Effect of sowing time and crop geometry on the Capsaicinoid content in Bhoot Jolokia (*Capsicum chinense* Jacq.)

Sanju Singh Moirangthem · Sainen Gogoi ·
Premila Devi Thongbam · K. T. Ramya ·
R. Abdul Fiyaz · D. S. Pandey

Revised: 27 October 2011 / Accepted: 14 March 2012 / Published online: 30 March 2012
© Association of Food Scientists & Technologists (India) 2012

Abstract *Capsicum* species are not only cultivated as vegetable and condiment crop but are also incorporated into a number of medicinal preparations in the ancient literature around the world. ‘Naga chilli’ or ‘Bhoot Jolokia’ (*Capsicum chinense* Jacq.) is a chilli variety indigenous to the northeast region of India and has been recognized as one of the hottest chilli in the world. It has also been used conventionally in treating various human ailments since time immemorial by the indigenous people of the northeast India. Capsaicin and dihydrocapsaicin are the two major members of the so-called capsaicinoid family, which includes other minor analogues, and usually account for at least 90 % of the pungency trait in chilli fruits. In the present study, determination of Capsaicinoid content of Bhoot Jolokia (*Capsicum chinense* Jacq.) under different sowing time and spacing was done. In the Scoville Organoleptic test, the highest Scoville Heat Unit (SHU) value was found in September 15 sowing fruits and the lowest in the February 15 sowing fruits. Fruit produced from spacing 105 cm × 105 cm showed highest Scoville Heat Unit. In High Performance Liquid Chromatography analysis, highest amount of capsaicinoid was also found in September 15 sowing while fruits produced from spacing 105 cm × 105 cm showed highest amount of capsaicinoid. Identification of Nordihydrocapsaicin, Capsaicin and Dihydrocapsaicin was done by comparing the retention time of sample with those of standard.

Keywords Bhoot Jolokia · Hottest chilli · Capsaicinoid · HPLC · Spacing · Planting time

Introduction

Chilli or hot pepper is a commonly cultivated Solanaceous crop of India. Different cultivars of chilli are being grown for vegetables, spices, pickles, condiments etc. It is mainly used for its pungency and colouring properties. India is the largest producer and consumer of chilli among the other major producer of the world. India contributes about 25 % to the total world production and remained at the first position in terms of international trade by exporting 20 % from its total production. India exports different forms of hot peppers like chilli powder, dried chilli, pickled chillies and chilli oleoresins. Dietary spices such as fruit of *Capsicum chinense* are important ingredients which are sources of the highly pungent capsaicinoids and of antioxidants, which may play a role in preventing or reducing chronic and age-related diseases. The genus *Capsicum* (Solanaceae) consists of five domesticated species: *Capsicum annuum* L., *Capsicum baccatum* L., *Capsicum chinense* Jacq., *Capsicum frutescens* L. and *Capsicum pubescens* Ruiz & Pav as well as around 25 wild species (IBPGR 1983). *Capsicum annuum* is one of the major vegetable and spice crops cultivated worldwide. However, the ‘Naga chilli’, which is native to the northeastern part of India, has received the attention of world scientific community due to its extremely high pungency and unique aroma. It is known by various names in different regions such as ‘Bhoot Jolokia’ or ‘Bih Jolokia’ in Assam, ‘Naga king chilli’ in Nagaland, ‘Omorok’ in Manipur and ‘Ghost pepper’ by the western media. It is also known by the names, ‘Saga Jolokia’, ‘Indian mystery chilli’ and ‘Indian rough chilli’ (after the chilli’s rough skin). It has been

S. S. Moirangthem · S. Gogoi
Department of Horticulture, Assam Agricultural University,
Jorhat, Assam 785013, India

P. D. Thongbam (✉) · K. T. Ramya · R. A. Fiyaz · D. S. Pandey
Division of Plant Breeding,
ICAR Research Complex for NEH Region,
Umiam, Meghalaya 793103, India
e-mail: pthongbam2000@yahoo.co.in

acknowledged as the hottest chilli in the Guinness Book of World Records (2006). It has also been used conventionally by different ethnic communities of the northeastern India in treating various human ailments (Bhagowati and Changkija 2009). In Nagaland, *Capsicum* spp. including Naga chilli are used to tone up body muscles after heavy workouts whereas hot infusions are used for toothache and muscle pain (Bhagowati and Changkija 2009).

Ethnobotanical literature around the world contains a wide array of information on traditional uses of different *Capsicum* species in treating various maladies. The dried fruits of *Capsicum frutescens* are used by the ‘Garó’ ethnic community of Bangladesh in preparation of fermenting medium for their traditional liquor (Anisuzzaman and Rahman 2007). The extremely hot or burning sensation of chilli is due to the presence of capsaicinoids found only in *Capsicum* (Hoffman et al. 1983). The capsaicinoids present in the *Capsicum* fruit are predominantly capsaicin and dihydrocapsaicin making up 80–90 %. Other minor compounds are Nordihydrocapsaicin, Homocapsaicin and homodihydrocapsaicin (Fig. 1). The ratio of capsaicin to dihydrocapsaicin ranges between 1:1 and 2:1 (Govindarajan and Sathyanarayana 1991). In northeastern India, the Naga chilli has been cultivated in a traditional manner since time immemorial and there exists a considerable amount of genetic variability among the landraces (Bhagowati and Changkija 2009). The capsaicin content of fruits of *Capsicum chinense* has been found to be very high in comparison to the fruits of the other chilli species (Sanatombi and Sharma 2008). The pharmaceutical applications of capsaicinoids are attributed to their analgesic, antiarthritic, anticancer, and antioxidant properties (Prasad et al. 2005). In fact, capsaicin has been at the centre of intense research for elucidating the basis of its pharmacological properties and exploiting the therapeutic potential (reviewed by Prasad et al. 2005). Capsaicin has become a promising

molecule for the development of a new generation of analgesic-anti-inflammatory agents targeting the nociceptive primary afferent neurons (Szolcsanyi 2003). It has also been reported to inhibit the growth of prostate cancer cells (Mori et al. 2006). The antioxidative capacity of chilli is higher than ginger, garlic, mint and onion (Shobana and Naidu 2000), which may play an important role in the process of chemoprevention (Yu et al. 2002).

Capsaicinoids are alkaloids produced by a condensation reaction between an aromatic moiety and a C9–C11 branched chain fatty acid. The aromatic moiety is vanillylamine, a phenylalanine derivative. Branched-chain fatty acids are biosynthesized from valine and leucine, and in the case of capsaicin and dihydrocapsaicin those acids differ in the degree of unsaturation of the 9-carbon fatty acid side chain (Bennett and Kirby 1968; Suzuki et al. 1981). The degree of pungency depends on the *Capsicum* species and cultivars, and the capsaicin and dihydrocapsaicin contents can be affected by different factors such as the developmental stage of the fruit (Sukrasno and Yeoman 1993) and the environmental growth conditions (Lindsey and Bosland 1995; Estrada et al. 1998; Zewdie and Bosland 2000). The content of capsaicinoids in a given cultivar may differ by as much as 90 mg per fruit depending on environmental factors (Kozukue et al. 2005). The capsaicin is produced by the glands at the junction of placenta and the pod wall, distributed unevenly throughout the pod and found maximum in placental tissues.

This study was aimed to develop and validate a selective and sensitive HPLC-Breeze system equipped analytical method, allowing the identification and accurate quantification of nordihydrocapsaicin, capsaicin and dihydrocapsaicin, the three major capsaicinoids in *Capsicum* fruits. The method would be particularly suitable to analyze capsaicinoids and may be used to determine the concentrations of other minor

Fig. 1 Structure of capsaicinoids and its molecular weight

Common name	Structure	Mol. wt.
Nordihydrocapsaicin		291
Capsaicin		303
Dihydrocapsaicin		305
Homocapsaicin		317
Homodihydrocapsaicin		319

capsaicinoids. So far, no reports on the determination of capsaicinoids in *Capsicum* fruits in respect of different sowing time and spacing was done.

Materials and methods

Experimental site and treatments

The experiment was laid out in the Horticultural Farm, Department of Horticulture, Assam Agricultural University, Jorhat, India during the year 2007–2009. The experimental site was situated at 26° 47' N latitude, 94° 12' E longitude and at an altitude of 86.6 m above sea level. The topography of land was uniform. Jorhat experiences a sub-tropical climate with hot humid summer and relatively dry and cool winter. The mean monthly rainfall is about 160.93 mm unevenly distributed throughout the year. The pre-monsoon showers commences from mid March and normal monsoon rain starts from June and continues upto September. Generally from October onwards, the rainfall intensity decreases and it reaches the minimum during December and January. Temperature variation is not extreme, the maximum being around 32.90 °C during summer and minimum around 9.8 °C during winter. The meteorological data during the period of experimentation are presented in Fig. 2. The soil of the experimental area was characterized by acidic in nature.

The variety taken for the experiment was the local cultivar of Assam known as Bhoot Jolokia. The seeds were obtained from All India Coordinated Research Project on Vegetable Crops, Assam Agricultural University, India. In the nursery bed, seedlings were raised under the plastic tunnels and 60–70 days old seedlings (sown in 4 different

date) were selected and transplanted in individual plots at 4 different dates viz., September (P₁), October (P₂), January (P₃), February (P₄) with 3 spacing of 75 cm×75 cm (S₁), 90 cm×90 cm (S₂) and 105 cm 105 cm (S₃). Timely inter-cultural operations were carried out to loosen the soil, as well as to control the weeds taking care not to inflict much damage to the roots. To maintain soil moisture, irrigation was provided during the crop period as per the recommendation. To protect the crop from insects 4 sprays of Malathion 50EC 1.5 ml/lit of water at 15 days interval during vegetative stage was given. To control rotting, Bavistin 0.5 % + Streptocycline 200 ppm was sprayed 4 times alternatively at 15 days interval during the fruiting period. Liming was done at the rate of 1.7 t/ha 30 days before sowing as soil was acidic in nature. Fertilizer used was Farm Yard Manure: 10 t/ha, nitrogen: 120 kg/ha, phosphorus: 60 kg/ha and potash 60 kg/ha. All other cultural practices were uniform for all the planting times.

Biochemical parameters

Fruits at mature ripe stage were picked from the each treatment and were dried in oven for 48 hours at 60 °C and brought to the laboratory for chemical analysis of the following parameters.

Scoville organoleptic test

It is a subjective dilution-taste procedure in which water mixed with sugar (sucrose) is added to the extract (blended pure ground chillies) and given to the human subjects to taste the sample for pungency. Usually, there are five people who taste the sample. To achieve the rating, three people out

Fig. 2 Meteorological data of the experimental site

of five must agree on the taste. If there is still pungency found, then the samples are further diluted with solution of alcohol and sweetened water until there is no heat felt by the tasters. The pungency (or heat factor) of chilli peppers is measured in multiples of 100 units. One part of chilli “heat” per 1,000,000 drops of water is rated at only 1.5 Scoville Units. 2 g of material was weighed and mixed with 50 ml of ethyl alcohol. The mixture was allowed to stand for 24 hours at room temperature, with occasional shaking. Serial dilution of clear supernatant with 5 per cent solution of sugar (sucrose) in distilled water was prepared. Then 5 ml diluted solution was swallowed and the presence or absence of a distinct pungency in the throat or mouth was noted (Scoville 1912).

Capsaicin content

The capsaicin content was determined with HPLC (High Performance Liquid Chromatography) using the AOAC Official Method 995.03 (AOAC 1995).

Sample preparation

2.00 g of ground chilli powder was extracted in warm ethanol for 3 hour and dissolved in 1 ml of acetone and finally volume was made upto 25 ml using ethanol. Extract was filtered through sample clarify kit (centrifugal filtration device) equipped with 0.45 mm PTFE Membrane (Pall Corp) and injected into the liquid chromatography.

High performance liquid chromatography (HPLC) condition

Separation of the capsaicinoid was accomplish on a HPLC—Breeze system equipped with a) Uni HPLC pump (Waters 600), b) Symmetry C₁₈ column 300×4.6 mm and packed with

5 µm particles, c) Dual λ absorbance detector (Waters 2489) with detection at 280 nm. The mobile phases (isocratic type) were A) 1 percent Acetic acid water and B) Acetonitrile (40:60 ratio). The flow rate was 1.5 ml/min with an injection volume of 20 µl of sample solution at ambient temperature.

Individual components are identified on the basis of retention time (RT) of sample with those of standard. Identification of nordihydrocapsaicin, Capsaicin and dihydrocapsaicin was done by comparing the retention time. Peak 1 at retention time 8.036 min identifies nordihydrocapsaicin, peak 2 at 8.732 min identifies capsaicin and peak 3 at 13.347 min identifies dihydrocapsaicin (Fig. 3).

Standard Solution

8-Methyle-N-Vanillyle-6-Nonenamide standard ((98 %, catalog no. M2028) was taken as standard capsaicin from Sigma Chemical Co. (New Delhi, India). Other component contains approximately 30 % of dihydrocapsaicin and other naturally occurring capsaicinoids. 0.01 mg/ml of standard capsaicin solution was taken for analysis.

Calculation

Capsaicinoid contains 3 major compounds Nordihydrocapsaicin (N), Capsaicin (C) and Dihydro capsaicin (D). The capsaicinoid content was calculated as sum of these compounds (i.e., N+C+D) in Scoville Heat Units (SHU). N, C and D are calculated with the following formula in UV- detection:

$$\text{Microgram total capsaicinoid/g} = 16 \text{ SHU}$$

For ground sample –

$$N = (P_n/P_s) \times (C_s/W_t) \times (200/0.98) \times 9300$$

Fig. 3 HPLC chromatogram showing separation of capsaicinoids in standard 0.01 mg/ml. Peaks: 1, Nordihydrocapsaicin (N); 2, Capsaicin (C); 3, Dihydrocapsaicin (D) Conditions: C₁₈ column; 300×4.6 mm; column temp, 30 °C; mobile phase; acetonitrile:1 % acetic acid ; detector; UV at 280 nm; flow rate; 1.5 ml/min

$$C = (P_c/P_s) \times (C_s/W_t) \times (200/0.89) \times 16100$$

$$D = (P_d/P_s) \times (C_s/W_t) \times (200/0.93) \\ \times 16100(\text{AOAC 1995})$$

Where

P_n , P_c and P_d average peak areas for nordihydrocapsaicin, capsaicin and dihydrocapsaicin respectively, from duplicate injections
 P_s average peak area of standard solution
 C_s concentration of standard solution (mg/ml)
 W_t weight of test sample (g)

Statistical analysis

The data obtained from different observations during field experiment (randomised block design with three replications) were subjected to the analysis of variance. Significance and non-significance of the variance due to different treatments were determined by calculating the respective F values (Panse and Sukhatme 1967). The significance of difference between mean values of the characters of the treatments was tested by computing critical difference (CD).

Results and discussion

Pungency rating

Scoville organoleptic test

The first method developed to assess the pungency of fruits was the Scoville organoleptic test (Scoville 1912) which estimates pungency (total capsaicinoid concentration) from the burning mouth sensation, using diluted samples and a tasting panel of five individuals. This test is subjective and does not permit the determination of the levels of individual capsaicinoids. Table 1 shows the capsaicinoid contents of the sample obtained by Scoville Organoleptic Test. Capsaicinoid content was highest in September 15 sowing (P_1) of 553889.0 SHU and the lowest was recorded in February 15 sowing (P_4) i.e. 302777.8 SHU which is closely to the P_3 (January 15) sowing. Influence of spacing on capsaicinoid content showed non significant. The capsaicinoid content was highest in the September 15 sowing and was decreased as the date of sowing delayed.

Among spacing also higher the density lower the capsaicinoid but highest was recorded in wider spacing. This might be due to the reason that fruit with thin skin contains

Table 1 Pungency rate of capsaicin content in Bhoot Jolokia by Scoville organoleptic test and HPLC method

Treatment	Scoville Organoleptic Test	HPLC method	
	SHU	SHU	µg g ⁻¹
Sowing time			
P ₁	553,889.0	760,855	47,553
P ₂	422,777.7	448,871	28,054
P ₃	323,333.3	362,769	22,672
P ₄	302,777.8	394,036	24,626
S.Ed(±)	10,137.2	16,354.9	
CD (0.05)	21,023.4	33,918.1	
Spacing			
S ₁	393,749.9	493,172	30,822
S ₂	395,833.4	485,578	30,348
S ₃	412,500.1	496,148	31,008
S.Ed(±)	8,779.1	14,163.8	
CD (0.05)	NS	NS	
Interaction			
P ₁ X S ₁	553,333.0	778,779	48,673
P ₁ X S ₂	541,667.0	733,639	45,852
P ₁ X S ₃	566,667.0	770,149	48,134
P ₂ X S ₁	410,000.0	442,916	27,682
P ₂ X S ₂	425,000.0	449,986	28,124
P ₂ X S ₃	433,333.3	453,711	28,356
P ₃ X S ₁	311,666.7	357,707	22,356
P ₃ X S ₂	325,000.0	364,984	22,811
P ₃ X S ₃	333,333.3	365,617	22,851
P ₄ X S ₁	300,000.0	393,287	24,580
P ₄ X S ₂	291,666.7	393,705	24,606
P ₄ X S ₃	316,666.7	395,117	24,694
S.Ed(±)	17,558.2	28,327.6	
CD (0.05)	36,413.5	58,747.8	

P_1 -September 15 sowing; P_2 -October 15 sowing; P_3 -January 15 sowing; P_4 -February 15 sowing; S_1 -75 cm×75 cm; S_2 -90 cm×90 cm; S_3 -105 cm×105 cm; SHU- Scoville Heat Unit; $n=9$

higher capsaicinoid content as compared to the fruit with thick skin (Deb et al. 1963). More number of seeds and bigger size was obtained in September sowing and wider spacing. Bigger size fruit had thin skin but smaller size had thicker skin. It is also reported that the dissepimental tissue content is directly proportional to the capsaicinoid content (Ramanujan and Tirumalachar 1966). Bigger fruit size contains larger dessipiment and more number of seeds. Interaction effects of P_1S_3 were recorded the maximum capsaicinoid (566667.0 SHU) which was at par with P_1S_2 content while the lowest capsaicinoid (291666.7 SHU) content was recorded in interaction effects of P_4S_2 which was at par with P_4S_1 .

Table 2 Individual Capsaicinoid content of the Bhoot Jolokia fruit

SAMPLE	N		C		D	
	SHU	$\mu\text{g g}^{-1}$	SHU	$\mu\text{g g}^{-1}$	SHU	$\mu\text{g g}^{-1}$
Sowing time						
P ₁	10,153	676	594,764	39,680	287,834	19,188
P ₂	8,591	572	380,576	25,371	89,270	5,951
P ₃	5,915	393	274,856	18,396	55,711	3,713
P ₄	7,745	516	76,833	5,122	94,276	5,840
S.Ed($\pm$)	81.69		6,014.08		645.02	
CD (0.05)	169.41		12,472.45		1,337.70	
Spacing						
S ₁	7,887	525	348,436	23,283	76,848	4,789
S ₂	8,360	557	402,095	26,805	75,120	5,007
S ₃	6,185	536	402,991	26,865	85,100	5,673
S.Ed($\pm$)	70.74		5,208.35		558.61	
CD (0.05)	146.71		10,801.46		1,158.48	
Interaction						
P ₁ X S ₁	9,297	619	491,388	32,759	279,730	18,648
P ₁ X S ₂	11,087	739	644,458	42,963	290,622	19,374
P ₁ X S ₃	10,077	671	648,446	43,229	293,151	19,543
P ₂ X S ₁	7,597	506	359,252	23,950	78,093	5,206
P ₂ X S ₂	8,990	599	392,229	26,148	78,093	5,206
P ₂ X S ₃	9,186	612	390,249	26,016	111,625	7,441
P ₃ X S ₁	6,660	444	263,375	17,778	57,004	3,800
P ₃ X S ₂	5,528	358	281,074	18,738	52,227	3,481
P ₃ X S ₃	5,558	370	280,120	18,674	57,903	3,860
P ₄ X S ₁	7,995	533	76,336	5,087	95,959	5,063
P ₄ X S ₂	7,837	522	76,072	5,071	94,091	6,272
P ₄ X S ₃	7,404	493	78,093	5,206	92,780	6,185
S.Ed($\pm$)	141.49		10,416.70		1,117.22	
CD (0.05)	293.43		21,602.92		2,316.97	

P₁ -September 15 sowing; P₂ -October 15 sowing; P₃ -January 15 sowing; P₄ -February 15 sowing; S₁ -75 cm×75 cm; S₂ -90 cm×90 cm; S₃ -105 cm×105 cm; N-Nordihydrocapsaicin; C-Capsaicin; D-Dihydrocapsaicin; SHU- Scoville Heat Unit; (n=9)

Fig. 4 HPLC chromatogram showing different peaks of capsaicinoids of Bhoot Jolokia. Peaks: 1, Nordihydrocapsaicin (N); 2, Capsaicin (C); 3, Dihydrocapsaicin (D). Conditions: C₁₈ column; 300×4.6 mm; column temp, 30 °C; mobile phase; acetonitrile:1 % acetic acid ; detector; UV at 280 nm; flow rate; 1.5 ml/min

Capsaicinoid content measured by HPLC

The result from the Table 1 shows the capsaicinoid content of the sample obtained by HPLC. The highest level of capsaicinoid content of the sample was recorded in September 15 sowing (P_1) of $47,553 \mu\text{g g}^{-1}$ and the lowest amount was recorded in January 15 sowing (P_3) i.e. $22,672 \mu\text{g g}^{-1}$. It is apparent that September 15 sowing contains more capsaicinoid as compared to January 15 and February 15 sowing. In the present investigation, capsaicinoid contains was the highest in the early sowing due to bigger fruit size and more number of seeds which ultimately produce larger dessipiment compared to smaller fruit which are produced by late planting February and January 15 sowing. Generally it is agreed that the dessipiment contains the highest capsaicinoid among all parts of the chilli fruit (Purseglove et al. 1981). Data showed that there was no significant difference among spacing. But the highest capsaicinoid ($31,008 \mu\text{g g}^{-1}$) was recorded in S_3 ($105 \text{ cm} \times 105 \text{ cm}$). Interaction effects due to sowing time cum spacing were found to be significant influence on capsaicinoid content. P_1S_1 was recorded the highest capsaicinoid ($48,673 \mu\text{g g}^{-1}$) content which was at par with P_1S_3 . While the lowest capsaicinoid ($22,356 \mu\text{g g}^{-1}$) content was recorded in P_3S_1 which was at par with P_3S_2 .

Table 2 shows the chromatographic separation of capsaicinoids of the sample and Fig. 3 shows the standard separation and Fig. 4 shows the capsaicinoids content of Bhoot Jolokia under HPLC. The highest amount of nordihydrocapsaicin, capsaicin and dihydrocapsaicin were recorded in September sowing (P_1) of $676 \mu\text{g g}^{-1}$, $39,680 \mu\text{g g}^{-1}$ and $19,188 \mu\text{g g}^{-1}$ while the lowest nordihydrocapsaicin and dihydrocapsaicin was recorded in January sowing (P_3) of $393 \mu\text{g g}^{-1}$ and $3,713 \mu\text{g g}^{-1}$ which was at par with February sowing (P_4) but lowest capsaicin ($5,122 \mu\text{g g}^{-1}$) was found in February sowing (P_4). Among spacing the highest amount of capsaicin and dihydrocapsaicin was recorded in S_3 ($105 \text{ cm} \times 105 \text{ cm}$) of $26,865 \mu\text{g g}^{-1}$ and $5,673 \mu\text{g g}^{-1}$ while the lowest was recorded in S_1 ($75 \text{ cm} \times 75 \text{ cm}$) of $23,283 \mu\text{g g}^{-1}$ and $4,789 \mu\text{g g}^{-1}$. But in case of Nordihydrocapsaicin, S_2 ($90 \text{ cm} \times 90 \text{ cm}$) was found to be highest ($557 \mu\text{g g}^{-1}$) and the lowest was recorded in S_1 ($75 \text{ cm} \times 75 \text{ cm}$) i.e. $525 \mu\text{g g}^{-1}$. In case of interaction effect, September sowing (P_1) with spacing of $90 \text{ cm} \times 90 \text{ cm}$ (S_2) shows highest content of nordihydrocapsaicin ($739 \mu\text{g g}^{-1}$), but highest capsaicin ($43,229 \mu\text{g g}^{-1}$) and dihydrocapsaicin ($19,543 \mu\text{g g}^{-1}$) was found in P_1S_3 . Lowest capsaicin was found in P_4S_2 and dihydrocapsaicin in P_3S_2 . In the present investigation, the pungency of Bhoot Jolokia fruits sown in September 15 was 760,855 SHU (Table 1) which is about 1.2 times higher than Red Savina Habanero which has a pungency of 577,000 SHU (Mathur et al. 2000) and about 0.8 times lower than Tezpur chilli which had a pungency level of 855,000 SHU. In case of later sowing was found that 0.7,

0.4 and 0.6 times lower than Red Savina Habanero and about 0.5, 0.3 and 0.4 times lower than Tezpur chilli.

Conclusions

The crops which are sown in September and with a spacing of $105 \text{ cm} \times 105 \text{ cm}$ showed better performance for all the parameters studied. The present study could conclude September 15 to be the optimal date of sowing as concerning environmental temperature and rainfall. The productivity shown for 15 September sowing is much higher than the ones produced traditionally. Scoville organoleptic test and HPLC method showed highest amount of capsaicinoid content in the fruits produced from a spacing of $105 \text{ cm} \times 105 \text{ cm}$ and the crops sown in September 15. Sowing on earlier dates may not be effective as the crop is sensitive to high rainfall which is prevalent situation in north eastern part of India. The 15 September sowing can be considered basis for studying the effects of other agronomic practices.

References

- Anisuzzaman M, Rahman AHMM (2007) An ethnobotanical study of Madhupur, Tangail. *J Appl Sci Res* 3:519–530
- AOAC (1995) AOAC Official Method 995.03 Capsaicinoids in capsicums and their extractives Liquid chromatographic method. http://www.pinkstinker.com/docs/AOAC_Official_Method_995.pdf. Retrieved November 16, 2008
- Bennett DJ, Kirby GW (1968) Constitution and biosynthesis of capsaicin. *J Chem Soc C* 442
- Bhagowati RR, Changkija S (2009) Genetic variability and traditional practices in naga king chili landraces of Nagaland. *Asian Agri-Hist* 13:171–180
- Deb AR, Ramanujam S, Krishnamurthy GSR, Tirumalachar DK (1963) Capsicum content of some new pure varieties of chilli. *Ind J Technol* 1:59–60
- Estrada B, Pomar F, Dr'az J, Merino F, Bernal MA (1998) Effects of mineral fertilizer supplementation on fruits development and pungency in "Padro'n" peppers. *J Hortic Sci Biotechnol* 73:493–497
- Govindarajan VS, Sathyanarayana MN (1991) Capsicum-production, technology, chemistry, and quality. Part V Impact on Physiology, Pharmacology, Nutrition, and Metabolism; Structure, Pungency, Pain, and Desensitization Sequences. *CRC Crit Rev Food Sci Nutr* 29:435–473
- Guinness Book of World Records (2006) Hottest chilli. http://en.wikipedia.org/wiki/Bhoot_Jolokia_chili_pepper. Retrieved February 20, 2011
- Hoffman PG, Lego MC, Galetto WG (1983) Separation and quantitation of red pepper major heat principles by reverse-phase high pressure liquid chromatography. *J Agric Food Chem* 31:1326–1330
- IBPGR (1983) Genetic resources of capsicum. International Board for Plant Genetic Resources, Rome
- Kozukue N, Han J, Lee S, Kim J, Lee K, Park M, Levin CE, Friedman M (2005) Analysis of seven capsaicinoids in peppers and pepper-containing foods by liquid chromatography-mass spectrometry. *J Agric Food Chem* 53:9172–9181
- Lindsey K, Bosland PW (1995) A field study of environmental interaction on pungency. *Capsicum Eggplant Newsl* 14:36–38

- Mathur R, Dangi RS, Das SC, Malhotra RC (2000) The hottest chilli variety in India. *Curr Sci* 79:287–288
- Mori A, Lehmann S, O’Kelly J, Kumagai T, Desmond JC, Pervan M, McBride WH, Kizaki M, Koeffler HP (2006) Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. *Cancer Res* 66:3222–3229
- Panse VG, Sukhatme PV (1967) Statistical method of agricultural workers (2nd edited). ICAR, New Delhi
- Prasad NBC, Shrivastava R, Ravishankar GA (2005) Capsaicin: a promising multifaceted drug from *Capsicum* spp. *Evid Base Integr Med* 2:147–166
- Purseglove JW, Brown EG, Green CL, Robbies SRJ (1981) Spices, vol I. Longman, London
- Ramanujan S, Tirumalachar DK (1966) Component analysis of *Capsicum* content of chilli. *Ind J Genet* 26:227–9
- Sanatombi K, Sharma GJ (2008) Capsaicin content and pungency of different *Capsicum* spp. cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 36:89–90
- Scoville WL (1912) Note capsicum. *J Am Pharm Assoc* 1:453
- Shobana S, Naidu KA (2000) Antioxidant activity of selected Indian spices. *Prostaglandins Leukot Essent Fatty Acids* 62:107–110
- Sukrasno N, Yeoman MM (1993) Phenylpropanoid metabolism during growth and development of *Capsicum frutescens* fruits. *Phytochemistry* 32:839–844
- Suzuki T, Kawada T, Iwai K (1981) Biosynthesis of acyl moieties of capsaicin and its analogues from valine and leucine in *Capsicum* fruits. *Plant Cell Physiol* 22:23–32
- Szolcsanyi J (2003) Future perspectives of capsaicin research. In: De, A.K. (Ed.), *The Genus Capsicum*. Taylor and Francis London pp 248–269
- Yu R, Choi M, Kawada T, Kim B, Han S, Yoo H (2002) Inhibitory effect of capsaicin against carcinogen-induced oxidative damage in rats. *Int J Food Sci Nutr* 7:67–71
- Zewdie Y, Bosland PW (2000) Evaluation of genotype, environment, and genotype-by-environment interaction for capsaicinoids in *Capsicum annuum* L. *Euphytica* 111:185–190