www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(10): 458-461

www.thepharmajournal.com
Received: 10-08-2023
Accepted: 14-09-2023

V Jayasree
Ph.D. Scholar, Department of Vegetable Science, College of Horticulture, Bengaluru, University of Horticultural Sciences, Bagalkot, Karnataka, India

V Keshavarao
Principal Scientist, Division of Basic Sciences, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India

S ShankaraHebbar
Principal Scientist, Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India

K Madhavi Reddy
Principal Scientist, Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India

S Sriram
Principal Scientist, Division of Plant protection, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India

R Uma Maheshwari
Senior Scientist, Division of Plant protection, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India

Lakshmana Reddy DC
Principal Scientist, Division of Basic Sciences, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India

M Anjanappa
Professor and Head, Department of Vegetable Science, College of Horticulture, Kolar, University of Horticultural Sciences Bagalkot Karnataka, India

Jyothi Kattegouder
Assistant Professor, Department of Vegetable Science, College of Horticulture, Kolar, University of Horticultural Sciences Bagalkot, Karnataka, India

CN Hanchinmani
Professor and Head Department of Vegetable Science College of Horticulture, Bengaluru, University of Horticultural Sciences, Bagalkot, Karnataka, India

Corresponding Author:
K Madhavi Reddy
Principal Scientist, Division of Vegetable Crops, ICAR-IIHR, Bengaluru, Karnataka, India

Studies on effect of chilli root stock on pungency of grafted bell pepper

V Jayasree, V Keshavarao, S Shankara Hebbar, K Madhavi Reddy, S Sriram, R Uma Maheshwari, Lakshmana Reddy DC, M Anjanappa, Jyothi Kattegouder and CN Hanchinmani

Abstract
The commercial grade of peppers relies heavily on their capsaicin levels, a crucial determinant. Capsaicin and dihydrocapsaicin, which are the pungent alkaloids, are synthesized directly from two precursors: vanillylamine and a 9–11 carbon fatty acid. Assessing capsaicinoid content in mature fruit holds great importance for both chili and bell pepper quality. This study was conducted between July 2022 and January 2023 at the Division of Vegetable Crops, ICAR Indian Institute of Horticultural Research (IIHR) in Hesaraghatta, Bengaluru. In this grafting study, chili hybrids, namely Scion on H33, Scion on H19, Scion on H39, and Scion on H12, were employed as rootstocks, while the bell pepper hybrid Arka Athulya served as the common scion. As a result of grafting, there was no accumulation of compounds related to pungency, such as capsaicin and dihydrocapsaicin. Some variations in the HPLC areas of significant peaks were observed in the fruits of grafted plants, possibly as a consequence of the grafting process.

Keywords: Capsicin, dihydrocapsaicin, bell pepper, chilli, grafting, pungency and hybrid

Introduction
Capsaicinoids are the compounds responsible for the pungency found in pepper fruits and their derived products. Peppers, which are the fruits of plants belonging to the Capsicum genus and the Solanaceae family, encompass various domesticated species, including *Capsicum annuum, C. frutescens, and C. chinense*, each comprising numerous common varieties. These diverse peppers are widely cherished across many regions of the world for their valuable and distinct sensory attributes, encompassing color, pungency, and aroma. Among these attributes, pungency, which holds significant commercial importance, arises from the presence of molecules belonging to the capsaicinoids group. Capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide) and dihydrocapsaicin represent the predominant capsaicinoids in peppers, constituting approximately 90% of the total capsaicinoid content in most spicy pepper types (Othman *et al*., 2011) [4]. The capsaicin content in peppers plays a pivotal role in determining their commercial grade, but its significance extends beyond that, as capsaicin is regarded as a key bioactive compound responsible for the medicinal properties of peppers. It has been utilized as an analgesic in the treatment of arthritis-related pain and inflammation, showcasing its therapeutic potential. Moreover, capsaicin has been suggested to possess anticancer properties and efficacy against neurogenic inflammation, which includes the relief of sensations like hand, mouth, and eye burning and stinging. This very attribute is why capsaicin is employed in defensive pepper sprays. Additionally, capsaicin has demonstrated protective effects against elevated cholesterol levels and obesity. Furthermore, capsaicin and other capsaicinoids exert a wide array of physiological and pharmacological influences on various bodily systems, encompassing the gastrointestinal tract, cardiovascular and respiratory systems, as well as sensory and thermoregulation systems. These effects are primarily attributed to capsaicinoids' unique impact on primary afferent neurons of the C-fiber type. This distinctive effect clarifies their application in the management of certain peripheral painful conditions, such as rheumatoid arthritis. However, it's worth noting that excessive consumption of capsaicin can have adverse effects on health (Hachiya *et al*., 2007) [3].

In a case-control study conducted in Mexico City, individuals who consumed chili peppers had a 5.5-fold higher risk of developing gastric cancer compared to non-consumers. The study involved 220 cases of gastric cancer and 752 randomly selected controls from the general population. Notably, those who self-identified as heavy chili pepper consumers faced a significantly greater 17-fold increased risk. However, when evaluating chili pepper consumption in terms of daily frequency, no significant dose-response association was observed. It's important to note that the capsaicin content in a particular variety can vary due to factors such as light intensity, temperature, fruit age, and fruit position on the plant (Govindarajan *et al.*, 1991) [2]. This study aimed to investigate changes in capsaicinoid levels in interspecific grafting, utilizing bell pepper as the scion and chili pepper (*Capsicum annum*) hybrids as the rootstocks.

Material and Methods

Present investigation was carried out during July 2022 to January 2023 at Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta, Bengaluru. Chilli hybrids *viz,* IHR4392 × IHR3291, IHR4563× IHR3291, IHR4392 × IHR4602 and IHR3226 × IHR3291 were used as rootstocks and bell pepper hybrid Arka Athulya was used as a common scion for the grafting studies.

S. No.	Hybrids (F_1s)	Mentioned as
1	IHR4392 × IHR3291	Scion on H33
2	IHR4563 × IHR3291	Scion on H19
3	IHR4392 × IHR4602	Scion on H39
4	IHR3226 × IHR3291	Scion on H12
5	Arka Athulya	Scion on own roots

Sample preparation

To prepare the samples, mature air-dried pods were subjected to a 48-hour drying process in a hot air oven set at 60 degrees Celsius. The dried samples were subsequently ground into a fine powder using a RETSCH Mixer mill MM 400 and sieved. For further analysis, 200-400 mg of the sample powder was carefully measured and placed in a 50 mL volumetric flask. Ethanol (25 mL) was then added, and the mixture was heated in a shaking water bath at 90 °C for 30 minutes. Afterward, distilled ethanol was used to adjust the volume to 50 mL. The flask was thoroughly shaken and allowed to cool to room temperature. Prior to analysis, the ethanol extract was filtered through a 0.2µm Nylon membrane filter (Pall Corporation) and subsequently subjected to HPLC.

HPLC analysis

The HPLC analysis was conducted using a Shimadzu Series LC-10A system (Shimadzu, Kyoto, Japan), which consisted of a liquid chromatography setup coupled with a UV-VIS detector (model 10A), a binary pump, and Shimadzu LC solutions software. The chromatographic separation was achieved using an Onyx 100 x 4.6 mm monolithic C18 column (Phenomenex, USA) along with an Onyx monolithic C18 guard cartridge (part no. KJO-7651, Phenomenex, CA, USA). Sample injection was carried out using an autosampler (model: SIL 20 A HT, Shimadzu, Kyoto, Japan). The column and guard column were temperature-controlled at 30 °C throughout the analysis. The flow rate was set at 1.8 ml/min, and the mobile phase consisted of water (solvent A) and acetonitrile (solvent B). The instrument was configured to operate in linear gradient mode, with the following gradient conditions: 0-8 minutes, transitioning from 30% to 78% B; and 8-12 minutes, maintaining a constant 78% B concentration. Detection was carried out at 205 nm. Nordihydrocapsaicin, capsaicin, and dihydrocapsaicin exhibited retention times of 5.41, 5.67, and 6.33 minutes, respectively. Filtered samples (20 µL) were introduced into the HPLC system for analysis. Capsaicin and dihydrocapsaicin standards were sourced from Sigma-Aldrich Co., USA.

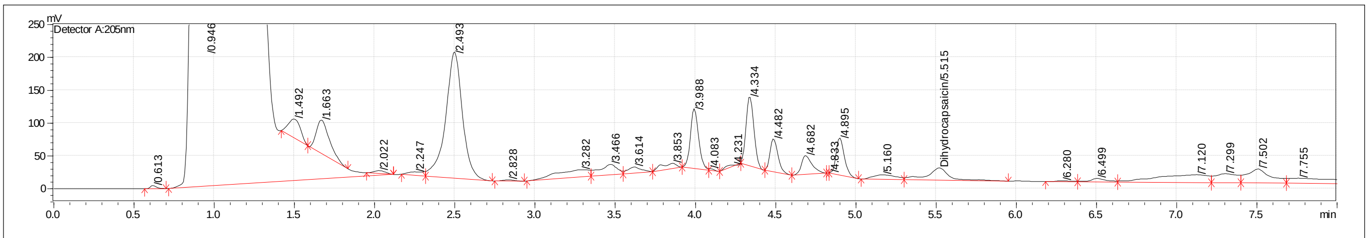
Results and Discussion

Five prominent peaks were observed in the chromatograph in the samples. These peaks were tagged with respective retention times and the areas of these peaks were noted in all the samples and the peak areas of these tagged peaks were compared. Some significant variations were observed in the peak areas among the samples which might have a resulted due to grafting with different root stocks Table 1

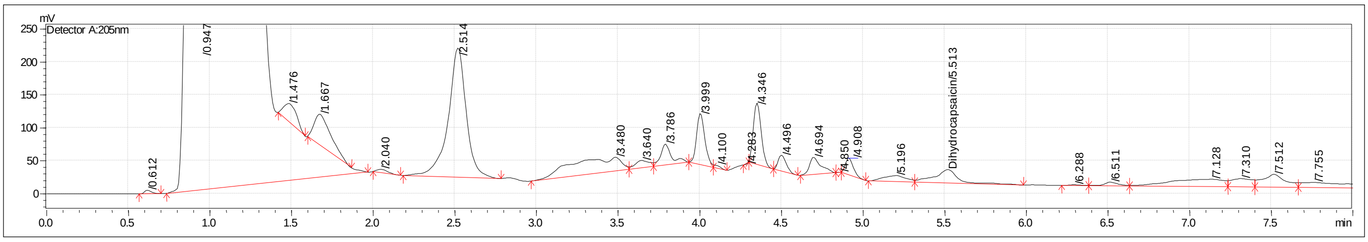
Table 1: Prominent peaks and retention times of grafted Bell pepper

Sl. No.	Treatments		1	2	3	4	5
1	Scion on H33	RTS	2.42	3.97	4.32	4.47	4.88
		Peak area	895398.5	356794.5	386989.5	169261	168610
2	Scion on H19	RTS	2.50	3.99	4.34	4.49	4.90
		Peak area	1387510	307272.5	367396	173701	1118727
3	Scion on H39	RTS	2.49	4.00	4.35	4.50	4.91
		Peak area	1427070	293653	338152	84419.5	94779.5
4	Scion on H12	RTS	2.52	4.01	4.35	4.50	4.92
		Peak area	1697293	369187.5	433318.5	197024	106613.5
5	Own root seedling	RTS	2.55	4.02	4.37	4.52	4.93
		Peak area	1123475	202791.5	214892	75756	74717

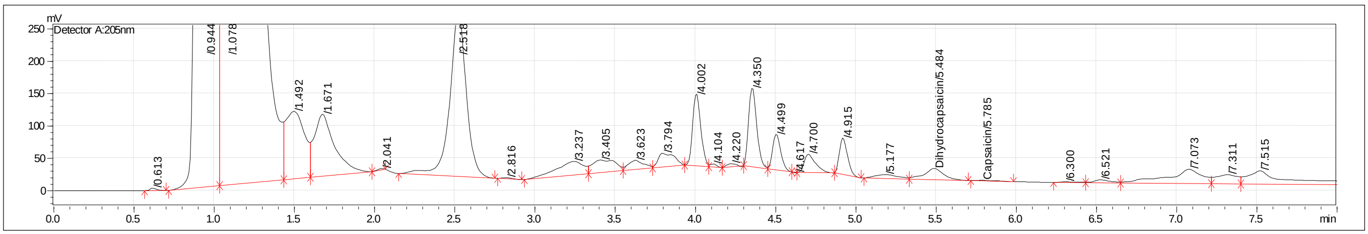
1, 2, 3, 4 and 5- Un Identified Secondary Metabolities.
RTS: Retention times



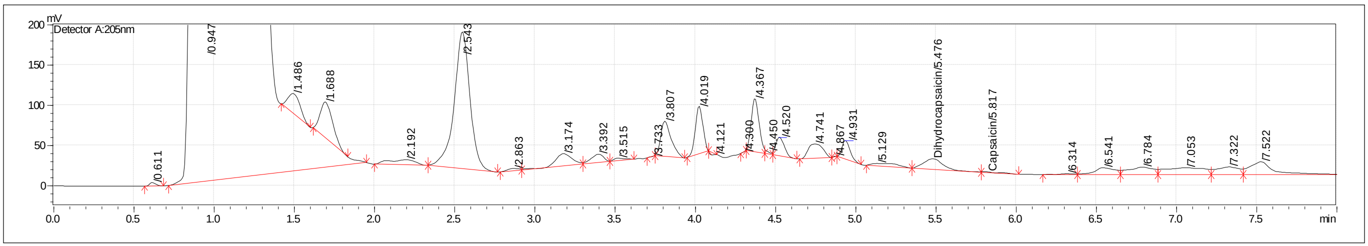
(a) Scion on H33



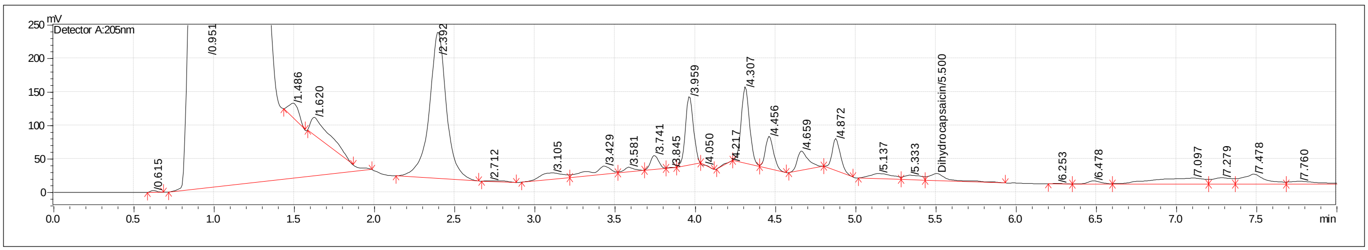
(b) Scion on H19



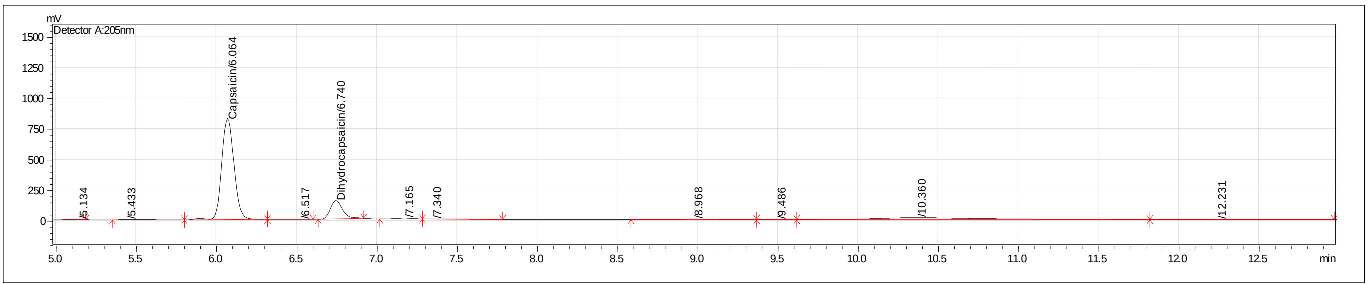
(c) Scion on H39



(d) Scion on H12



(e) Scion on own roots



(f) Standard

Fig 1: Chromatogram of the grafted bell pepper

Whether using own-root seedlings or grafted seedlings on chili rootstock, bell pepper fruits exhibited an absence of capsaicinoids, indicating that there was no discernible rootstock effect on scion fruit capsaicinoid content. Notably, the presence of capsaicin and dihydrocapsaicin, the compounds responsible for pungency, was not detected in the fruits due to the influence of the rootstock. In the fruits of grafted plants, some variations were observed in the HPLC peak areas of certain significant peaks, potentially attributable to the grafting process (Fig. 1). This finding aligns with similar reports provided by Andrey *et al*. (2021) [1]. In accordance with previous research, it is well-documented that most non-pungent peppers possess a four-base mutation in Pun1, the gene responsible for capsaicinoid synthesis in the interlocular septa (placenta) during fruit development (Stewart *et al*., 2005 and 2007) [5, 6]. The absence of capsaicin and dihydrocapsaicin in scion fruit, regardless of the rootstock used, supports the hypothesis that the bell pepper scion carries a mutation in Pun1 or another gene responsible for capsaicinoid synthesis or accumulation. Importantly, this study suggests that no graft transmissible metabolite compensated for the observed mutation (Yagishita *et al*., 1985 and 1990) [7, 8]. The data from the current study suggest that interspecific grafting of nonpungent bell pepper hybrid onto pungent chilli rootstocks does not compromise pungency of the scion. As expected, capsaicin and dihydrocapsaicin were not detected in fruit harvested from nonpungent scion grafted onto pungent rootstocks. To support the adoption of grafting technology in peppers additional research efforts and economic analysis are required to clarify the potential benefits of using chilli hybrids as rootstocks to manage biotic and abiotic stress.

Conclusion

Scion on own roots and regardless of rootstock, no capsaicinoids were detected in scion fruit and there were no rootstock by scion interactions for fruit capsaicinoid content. Due to grafting there is no accumulation of pungency related compounds i.e. capsaicin and dihydrocapsaicin. In fruits of grafted plants some variations were observed in the hplc areas of some significant peaks which might have resulted as consequence of grafting.

References

1. Andrey VA, Paul CB, James N. Effects of Interspecific Grafting Between Capsicum Species on Scion Fruit Quality Characteristics Hort. Science. 2021;56(11):1347-1353.
2. Govindarajan VS, Sathyanarayana MN. Capsicum: Production, technology, chemistry, and quality. Part V. Impact on physiology, pharmacology, nutrition, and metabolism; structure, pungency, pain, and desensitization sequences. Crit. Rev. Food Sci. Nutr. 1991;29(1):435-473.
3. Hachiya S, Kawabata F, Ohnuki K, Inoue N, Yoneda H, Yazawa S, *et al*. Effects of CH-19 Sweet, a non-pungent cultivar of red pepper, on sympathetic nervous activity, body temperature, heart rate, and blood pressure in humans. Biosci. Biotechnol. Biochem. 2007;71(1):671-676.
4. Othman ZA, Hadj Ahmed YB, Habila MA, Ghafar AA. Determination of Capsaicin and Dihydrocapsaicin in Capsicum Fruit Samples using High Performance Liquid Chromatography. Molecules. 2011;16(1):8919–8929.
5. Stewart C, Kang B, Liu K, Mazourek M, Moore SL, Yoo EY, *et al*. The Pun1 gene for pungency in pepper encodes a putative acyltransferase. Plant J. 2005;42:675–688.
6. Stewart C, Mazourek M, Stellari GM, MO’ Connell, Jahn MM. Genetic control of pungency in C. chinense via the Pun1 locus. J Expt. Bot. 2007;58:979–991.
7. Yagishita N, Hirata Y, Mizukami H, Ohashi H, Yamashita K. Genetic nature of low capsaicin content in the variant strains induced by grafting in *Capsicum annuum* L. Euphytica. 1990;46(1):249–252.
8. Yagishita N, Hirata Y, Okochi K, Mimura K, Mizukami H, Ohashi H. Characterization of graft-induced change in capsaicin content of *Capsicum annuum* L. Euphytica. 1985;34(1):297–301.