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Genetic variability based on Morphological and Biochemical characterization of Naga King Chilli Genotypes (*Capsicum chinense* Jacq.)

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ABSTRACT: The state of Nagaland has tremendous diversity for Naga king chilli. With a view to characterize different genotypes morphologically and biochemically the present study was conducted for two consecutive years with 11 Naga King chilli (*Capsicum chinense* Jacq.) genotypes procured from different parts of Nagaland. The experiment was conducted at the research farm of department of Genetics and Plant Breeding, Nagaland University, SASRD, Medziphema during February-October 2018-2019. It is also known as world's hottest chilli. The principal ingredient Capsaicin is the main biochemical constituent is responsible for hotness. Naga King Chilli besides being used as a spice and a food additive, has a wide spectrum of application including, pain therapy, anti-obesity, anticancer, antioxidant, antimicrobial, anti-diabetic, relieving arthritis, frostbite, respiratory ailments, lachrymatory agent *etc*. The fresh fruit also have significant amount of vitamin B, vitamin C, vitamin E and pro vitamin A. In the present investigation sixteen genotypes of king chilli from different regions of Nagaland, India have been collected and analyzed for beta-carotene, ascorbic acid content, moisture content and capsaicin content. The result indicated that beta-carotene (0.58-4.88 mg/100g), ascorbic acid contents (154.67-198.00 mg/100g), moisture content (80.97-85.20 percent) and capsaicin content (0.61-1.96) ranged between the eleven genotypes and showed highly significant among them.

Keywords: Ascorbic acid, Beta-carotene, Capsaicin and King chilli.

INTRODUCTION

In India, Naga King Chilli is appraised as India's hottest chilli and was previously regarded as the world's hottest chilli having Scoville heat units (SHU's) rating of 1,001,304 (Bosland and Baral, 2007). The pungent principle of chilli fruit is capsaicinoids, a family of compounds that give them the characteristic pungent taste. The chilli was recorded to be the hottest chilli in the world in 2006 with a Scoville heat unit (SHU) rating of 1,001,304. Currently it occupies the fifth position among the hottest chillies in the world. The fruits are also sources of vitamins A, complex B1 and B2 and minerals such as dietary calcium, iron and phosphorus. The content of vitamin C in the Capsicum fruit is higher than in Citrus. The moisture estimation in king chilli was 86.75 ±0.82 in green stage and 83.26 ±0.56 in red stage (mature) (Malakar, 2019). This chilli is grown mainly in the state of Nagaland, Assam and Manipur and to some extent in Mizoram, Arunachal Pradesh and Meghalaya. It is also cultivated in the north eastern region of Bangladesh (Bhuyan *et al*., 2015). Because of its commercial importance, the Nagaland Government obtained the Geographical Indication (GI) of Goods tag for Naga King Chilli in the year (Registration and Protection) Act 1999, to provide some safety net to Naga farmers in the cultivation of the King Chilli. Nagaland Government has obtained the GI rights for this product in 2008.

King Chilli (*Capsicum chinense* Jacq.) is grown widely in North Eastern Region of India. The chromosome number of king chilli is 2n=24 and it is a self-pollinated plant; however, considerable cross pollination (upto 10 percent) may occur when insect population is high. The word "Capsicum" was possibly derived from Greek word "Kepso" meaning "to bite" with reference to the dominant pungency stimulated by the spice chilli. A number of chilli variants in the north-eastern region of India (Kumar *et al*., 2011) are

distinguished with different local names such as Naga chilli in Nagaland, Bhut Jolokia in Assam and U-Morok in Manipur (Sanatombi *et al*. 2010; Verma *et al*. 2013). It was first introduced by the Portuguese towards the end of 15th Century (Indira *et al*., 2007). Naga King Chili (*Capsicum chinense* Jacq.) was certified as "the World's Hottest Chilli" by the Guinness World Records in September 2006, measuring 855,000 scoville heat units (SHUs), beating the "Mexican red savanna habaneros" (5,77,000 SHUs) (Sharma, 2014). Officially it was declared as the hottest of all spices on 9th September 2006 by Guinness world record limited. (Kalita, 2007). The King chilli with its high pungency renders an immense scope both in domestic and international market. Though, it has been reported that about 1000 tonnes of King chilli is produced every year in North Eastern region, however in Nagaland no authenticated estimates are available as the crop is cultivated in an unorganized sector. The amount of capsaicinoids in a chili pepper is dependent upon the genetic makeup of the plant and the environment where it is grown Zewdie and Bosland, (2000). King Chili is also known for its richness in ascorbic acid, avery essential antioxidant for human nutrition and proper functioning of body (Igwemmar *et al*., 2013).

Keeping in mind that Naga king chilli is a potential crop for North Eastern region of India for domestic and export purpose, an attempt has been made to study the morphological and biochemical characterization of Naga king chilli genotypes.

MATERIALS AND METHODS

The present investigation was conducted for two growing Seasons *i.e.* 2018 and 2019 under open field condition located in the Experimental Farm of Genetics and Plant Breeding NU: SASRD. The experiment was conducted in Randomized Block Design (RBD) with three replications accommodating 12 plants in each plot of (3 × 2) m^2 with a spacing of 75 cm between the plants and rows. The experimental materials in the present study comprise of eleven genotypes of Naga King Chilli procured from different hotspot growing locations in Nagaland. The particulars of the landraces are presented in Table 1. The observations were recorded for the characters such as Stem colour, plant growth habit, branching habit, leaf size, leaf shape, leaf colour, ripe fruit colour, fruit shape, anther colour, seed colour and fruit surface were recorded for each landrace (Table 2).

Table 1: Specification of genotypes.

Code	Placeofcollection	District
T1	Azailong	Peren
T2	Pongkong	Mon
T3	Medziphema	Dimapur
T4	Tenyiphe	Dimapur
T5	Deukoram	Peren
T6	Peren	Peren
T7	ChÜmoukedima	Dimapur
T8	Jalukie	Peren
T9	Sochunoma	Dimapur
T10	Athibung	Peren
T11	Thekrejuma	Kohima

Beta-carotene (mg per 100 g). The Beta–Carotene was determined by the method described by Ranganna (1997). The sample of 2 g of fresh ripen Bhut jolokia fruits was taken and crushed with acetone and decanted into conical flask and continued the extraction till the residue was colourless. The collected extract was then transferred into a separating funnel and 25-30 ml of petroleum ether (60-80%) was added with 5-10 ml of 5 % $(Na)_2SO_4$ petroleum ether was repeatedly used until all the colour got transferred into the petroleum ether layer. Volume was made upto 25 ml with petroleum ether and colour intensity was measured at 460 nm using a spectrophotometer. The ß- Carotene content was calculated using the following formula.

$$\text{Carotene (mg/100mg)} = \frac{3.87 \times \text{Volume made up}}{\text{Weight of the sample}}$$

Ascorbic acid (mg per 100 g). Ascorbic acid was determined by using 2, 6-Dichlorophenol indophenols dye (Fred and Walter 1996). The sample of 5 gram was taken and volume was made upto 50 ml with 4% oxalic acid. 10 ml of filtrate was mixed with 10 ml of 4% oxalic acid and titrated against the standard dye. Amount of ascorbic acid was calculated and expressed as mg/100g.

Moisture content (percentage). The moisture of the sample represents the most abundant constituent *i.e.* the water ranging from nearly 50–90% of the total chemical composition and is expressed in percentage (%). The moisture content on fresh weight basis was determined by drying to constant weight with the help of moisture meter/Oven drying method. The estimation was done in triplicate and the mean of the three estimations was recorded as percentage of moisture content. Moisture was determined by drying a representative of five grams per sample in an oven with air circulation at 100-105°C for six hours and the percentage was calculated where MC (moisture content), IW (initial weight) and DW (dry weight).

$$MC = \frac{IW - DW}{DW} \times 100$$

Capsaicin (mg per 100 g)

Capsaicinoids extraction for uHPLC. Fully matured fresh fruits of *Capsicum chinense* Jacq. was dried in oven (Yorco hot air sterilizer) at 60˚C for two hours and crushed into powder using mortal pestle. Ethanol solvent was used for capsaicinoids extraction for the preparation of chilli extract. For preparation of extract, two grams of dried chilli powder was dissolved in 4 mL of ethanol solvent and kept in water bath at 80˚C for three hours, manually inverted after every hour. The samples were then kept in room temperature for cooling. The supernatant layer of each sample was filtered through Nylon 33mm0.45 µm filter (Axiva Schem. Pvt. Ltd.) The filtered extract was then stored at 4˚C.

High Performance Liquid Chromatography analysis. uHPLC Ultimate 3000 (Thermo scientific) system equipped with a Finnigan Surveyor Auto Sampler Plus, a Finnigan Surveyor LC Plus quaternary pump and a surveyor photodiode array (PDA) detector was used for uHPLC analysis. Betasil C_{18} column (particle size 3µm, dimension 150 × 4.6 mm) from Thermo Scientific was used. The column temperature was maintained at 60˚C, sampler temperature was at 20˚C and sample volume: 5 µl. Binary mixture of water- acetonitrile at 50:50 ratio was used as mobile phase and the flow rate was 1.5 ml/min resulting in a total run time of 15 min per injection. UV detection wavelength was set as 222 nm. The standard solutions of 20, 40, 60, 80, 100 µg/g of 21750- 100MG-F Capsaicin (Sigma, Life Sciences) was used to prepare calibration curve by injecting in triplicate the five - increasing concentrations of standard.

Capsaicinoid quantification. The major capsaicinoids were determined by comparing the samples with that of external reference standards which were run under the same conditions as that of samples. On the basis of retention times identification was done while the peak areas were used for quantitative determination. The concentrations of capsaicinoid in the sample are expressed as percentage.

Statistical Analysis. Statistical analysis of the data was carried out by using Indo Stat and for significant results mean was done using LSD at 5%.

RESULTS AND DISCUSSION

Eleven qualitative characters were deliberated based on phenotypic observations during the growing season as shown in Table 2.

Beta-Carotene (mg per 100g). Highly significant differences were observed for Beta-Carotene. The Beta-Carotene per plant was recorded highest in genotype T1 (4.88) mg per 100g followed by the genotype T9 (4.17) mg per 100g and T3 (3.67) mg per 100g, while the lowest was observed in genotype T4 (0.58) mg per 100g followed by T11 (1.35) mg per 100 g and T10 (1.49) mg per 100g. The general mean for Beta-carotene was recorded 2.51 mg per 100g. Ngozi *et al.* (2020) also found similar findings while studying ten genotypes of pepper (*Capsicum annuum* L.) and according to the nutrients analyzed, total beta-carotene ranged from 4-7 mg per 100 g.

Ascorbic acid (mg per 100g). Significant differences were found for Ascorbic acid. The Ascorbic acid per plant was recorded highest in genotype T8 (198.00) mg per 100g followed by the genotype T7 (196.00) mg per 100g and T11 (193.00) mg per 100 g, while the lowest was observed in genotype T9 (154.67) mg per 100g followed by T6 (169.67) mg per 100 g and T10 (175.00) mg per 100 g. The general mean for Ascorbic acid was recorded 181.15 mg per 100g.Similar findings have been obtained by Orobiyi *et al.* (2015) while studying in 22 high yielding chili pepper landraces of northern Benin, the ascorbic acid (vitamin C) content varied from 84.64mg to 192.64 mg per 100g of fresh weight with an average of 125.70 mg.

Moisture content (percent). Significant differences were found for Moisture content. The moisture content per fruit of the plant was recorded highest in genotype T7 (85.20) per cent followed by the genotype T9 (84.40) per cent and T10 (84.23) per cent. The lowest was recorded in genotype T4 (80.97) per cent. The general mean was recorded 83.56 per cent. Similar results have been obtained by Malakar (2019) which reported that composition of king chilli the moisture estimation was 86.75 ±0.82 in green stage and 83.26 ±0.56 in red stage (mature).

Capsaicin content (percent). Significant differences were recorded for the capsaicin content. The highest capsaicin content was recorded in the genotype T7 (1.96) per cent followed by the genotype T5 (1.77) per cent and T11 (1.74) per cent. The lowest was recorded in the genotype T4 (0.61) per cent followed by T1 (0.87) per cent and T11 (1.74) per cent. The general mean for capsaicin content was 1.36 per cent. Our results have some similarity with Mena *et al.* (2018) for capsaicin content while studying sixteen genotypes of king chilli from different states of North East India were collected and analyzed to quantify their dry fruit yield, ascorbic acid contents, capsaicin content, α-Carotene and ß-carotene. The result indicated that dry fruit yield (0.01-0.04 kg/plant), ascorbic acid contents (92.07-301.11 mg/100g), capsaicin content (0.75-4.65 %).

Table 2: Results on qualitative characters of Naga King Chilli genotypes.

Sr. No.	Qualitative characters	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	Code
1.	Stemcolour	2	1	2	1	1	1	1	1	1	1	1	1. Light green with green patch 2. Dark green with purple patch 3. Others
2.	Plantgrowthhabit	1	1	1	2	1	3	1	1	3	1	3	1. Intermediate 2. Erect 3. Prostrate 4. Others
3.	Branching habit	2	2	1	1	1	1	2	1	1	1	1	1. Intermediate 2. Sparse 3. Others
4.	Leafsize	2	2	2	2	2	2	2	2	2	2	2	1. Large 2. Medium 3. Others
5.	Leafshape	1	1	1	1	1	1	1	1	1	1	1	1. Ovate 2. Lanceolate 3. Others
6.	Leafcolour	2	1	2	2	1	1	2	1	2	2	2	1. Green 2. Dark green 3. Others
7.	Ripe fruitcolour	1	2	1	1	1	1	1	2	1	1	2	1. Red 2. Lightred 3. Others
8.	Fruitshape	1	2	1	1	1	3	2	3	1	1	2	1. Campanulate 2.Triangular 3. Elongate 4. Others
9.	Anthercolour	2	2	2	2	2	2	2	2	2	2	2	1. White 2. Purple 3. Others
10.	Seedcolour	2	2	2	2	2	2	2	2	2	2	2	1. Red 2. Yellowish 3. Others
11.	Fruitsurface	2	2	2	2	3	2	2	3	2	2	3	1. Smooth 2. Semi-wrinkled 3. Wrinkled 4. Others

Table 3: Pooled analysis of variance (ANOVA).

Source	df	Mean sum of square			
		Beta-carotene	Ascorbic acid	Moisture content	Capsaicin content
Years	1	1.55	15.52	1.64	0.01
Replication	4	0.25	1.70	1.83	0.02
Genotype	10	10.69*	929.52*	7.09*	0.93*
Year × Genotype	10	0.06^{NS}	2.58^{NS}	0.14^{NS}	0.13*
Error	40	0.18	5.83	1.36	0.02

Note: *: Significant at 5% level of significance, NS: Non-significant at 5% level of significance.

Table 4: Mean performance of Naga King Chilli for yield and yield related characters during 2018 and 2019 (Pooled).

Genotypes	Beta carotene	Ascorbic acid	Moisture content	Capsaicin content
T1	4.88	182.33	83.60	0.87
T2	1.60	185.33	82.97	1.28
T3	3.67	177.67	84.13	1.35
T4	0.58	181.33	80.97	0.61
T5	2.51	179.67	83.87	1.77
T6	1.68	169.67	83.03	1.31
T7	2.47	196.00	85.20	1.96
T8	3.18	198.00	83.13	1.53
T9	4.17	154.67	84.40	1.34
T10	1.49	175.00	84.23	1.17
T11	1.35	193.00	83.60	1.74
General mean	2.51	181.15	83.56	1.36
SEm±	0.17	0.99	0.48	0.06
CD5%	0.49	2.82	1.36	0.17
CD1%	0.66	3.77	1.82	0.23

CONCLUSION

At present due to the occurrence of genetic erosion caused by interchanging of local cultivars of Naga King Chilli with improved ones, it has become immensely necessary to conserve genetic diversity. In Naga King Chilli, a wide diversity of plant and fruit character is quite apparent, which holds eminent potential for developing high yielding varieties with desirable characters through appropriate breeding methods. The analysis of variance indicated significant differences for all the traits under study. This revealed the presence of genetic variability in the breeding material under investigation. The genotype T1 recorded the highest beta-carotene content, genotype T8 for highest ascorbic contentT7 has the highest content of capsaicin and moisture and it can be utilized for successful breeding programmes. The study on morphological and biochemical characterization of different genotypes of Naga king chilli provided a clear picture of different variants of the species on the basis of morphological and biochemical variations. The crop is commercially very important as it has got GI tag in 2008. It has tremendous export potential and its various genotypes were needed to be characterized on these important aspects. The micro-climatic variations play a crucial role on morphological and biochemical attributes of the species. The present efforts paved the path for further evaluation of various genotypes over varying micro-climatic conditions of the state of Nagaland.

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Conflict of Interest. None.

REFERENCES

Bhuyan, M. H. M. B., Rahman, S. M. L., Ara, R. and Sarker, J. C. (2015). Evaluation of naga chilli (*Capsicum chinense* Jacq.) genotypes under North eastern region of Bangladesh. *Scientia Agricola, 12*(1): 40-45.

Bosland, P. and Baral, J. (2007). 'Bhut Jolokia'—The world's hottest known chile pepper is a putative naturally occurring interspecific hybrid. *Horticultural Science, 42*(2): 222-224.

Mena, Ephilo & Ansari, Mohd & Ramjan, Md & Warade, S. (2018). Evaluation of capsaicin, Ascorbic acid, α-Carotene and ß-Carotene in Bhut Jolokia (*Capsicum chinense* Jacq.) genotypes from North East India. 93-97.

Fred, G. S. and Walter, L. N. (1996). Determination of ascorbic acid. *Industrial and Engineering Chemistry Analytical Edition, 17*(12): 754-756.

Igwemmar, N. C., Kolawole S. A. and Imran I. A. (2013). Effect of Heating on Vitamin C Content of Some Selected Vegetables. *International Journal of Scientific and Technology Research, 2*(11): 209-212.

Indira, P., Rajalekshmi, V. S. and Peter, K. V. (2007). All about capsicum spices. *Indian Spices, 34*: 10-20.

Kalita, J. (2007). Bhut Jolokia-the hottest of all species. *Spice India, 20*: 9-12.

Kumar, S., Kumar, R., Kumar, S., Singh, M., Rai, A. B., Rai, M. (2011). Incidences of leaf curl disease on Capsicum germplasm under field conditions. *Indian Journal of Agricultural Sciences, 81*: 187–189.

Malakar, S. (2019). A review on strategic production management and utility potential of king chilli (*Capsicum chinense* Jacq): The hottest chilli in India. *Academia Journal of Agricultural Research, 7*(10): 251-256.

Ngozi, E. Abu, Grace, O. Chimdi and Eugene, O. Ojua (2020). Nutritional and anti-nutritional evaluation of ten genotypes of pepper (*Capsicum annuum* L.) grown in a derived savanna ecology of Nigeria. *Ethiopian Journal of Science and Technology. 13*: 17-30.

Orobiyi, A., Ahissou, H., Gbaguidi, F., Sanoussi, F., Houngbèmè, 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. *International Journal Current Microbiology and Applied Science, 4*(9): 394-403.

Ranganna, S. (1997). Manual of analysis of fruits and vegetables products. Tata McGraw hill publication company Ltd. New Delhi, India.

Sanatombi, K., Sharma, G. J. and Mandi, S. S. (2010). DNA profiling of Capsicum landraces of Manipur. *Scientia Horticulturae, 124*(3): 405-408.

Sharma, A. (2014). Sustainable Economic Analysis and Extent of Satisfaction Level of King Chilli Growers in Nagaland. *Agriculture for Sustainable Development, 2*(1): 71-74.

Verma, P. K., Rawat, K. K., Das, N. and Pradhan, B. (2013). A botanical enigma of India's hottest chilli Bhoot Jolokia (*Capsicum chinense* Jacq). *New York Science Journal, 6*: 49–51.

Zewdie, Y. and Bosland, P. (2000). Capsaicinoid inheritance in an interspecific hybridization of *Capsicum annuum* × *C. chinense*. *Journal of the American Society for Horticultural Science, 125*(4).

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