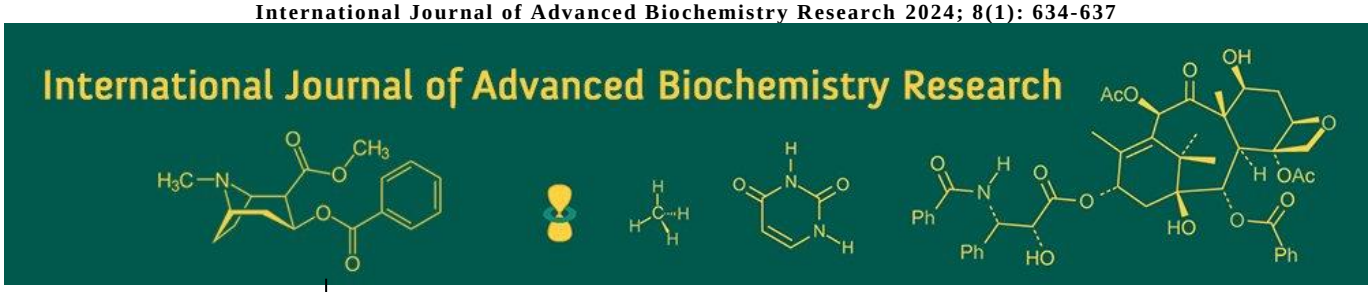

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(1): 634-637
www.biochemjournal.com
Received: 02-12-2023
Accepted: 04-01-2024

Pushpa Hulagannavar
Department of Biotechnology and Crop Improvement, KRC College of Horticulture, Arabhavi, UHS, Bagalkot, Karnataka, India

Dileepkumar A Masuthi
Department of Biotechnology and Crop Improvement, KRC College of Horticulture, Arabhavi, UHS, Bagalkot, Karnataka, India

Lakshmidevamma TN
Dept. of Biotechnology and Crop Improvement, College of Horticulture, Bengaluru, UHS, Bagalkot, Karnataka, India

Abdul Kareem M
Dept. of Plant Pathology, College of Horticulture, Sirsi, UHS, Bagalkot, Karnataka, India

Shivayogi Ryavalad
RHREC, Dharwad, UHS, Bagalkot, Karnataka, India

Ratnakar Shet
Dept. of BCI, College of Horticulture, Sirsi, UHS, Bagalkot, Karnataka, India

MH Tatagar
Dept. of Entomology, College of Horticulture, Sirsi, UHS, Bagalkot, Karnataka, India

Corresponding Author:
Dileepkumar A Masuthi
Department of Biotechnology and Crop Improvement, KRC College of Horticulture, Arabhavi, UHS, Bagalkot, Karnataka, India

Quantitative determination of Capsaicin, oleoresin and ascorbic acid content in Chilli (*Capsicum annuum* L.)

Pushpa Hulagannavar, Dileepkumar A Masuthi, Lakshmidevamma TN, Abdul Kareem M, Shivayogi Ryavalad, Ratnakar Shet and MH Tatagar

DOI: https://doi.org/10.33545/26174693.2024.v8.i1i.471

Abstract
Chilli (*Capsicum annuum* L.) are highly valuable crops, playing diverse roles as a spice, medicine and source of antioxidants. Capsaicin, the compound responsible for their heat, holds significant potential for appetite control, pain relief and even cancer prevention. While data on capsaicin and other important biochemical components is often limited, breeding efforts strive to enhance crop quality for both human health and economic benefit. This study investigated the capsaicin, oleoresin and ascorbic acid (vitamin C) content in diverse chilli parents and crosses. We observed remarkable variations in these key components among parent genotypes and hybrids. Interestingly, specific lines excelled in each domain: Byadagi Dabbi for oleoresin, KCA-44-2 for capsaicin, and KCA-24-1 for ascorbic acid. Notably, some hybrid crosses, particularly Byadagi dabbi × KCA-24-1, KCA-5-3× KCA-24-1, and Byadagi dabbi × KCA-24-1, displayed synergistic effects, exceeding parental levels of these compounds. These findings highlight the potential of cross-breeding to enhance desirable traits and create chilli varieties with even greater nutritional value, heat intensity, and aroma. Our study provides a valuable foundation for future research in chilli breeding, paving the way for the development of improved cultivars with significant benefits for consumers and farmers alike.

Keywords: Chilli, capsaicin, oleoresin, ascorbic acid, biochemical diversity, colorimeter

Introduction
Chilli (*Capsicum annuum* L.) is an highly valuable crop in India, serving diverse purposes as a spice, condiment, culinary aid, medicine, vegetable and even ornamental plant. Chilli peppers are used all over the world for their pungency, aroma, bright colors and vitamins. But chillies aren't just for cooking. For centuries, in places like India, China, and North America, traditional healers have used them to treat things like arthritis, muscle pain, stomach problems and even skin problems and bites (Meghvansi *et al*., 2010) [7]. Phytochemicals are chemical compounds produced by plants and play role in resistance against bacteria, fungi, and virus infections. Medicinal and spice crops are known to be rich in phytochemicals (Paramesha *et al*., 2023) [8]. Capsicum are packed with antioxidants like vitamin C, E, A, and carotenoids. These antioxidants, found alongside sugars, help protect our cells from damage. In fact, chilli peppers boast the highest content of vitamin C among vegetables, making them great for preventing cholesterol and fatty acid oxidation. Interestingly, chilli peppers are the only plants that produce capsaicin and which as a non-volatile, hydrophobic and crystalline compound. This colourless wonder, chemically known as 8-methyl-N-vanillyl-6-nonenamide (Vani 2017) [11]. Capsaicin isn't just for heat. It also shows promise in curbing your appetite, making it a helpful addition to both your meals and your medicine cabinet. Furthermore, Capsaicin plays a role in anthracnose resistance in some plants, particularly in chili peppers themselves. Capsaicin is one of the defense mechanisms that chili peppers have evolved to protect themselves against these pathogen and exhibit anticancer, anti-arthritic, and analgesic properties. Chilli extracts hold significant value in the food and pharmaceutical industries (Bhattacharya *et al*., 2010) [2].

With evolving lifestyles and health concerns, enhancing crop quality has become paramount. Improved quality not only bolsters human health but also strengthens farmers' incomes (Janaki *et al*., 2017) [4]. However, data on pungency and carotenoid levels within gene banks is often limited, hindering the selection of suitable genotypes for crop improvement programs. Therefore, recent breeding efforts prioritize quality traits alongside yield and resilience to both biotic and abiotic stresses. The present study was to use colorimetric method for the quantification of the capsaicin, oleoresin and ascorbic acid content in different lines and hybrids of chilli.

Materials and Methods

Experimental Materials

The experimental material for the present study comprised of 11 parents, 24 crosses and one commercial hybrid (Arka Meghana) of chilli (*Capsicum annum*. L) grown in the open field of Department of Biotechnology and Crop Improvement, Kittur Rani Channamma College of Horticulture, Arabhavi, University of Horticultural Sciences, Bagalkot, Karnataka, India.

Capsaicin content (%)

Capsaicin content in the samples was estimated by spectrophotometric measurement of the blue coloured component formed as a result of reduction of phosphomolybdic acid to lower acids of molybdenum following Ademoyegun *et al*. (2011) [1].

One gram (1 g) of each dry sample was extracted with 10 ml of dry acetone using pestle and mortar. The extract was 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 (60 °C). The residue was then dissolved in 0.4 ml of NaOH solution and 3 ml of 3% phosphomolybdic acid. The contents were shaken and allowed to stand for 1 h. The solution was filtered to remove any floating debris and centrifuged at 5,000 rpm for 15 min. 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 of the chili genotypes are analysed.

$$\text{Per cent capsaicin} = \frac{\mu g \text{ capsaicin}}{1000 \times 1000} \times \frac{10}{1.0} \times \frac{100}{0.5}$$

Oleoresin content (%)

Chilli oleoresin was extracted using Soxhlet method as described by Jensen William (2007) [5]. Ten grams of chilli was placed in a thumble and acetone was poured in the ratio of 1:10 and then heated at reflux. The process of heating and condensation was repeated until the complete oleoresin was extracted to organic acid and after the complete extraction, the solvent was allowed to vapourize, typically by means of a rotary evaporator. The extracted amount of oleoresin was calculated using the formulae.

$$\text{Oleoresin (\%)} = W_3 - W_2/W_1 \times 100$$

W_1-Weight of the sample taken
W_2-Weight of porcelain dish
W_3-Weight of porcelain dish + sample extract after drying

Ascorbic acid (mg/100g)

Ascorbic acid / Vitamin C was estimated by volumetric method. Principle behind the procedure is that the ascorbic acid reduces the 2, 6-dichlorophenol indophenol dye to a colourless leuco base, further due to oxidation it gets converted into dehydro-ascorbic acid. Dye initially is blue in colour, but at the end point it forms pink colour which persists for few seconds (Pugalendhi *et al*., 2010) [9].

1. Dye solution: 42 mg of sodium bicarbonate was weighed into a small volume of distilled water. 52 mg of 2-6 dichlorophenolindophenols was dissolved in it and volume was made up to 200 ml with distilled water.
2. Standard stock solution: 100 mg ascorbic acid was dissolved in 100 ml of 4% oxalic acid solution in a standard flask *i.e.*, 1 mg per ml.

Working standard: 10 ml of stock solution was diluted to 100 ml with 4% oxalic acid. The concentration of working standard was 100 mg per ml.

Procedure

Five ml of the working standard solution was taken into 100 ml of the conical flask. 10 ml of 4% oxalic acid was added and titrated against the blue dye (V1 ml) until the end point of pink colour was persisted for few seconds. Sample was extracted in 4% oxalic acid, volume was made to 100 ml and centrifuged in order to settle it. Supernatant of 5ml was taken and 10 ml of 4% oxalic acid was added, titrated against the dye (V2 ml). Then the ascorbic acid was calculated by the formula given below

$$\text{Amount of ascorbic acid (mg/100 g) sample} = \frac{0.5 \text{ mg}}{\text{V1 ml}} \times \frac{\text{V2}}{5 \text{ ml}} \times \frac{100 \text{ ml}}{\text{Weight of sample}} \times 100$$

Results and Discussions

Capsaicin (%)

Capsaicin is a natural compound found in chili peppers and it plays a role in anthracnose resistance in some plants, particularly in chili peppers themselves. Capsaicin has antifungal properties, which means it can inhibit the growth and development of fungal pathogens. Plants that produce capsaicin are more resistant to anthracnose because of its antifungal properties. This is an example of how secondary metabolites, like capsaicin, can contribute to a plant's resistance to various stresses, including diseases (Sinha *et al*., 2011) [10]. In the course of research, an investigation into the total capsaicin content within various parent genotypes and their crosses was conducted. The capsaicin content displayed a range from 0.56 to 1.25 per cent, with a mean value of 0.91 per cent. Among these testers, the highest capsaicin content was observed in KCA-44-2 (1.25%), closely followed by KCA-24-1 (1.22%). In contrast, among the lines, the highest capsaicin content was identified in KCA-19-4 (1.02%), with KCA-17-2 and KCA-26-2 both displaying content at 0.93 per cent. These findings indicate that there is considerable variation in capsaicin content between the different tester and lines. Furthermore, the crosses demonstrated slight variations in capsaicin content, with the highest recorded in the cross between KCA-5-3 × KCA-24-1 (1.13 %), closely followed by the cross between

Anugraha × KCA-24-1 (1.04%). In contrast, the cross between Byadagi Dabbi × BSS 414 displayed the lowest capsaicin content at 0.56 per cent. The results were depicted in table 1.

Oleoresin (%)

Oleoresin from chili peppers is crucial for the flavor, spiciness and aroma of chili-based dishes and products. The natural antimicrobial properties of chili oleoresin, especially capsaicin, have been utilized in food preservation. It can inhibit the growth of certain bacteria and fungi, helping extend the shelf life of food products**.** These compounds can inhibit the growth and development of the anthracnose-causing fungi, helping to reduce the severity of the disease (Kaur *et al*., 2011) [6]. The oleoresin content was observed to vary among different parent and crosses, ranging from 17.2 per cent to 9.3 per cent, with an average of 11.83 per cent. Notably, Byadagi Dabbi exhibited the highest oleoresin content at 16.8 per cent, followed by KCA-17-2 at 12.6 per cent, among the lines. Among the testers, KCA-24-1 had the highest content at 14.8 per cent, followed by BSS 414 at 12.2 per cent. In the crosses, Byadagi Dabbi×KCA-24-1 recorded the highest oleoresin content at 17.2 per cent, while Byadagi Dabbi×KCA-44-2 had a content of 16.2 per cent. The lowest content was observed in the cross KCA-5-3×BSS 414 at 9.4 per cent. The results are represented in table 1.

Ascorbic acid (mg/100 g) in green chilli

Ascorbic acid, also known as vitamin C, plays a role in the resistance of plants, including chili peppers, to various diseases, including anthracnose. Ascorbic acid is involved in various signaling pathways related to plant defense mechanisms. It can activate the expression of genes that are responsible for the synthesis of antimicrobial compounds and proteins. Ascorbic acid can activate enzymes involved in defense responses, such as peroxidases and polyphenol oxidases. These enzymes are responsible for breaking down pathogen cell walls and toxins and can limit the growth and spread of anthracnose fungi (Janaki *et al.*, 2018) [3]. The ascorbic acid content in green chillies varied across different varieties. The range of values was from 31.29 mg/100g for Arka Meghana to 243.16 mg/100g for KCA-24-1, with an average (mean) content of 94.76 mg/100g. Among the selected testers, KCA-24-1 had the highest ascorbic acid content at 243.16 mg/100g followed by KCA-44-2 at 124.49 mg/100g. When considering different lines, KCA-5-3 had the highest ascorbic acid content at 119.37 mg/100g, followed by Anugraha 3 at 112.22 mg/100 g. Among the crosses, Byadagi Dabbi × KCA-24-1 exhibited the highest ascorbic acid content at 179.37 mg/100 g. While, KCA-5-3 × KCA-24-1 had ascorbic acid content of 153.62 mg/100g. The lowest ascorbic acid content was found in the cross KCA-26-2 × BSS 414 at 46.93 mg/100g. The results are represented in table 1 and figure 1.

Table 1: *Per se* performance of chilli genotypes for biochemical parameters

Genotypes	Oleoresin content (%)	Capsaicin (%)	Ascorbic acid (mg/100g)
Anugraha (Lines)	12.6	0.80	112.22
KCA-17-2	12.6	0.93	54.78
KCA-26-2	11.6	0.93	41.24
KCA -19-4	11.7	1.02	90.19
KCA -32-5	10.1	0.85	91.73
Byadagi Dabbi	16.8	0.85	99.34
GPM-40	9.3	0.84	40.65
KCA-5-3	10.5	0.92	119.37
BSS 414 (Testers)	12.2	1.18	64.53
KCA-24-1	14.8	1.22	243.16
KCA-44-2	10.1	1.25	124.49
Anugraha× BSS 414	11.4	0.97	91.85
Anugraha× KCA-24-1	12.5	1.04	140.74
Anugraha× KCA-44-2	12.0	1.00	99.49
KCA-17-2× BSS 414	11.9	0.83	62.30
KCA-17-2× KCA-24-1	13.0	0.81	107.36
KCA-17-2× KCA-44-2	12.3	0.84	63.21
KCA-26-2× BSS 414	10.8	0.90	46.93
KCA-26-2× KCA-24-1	12.9	0.95	88.68
KCA-26-2× KCA-44-2	11.6	0.99	48.79
KCA -19-4× BSS 414	10.1	0.88	92.27
KCA -19-4× KCA-24-1	11.2	0.89	85.53
KCA -19-4× KCA-44-2	11.6	0.79	141.39
KCA -32-5× BSS 414	10.4	0.89	80.93
KCA -32-5× KCA-24-1	10.9	0.91	142.33
KCA -32-5× KCA-44-2	10.6	0.94	95.68
Byadagi Dabbi×BSS 414	15.5	0.56	93.89
Byadagi Dabbi× KCA-24-1	17.2	0.62	179.37
Byadagi Dabbi× KCA-44-2	16.2	0.60	101.68
GPM-40× BSS 414	9.5	0.85	60.74
GPM-40× KCA-24-1	10.4	1.04	52.90
GPM-40× KCA-44-2	10.1	0.83	56.73
KCA-5-3× BSS 414	9.4	1.01	111.48
KCA-5-3× KCA-24-1	10.9	1.13	153.62
KCA-5-3× KCA-44-2	10.0	1.02	100.83
Arka Meghana	11.5	0.86	31.29

Mean	11.83	0.91	94.76
S.Em+	0.14	0.070	0.8366
C.D. 1%	0.45	0.220	3.2177

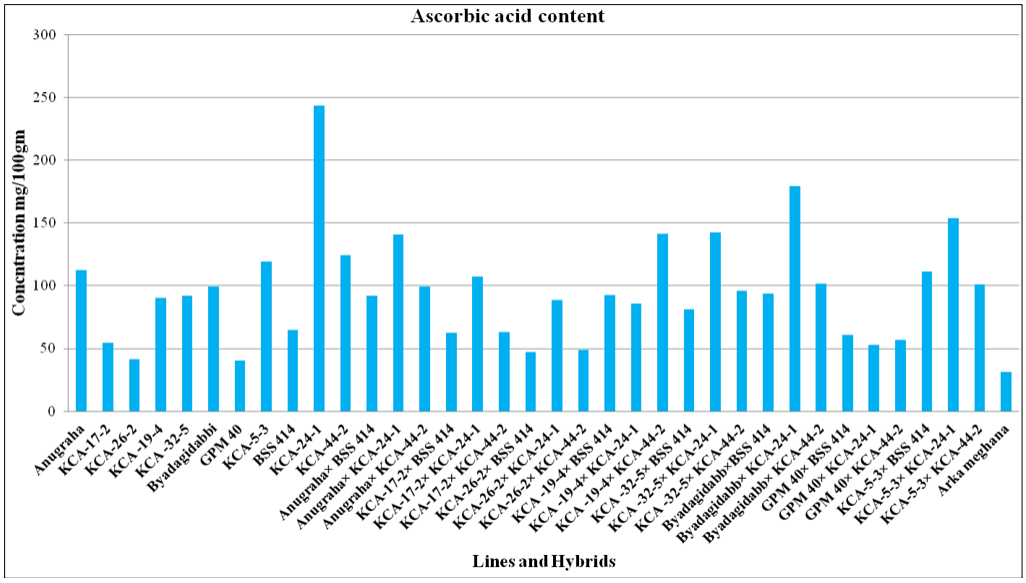

Fig 1: *Per se* performance of chilli genotypes for total ascorbic acid content

Conclusion

The present study has shed light on the remarkable diversity in the biochemical makeup of chili pepper. Specifically, observed that the significant variations in key components like ascorbic acid (vitamin C), capsaicin and oleoresin among both parent genotypes and their hybrid crosses. Within the studied genotypes, certain individuals emerged as champions in their respective biochemical domains. Byadagi Dabbi, KCA-44-2 and KCA-24-1 stood out among the parents, boasting the highest levels of oleoresin, capsaicin, and ascorbic acid, respectively. Notably, the hybrid crosses Byadagi Dabbi× KCA-24-1, KCA-5-3× KCA-24-1 and Byadagi Dabbi × KCA-24-1 also displayed impressive concentrations of these key components. These observations highlight the potential of cross-breeding to synergize desirable traits and potentially yield even higher levels of beneficial compounds. This study has provided a valuable glimpse into the remarkable biochemical diversity of chili peppers and the potential for harnessing this diversity to develop improved cultivars. The observed variations in ascorbic acid, capsaicin, and oleoresin content across genotypes and hybrids highlight the promising avenues for future research in chili pepper breeding, paving the way for the creation of pepper varieties with enhanced nutritional value, heat intensity and aroma, ultimately contributing to a healthier.

References

1. Ademoyegun OT, Fariyike TA, Aminu-Taiwo RB. Effects of poultry dropping on the biologically active compounds in *Capsicum annuum* L. (var. Nsukka yellow). Agri. Biol. J North America. 2011;2(4):665-672.
2. Bhattacharya A, Chattopadhyay A, Mazumdar D, Chakravarty A, Pal S. Antioxidant constituents and enzyme activities in chilli peppers. Intl. J of vegetable sci. 2010;16(3):201-211.
3. Janaki M, Naidu N, Ramana V, Rao MP. Assessment of genetic variability, heritability and genetic advance for quantitative traits in chilli (*Capsicum annuum* L.). Int. Quart. J Life Sci. 2018;10(2):729-733.
4. Janaki M, Ramana CV, Naidu LN, Babu JD, Rao KK, Krishna KU. Estimation of genetic variability parameters for yield and yield components in chilli (*Capsicum annuum* L.). J of Pharmacog. Phytochem. 2017;6(6S):685-689.
5. Jensen William. The Origin of the Soxhlet Extractor. J Ch. Ed. 2007;84(12):1913-1914.
6. Kaur N, Dhiman JS, Khurana DS. Physiological and biochemical trait analysis of *Capsicum annuum* L. germplasm for resistance to *Colletotrichum capsici*. J Cell Plant Sci. 2011;2(3):12-21.
7. Meghvansi MK, Siddiqui S, Khan MH, Gupta VK, Vairale MG, Gogoi HK, *et al*. Naga chilli: A Potential source of capsaicinoids with broad spectrum ethnopharmacological applications. J of ethnopharma. 2010;132(1):1-14.
8. Paramesha HT, Naika MB, Nishani S, Kantharaju V, Rathod V, Srikantaprasad D. Quantitative determination of phytochemical constituents in seeds of common methi and Kasurimethi. J Pharm. Innov. 2023;12(2):52-57.
9. Pugalendhi L, Veeraragavathatham D, Sathiyamurthy VA, Nataraja S. High yielding and moderately resistant to fruit rot disease chilli hybrid – CCH 1 (TNAU Chilli Hybrid CO 1). Electron. J Plant Breed. 2010;1(4):1049-1059.
10. Sinha DP, Saxena S, Kumar S, Singh M. Detection of pepper leaf curl virus through PCR amplification and expression of its coat protein in Escherichia coli for antiserum production. Afr. J Biotechnol. 2011;10(17):3290-3295.
11. Vani BK K. Estimation of capciacin content in different Red Chilli varieties by UV-Spectrophotometer. Caribb. J Sci. 2017;5(1):25-31.