., Downey, G., Rawson, A., Warriner, K. and Gernigon, G. 2011. Application of principal component and hierarchical cluster analysis to classify fruits and vegetables commonly consumed in Ireland based on in vitro antioxidant activity. *J. Food Composit. Anal*. **24**: 250-56.

16. Popelka, P., Jevinova, P., Karel, S. and Roba, P. 2017. Determination of capsaicin content and pungency level of different fresh and dried chilli peppers. *Folia Vet.* **61**: 11-16.

17. Scoville, W.L. 1912. Note on *Capsicum*. *J. Am. Pharm. Assoc.* **1**: 453-54.

18. Shotorbani, N.Y., Jamei, R. and Heidari, R. 2013. Antioxidant activities of two sweet pepper (*Capsicum annuum* L.) varieties phenolic extracts and effects of thermal treatment. *Avicenna J. Phytomed.* **3**: 25-34.

19. Sora, G.T.S., Haminiuk, C.W.I., Vieira da Silva, Zielinski A. A. F., Goncalves, G.A., Bracht, A. and Peralta, R.M. (2015). A comparative study of the capsaicinoid and phenolic contents and in vitro antioxidant activities of the peppers of the genus *Capsicum*: an application of chemometrics. *J. Food. Sci. Technol.* **52**: 8086–94.

20. Xu, R., Ye, H., Sun, Y., Tu, Y. and Zeng, X. 2012. Preparation, preliminary characterization, antioxidant, hepatoprotective and antitumor activities of polysaccharides from the flower of tea plant (*Camellia sinensis*). *Food Chem. Toxicol.* **50**: 2473-80.

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