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Estimation of capsaicin and capsaicinoid contents of high pungent chilli accessions of Andaman & Nicobar Islands and North-East India

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ABSTRACT

A native variety of chilli pepper from Andaman & Nicobar Islands (IC 553688), was characterized and the capsaicinoids content were estimated in terms of parts per million of heat (ppmH) by high performance liquid chromatography. The genotype IC 553688 had greenish-white petals, blue anthers, constriction between calyx and pedicel, fruits borne in clusters of 2-4 and had distinct aroma resembling to clarified butter. The leaf surface has characteristic crinkled pattern between the veins. One way ANOVA indicated that all the chilli accessions differed significantly in their capsaicin, Dihydrocapsaicin , CapDihcapsaicin and pungency levels (p<0.01). Among the varieties, IC 553688 was found to be more pungent with high capsaicinoids content (29,140.63 ppmH) and pungency value at 4, 37,109.52 Scovillie Heat Unit (SHU) as compared to that of *C. chinense* cv. Tezpur Local (10,170.60 ppmH and 1, 52,558.99 SHU), *C. frutescens* cv. Pusa Sadabahar (3,922.47 ppmH and 58,837.10 SHU) and *C. annuum* cv. Pusa Jwala (2,778.86 ppmH and 41,682.95 SHU) respectively. The genotype IC 553688 has become well adapted to hot and humid climate of Andaman and Nicobar islands where it has potential to be grown commercially for the extraction of nutraceuticals.

Key words: Capsaicin, dihydrocapsaicin, Scovillie heat unit, pungency, *Capsicum* species.

INTRODUCTION

The genus *Capsicum* consists of 25 wild and five cultivated species and grows well in tropical, sub-tropical, as well as temperate regions and is indigenous to the Western hemisphere. Chilli pepper was introduced into India from Brazil in the 16th century by the Portuguese and since then have adapted very well to the Indian sub-continent so much so that India is considered as its secondary centre of origin. In India, numerous landraces of chilli pepper differing in shape, size, colour and heat level can be found as farmers selected genotypes to fit their need (Boseland and Baral, 4). The fruits of *Capsicum* spp. are used as vegetable and spice in different parts of the world and have an international commercial importance owing to their pungency levels. Although chilli pepper is popular as food and spice, it was first used as a medicinal plant to treat respiratory ailments and for reducing pain. Presently they are used in ointments to relieve muscular pain and as the main ingredient for spray as a personal defense instrument or for crowd control too (Basu and De, 3).

The pungency in chilli fruits is estimated based on the contents of capsaicin and dihydrocapsaicin (Collins *et al*., 5). The capsaicinoids content of the chilli fruits greatly vary among the species, cultivars, and the growing conditions (Boseland and Baral, 4; Harvell and Bosland, 7; Kraikruan *et al*., 9; Lindsay and Bosland, 10; Sanatombi and Sharma, 12; Sukrasno and Yeoman, 14; Tiwari *et al*., 15) and accordingly are grouped as sweet to hot pepper. During the last few decades, there has been substantial increase in area under field crops with high nutraceutical values and accordingly there lies a constant scope for identification of genotypes with higher content of any active principle. Chilies and paprika are known since ages for their different nutritional and pharmaceutical values. It is the extracts which have a huge national and international market owing to their diverse use. Hence, hot chillies and peppers have huge potential in nutraceutical industry.

The native chilli variety 'Bhut Jolokia' found in northeastern regions of India was recently reported to be the hottest chilli pepper in the world (Mathur *et al*., 11). However, a locally grown chilli landrace, known as '*Jungli Mirch*', meaning wild chilli pepper in Hindi, used by the *Shompen* Tribe of Andaman & Nicobar Islands is also well known for its high pungency. It has not yet been analyzed for its pungency and hence not well documented. Therefore, the present study was undertaken to determine the species designation and to compare the heat levels of the genotype of Andaman & Nicobar Islands (IC 553688) with that of *C. chinense* cv. Tezpur Local, *C. annum* cv. Pusa Jwala, *C. frutescens* cv. Pusa Sadabahar.

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MATERIALS AND METHODS

Seeds of IC 553688 was collected from a place known as '47 Kilometre Point', south of Indira Point (6.75° N, 93.83° E) of Andaman & Nicobar Islands, in the Indian Ocean. The seeds of *C. frutescens* cv. Pusa Sadabahar, *C. annuum* cv. Pusa Jwala were collected from IARI, New Delhi and *C. chinense* cv. Tezpur Local was obtained from Tezpur, Assam, India. The seeds were sown in December 2008 in polypropylene trays fitted with a growing medium (80% decomposed coir dust coco-peat: vermiculite: river sand, 2:1:1), in a controlled glasshouse (day: 30 ± 1°C, night: 26 ± 1°C; RH, 65-70%). The seedlings (8-10 true leaf stage) were transplanted into polypropylene pots (23 cm × 23 cm). The pots were then maintained in a glass-house. The light intensity inside the glasshouse was 20% lower than the natural light and the conditions were maintained as stated earlier. Plant nutrients were added to the pots using a modified Hoagland's solution and also irrigated regularly to maintain a healthy crop. The capsicum descriptors developed by International Plant Genetic Resources Institute (IPGRI, 8) presently Bioversity International, were used for characterization of IC 553688. Fruits were harvested at red ripe stage and dried in diffused sunlight for 10-11 days.

The chilli fruits were dried in oven (55°C, 12 h) and powdered in a mortar with a pestle to get a fine powder. An aliquot of the powder (~1 g) was weighed accurately and transferred to a glass bottle with screw cap. Acetone (25 ml) was then added and shaken for four hours on an orbital shaker at 140 rpm under ambient conditions (18°C). The contents were allowed to settle and the supernatant was withdrawn using a syringe and filtered through a Millipore® membrane (0.2 µm). The filtrate was stored at 4°C in amber coloured glass vial with screw cap until further analysis by high performance liquid chromatography (HPLC). Capsaicin standard (Fluka, 65% by HPLC obtained from Sigma Aldrich, St. Louis, USA) was used as reference.

Capsaicin was estimated by using a HPLC fitted with Waters Model code 60F quaternary gradient dual head pump, Waters 600 pump controller, Waters 66E Multi-solvent delivery system, Waters 717 Plus auto-sampler, YMC30 stainless steel reverse phase column (250 × 4.6 mm, 5 µ sorbent), Waters 2998 PDA UV-VIS detector, and loaded with Empower 2 software was used. The mobile phase was methanol: water (65: 35) at 1 ml min^{-1}. The data acquired was analyzed at 229 nm. Aliquots (20 µl) of standard solutions were injected. The retention time (R_T) for capsaicin was 11.702 min and the minor peak at 17.211 min. was taken as that of dihydrocapsaicin (Deng *et al*., 2). Aliquot (20 µl) of the sample extracts were injected and the data were analyzed for capsaicinoid contents. The capsaicin content in the extract was calculated based on the purity of the reference standard, and the peak areas of capsaicin in the standard and sample. The total capsaicinoid content in the fruit was calculated as parts per million of heat (ppmH) using the procedure of American Spices Trading Association (ASTA, 1). The pungency of the fruits was calculated by multiplying the ppmH by a factor of 15 (Collins *et al*., 5).

Data were subjected to one-way ANOVA to identify significant mean differences using spss (SPSS Software for Windows release 17.0, SPSS Inc., Chicago, IL, USA). When significant differences in ANOVA ($p < 0.05$) were detected, the Tukey's HSD multiple comparison test was applied to compare the specific accession-wise differences.

RESULTS AND DISCUSSION

The plant characteristics of IC 553688 are summarized in Table 1. The leaf surface of the genotype IC 553688 had the characteristic crinkled pattern between the veins (Fig. 1a), had greenish-white petals, blue anthers (Fig. 1b), flowers borne in clusters of 2-4 (Fig. 1c), fruits had constriction between calyx and pedicel (Fig. 1d). The *Capsicum chinense* species

Table 1. Vegetative and reproductive characteristics of IC 553688 chilli.

Character	State	Character	State
Plant height (cm)	167.8	Flower position	Pendent
Leaf colour	Green	Fruit colour at maturity	Red
Leaf surface	Crinkled between the veins	Fruit shape	Conical
Leaf length (cm)	18.68	Fruit length (cm)	5.12
Leaf width (cm)	9.62	Fresh weight of fruit (g)	5.88
Corolla colour	greenish white	Dry weight of fruit (g)	0.68
Anther colour	Blue	Fruit surface	Unevenly crinkled
Anther filament colour	White	Seed colour	Straw
Annular constriction	present below calyx	100-seed weight (mg)	0.33
Clusters / axil	2-4	Number of seeds / fruit	43

Table 2. Capsaicin and capsaicinoids content, and pungency in some *Capsicum* species.

Species	Genotype	Capsaicin (ppm)	Dihydrocapsaicin (ppm)	CapDihcapsaicin (ppmH)	Pungency (SHU)	Capsaicin/ Dihydro capsaicin
C. chinense	IC 553688	23651.36 ± 6.68[a]	5476.19 ± 2.12[a]	29139.62 ± 3.57[a]	437109.40 ± 0.66[a]	4.32
C. chinense	Tezpur Local	7376.9467 ± 4.11[b]	2791.28 ± 0.96[b]	10169.2767 ± 2.86[b]	152556.00 ± 2.88[b]	2.64
C. annuum	Pusa Jwala	1029.5400 ± 0.45[c]	1749.52 ± 0.64[c]	2776.8533 ± 4.76[c]	41685.8400 ± 2.85[c]	0.59
C. frutescens	Pusa Sadabahar	2072.2200 ± 2.73[d]	1846.87 ± 2.80[d]	3918.0867 ± 4.83[d]	58839.96 ± 2.74[d]	1.12
F value		4330505.11**	2650137.94**	17337014.72**	16618669676.44**	

±Standard deviations; *Different letters in the same column indicate significant differences ($p < 0.01$; Tukey's HSD); **Significant at 0.01 level (2-tailed).

is characterized by distinct traits like crinkle leaf surface between the veins, presence of a calyx constriction, multiple flowers per node, greenish corolla, and blue anthers and the fruits have distinct aroma (Somos, 13; Baral and Bosland, 2; Boseland and Baral, 4; Basu and De, 3). The genotype 'IC 553688' under the study conformed to these features. The fruit had undulating surface and distinct aroma resembling clarified butter. The length of fruits ranged from 4.0-6.0 cm and weighed about 4.98-7.40 g.

The chromatograms of standard capsaicin solution (1,000 ppm) and the fruit extract of Tezpur Local and IC 553688 are depicted in Fig. 2 a-c. Data presented in Table II shows the capsaicin (ppm), dihydrocapsaicin (ppm), capsaicinoid (ppmH), the pungency as Scoville Heat Units (SHU) and the ratio of capsaicin to dihydrocapsaicin in fruits of different chilli species/varieties. The one way ANOVA showed that the there was significant mean difference among chilli accessions in terms of their capsaicin content [$F(3,8) = 4330505.11$, $p<0.01$]. Genotype IC 553688 recorded the highest capsaicin content (23,662.12 ppm) followed by the Tezpur Local (7,378.33 ppm), Pusa Sadabahar (2,072.87 ppm) and Pusa Jwala (1,029.5 ppm). Post-hoc analysis also showed that all the accessions differed significantly in their capsaicin content ($p<0.01$, Tukey's HSD). Similarly, significant mean differences were observed among chilli accessions in terms of Dihydrocapsaicin [$F(3,8) = 2650137.94$, $p<0.10$)], CapDihcapsaicin [$F(3,8) = 17337014.72$, $p<0.10$)] and pungency [$F(3,8) = 16618669676.44$, $p<0.10$)]. Among the *Capsicum* species studies, *C. chinense* had the highest Dihydrocapsaicin, CapDihcapsaicin and pungency content. Similar genotypic difference in capsaicin contents of different chilli cultivars has also been earlier reported (Kraikruan *et al*., 9; Deng *et al*., 6).

Fig. 1. Plant characteristics of chilli genotype IC 553688, (a) Characteristic crinkled pattern between the veins; (b) Corolla (greenish white, blue anthers); (c) Fruits (cluster bearing); and (d) Presence of annular constriction below the calyx.

However, the ratio of capsaicin to dihydrocapsaicin content varied widely among the species ranging from 0.58 in *C. annuum* (Pusa Jwala) to 4.31 in *C. chinense* (IC 553688). The capsaicin share among the capsaicinoids was highest (81.2%) in IC 553688 and that was lowest in Pusa Jwala (37.0%). The ratio of capsaicin to dihydrocapsaicin, a factor in the biogenetic pathway of the biosynthesis of capsaicinoids, plays an important role in the pungency level of a chilli fruit. Since capsaicin is more pungent than dihydrocapsaicin, capsaicin to dihydrocapsaicin ratio will be directly proportional to the fruit pungency. The capsaicin: dihydrocapsaicin in Thai *Capsicum* fruits in the range 1.18 to 2.25 for *C. annuum* and 1.43 to 2.31 for *C. frutescens* (Kraikruan *et al*., 9) and China *Capsicum* fruits the range was 2.41 to 2.56 for *C. annuum* and 3.01 to 3.20 for *C. frutescens* (Deng *et al*., 6). The fruits of *C. chinense* cv. Nagahari grown at Tezpur (26.37 °N,

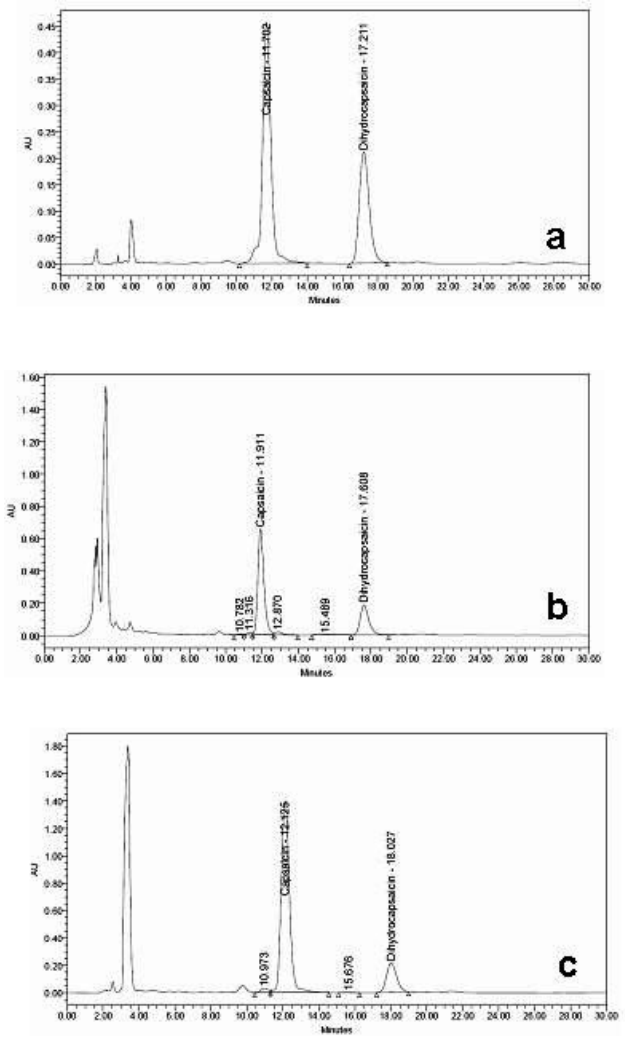

Fig. 2. HPLC chromatogram of (a) standard capsaicin (1000 ppm), (b) Tezpur Local, and (c) IC 553688.

92.50 °E) had higher capsaicin-dihydrocapsain (3.01) than the fruits of the same genotype (1.15) grown at Gwalior (Tiwari *et al*., 15). In concurrence, we also estimated a higher capsaicin-dihydrocapsaicin ratio in the fruits of *C. chinense* varieties; IC 553688 (4.3) and 'Tezpur Local' (2.64), followed by *C. frutescens* cv. 'Pusa Sadabahar' (1.12) and *C. annuum* cv. 'Pusa Jwala' (0.59).

Under similar growing conditions, IC 553688 was found to be superior to the *C. chinense* variety collected from Tezpur, the region where 'Bhut Jolokia' was identified as the hottest chilli in the world. In addition, IC 553688 has become well adapted to hot and humid climate during the long course of cultivation in Andaman and Nicobar Islands and therefore it can be successfully cultivated in hot humid climatic zones with better recovery of pungent principles. Hence, it enjoys good chances of commercial exploitation of the genotype in areas having similar environmental conditions.

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