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Evaluation of capsaicin, Ascorbic acid, α-Carotene and ß-Carotene in *Bhut Jolokia* (*Capsicum chinense* Jacq.) genotypes from North East India

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Abstract
Bhut Jolokia has great demand in the pharmaceutical industries for its richness in capsaicin, caretenoids and ascorbic acid content. Its nutritional content can vary between different genotypes and area. In the present study 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 %), α-Carotene (1.02-5.26 mg/L) and ß-carotene (0.97-4.45 mg/L) ranged between the sixteen genotypes. The genotype CHFKC-6 showed maximum content of capsaicin content and it can be utilized for pharmaceutical use. While the genotype CHFKC-1 was good for ascorbic acid content and β-Carotene. CHFKC-15 has average for ascorbic acid, α-Carotene and β-Carotene content.

Keywords: Ascorbic acid, *Bhut Jolokia*, capsaicin, α-carotene and β-carotene

Introduction
Bhut Jolokia (*Capsicum chinense* Jacq.) is the important chilli crop grown extensively under North East India. Pungency and flavour are fruit attributes of *Capsicum chinense* [1] due to the presence of capsaicin and dihydrocapsaicin, which are components of an alkaloid complex responsible for 90% of the intense organoleptic sensation of heat [2]. The main pungency principle of *Bhut Jolokia* (*Capsicum chinense* Jacq.) is capsaicin (8-methyl-N-vanillyl-6-nonenamide) and its analogs collectively known as capsaicinoids synthesized in the epidermal cells of placenta of the fruit, and possesses anti-inflammatory and antioxidant activities [3]. The fruits are also sources of vitamins A, complex B1 and B2 and minerals such as dietary calcium, iron and phosphorus [4]. The content of vitamin C in the *Capsicum* fruit is higher than in *Citrus* [5].

In 2007, Guinness World Records certified that the *Bhut Jolokia* or ghost pepper was the world's hottest chilli pepper, 401.5 times hotter than Tabasco sauce, the ghost chilli is rated at more than 1 million Scoville Heat Units (SHUs). Classic Tabasco sauce ranges from 2,500 to 5,000 SHUs. However, the *Bhut Jolokia* was shortly superseded by the infinity chilli in early 2011, followed by the Naga Viper, then later the Trinidad moruga scorpion in 2012, and finally the "Carolina Reaper" on August 7, 2013 [3].

King chilli has been used conventionally by different ethnic communities of Northeastern India in treating various human ailments. In Nagaland, *Capsicum* spp. including Naga chilli is used to tone up body muscles after heavy workouts whereas hot infusions are used for toothache and muscle pain [6]. Ethnobotanical literature around the world contains a wide array of information on traditional uses of different *Capsicum* species in treating various maladies. Considering the importance of capsaicin in human health and its commercial implications in the pharmaceutical and food industry, *Bhut Jolokia* (*Capsicum chinense* Jacq.) offers great potential for future exploitation due to its high capsaicinoid content.

Materials and Method
The experiment was carried out at polyhouse complex of College of Horticulture and Forestry, Central Agricultural University, Pasighat - 791102, Arunachal Pradesh in the year 2015-2016. The geographical location of the research farm is having an altitude of 153 m above mean sea level, latitude of 28°04`N and longitude of $95^0$22`E.

Experimental Materials

The experimental material for the present study comprised of 16 genotypes of king chilli (*Capsicum chinense* Jacq.) collected from different states of North East India (Table 1).

Tables and Figures

Table 1: List of chilli genotypes with their sources of collection

S. No	Genotype	Source
1	CHFKC-1	A landrace of Along (Arunachal Pradesh)
2	CHFKC-2	A landrace of Palin (Arunachal Pradesh)
3	CHFKC-3	A landrace of Yazali (Arunachal Pradesh)
4	CHFKC-4	A landrace of Kurungkumey (Arunachal Pradesh)
5	CHFKC-5	A landrace of Mebo (Arunachal Pradesh)
6	CHFKC-6	A landrace of Pasighat (Arunachal Pradesh)
7	CHFKC-7	A landrace of Kiyit (Arunachal Pradesh)
8	CHFKC-8	A landrace of Imphal (Manipur)
9	CHFKC-9	A landrace of Tseipama (Nagaland)
10	CHFKC-10	A landrace of Daporijo (Arunachal Pradesh)
11	CHFKC-11	A landrace of Mariyang (Arunachal Pradesh)
12	CHFKC-12	A landrace of Pasighat (Arunachal Pradesh)
13	CHFKC-13	A landrace of Dimapur (Nagaland)
14	CHFKC-14	A landrace of Mariyang (Arunachal Pradesh)
15	CHFKC-15	A landrace of Pasighat (Arunachal Pradesh)
16	CHFKC-16	A landrace of Along (Arunachal Pradesh)

Ascorbic acid content (mg/100g)

The ascorbic acid content was determined by the method described by Jagota and Dani (1982) [7]. Fruit sample (2 g) was taken into mortar and small amount of neutral glass powder was added to it. Mixture was exposed to grinding with equal volume of 6% metaphosphoric acid EDTA solution and the volume was made up to 50 ml with 3% metaphosphoric acid. The above mixture was centrifuged at 5000 rpm for 10 minutes and then filtered through Whatman No.1 filter paper and collected in volumetric flask. An aliquot of the sample extract (0.1 ml) was diluted to 1.2 ml with 3% metaphosphoric acid and final volume was made up to 4 ml with distilled water. To each tube 0.4 ml Folin-ciocalteau was added and mixed well. The tubes were incubated for 10 minutes at room temperature and centrifuged at 3000 rpm for 10 minutes. After centrifugation, supernatant was read against the blank solution in a UV-visible spectrophotometer at 760nm. The concentration of ascorbic acid in the sample was calculated from the slope of the ascorbic acid standard curve. The ascorbic acid content (mg/100g) was calculated by using the formulae:

$$\text{Vit.C (mg/100g)} = \frac{\text{Total volume of the sample x Conc.of the Vit.C x100 x 1}}{\text{Weight of sample x Amount of sample x 1000}}$$

Capsaicin content (%)

The capsaicin content was determined by the method as described by Balasubramaniam *et al.* (1982) [8]. Capsaicin content in king chilli genotypes was estimated by spectrophotometric method. Dry powdered sample (500 mg) was taken in volumetric flask and mixed with 10 ml of dry was subjected to 3 hours continuous shaking. The content was allowed to settle down and then centrifuged at 10000 rpm for 10 minutes and 1 ml of clear supernatant was taken out in a test tube and allowed to evaporate to dryness in hot water bath. Residue was dissolved in 5 ml of 0.4% sodium hydroxide solution, followed by addition of 3 ml of 3% phosphomolybdic acid. The content was shaken and allowed to stand for 1 hour. The solution was filtered and centrifuged at 5000 rpm for 10-15 minutes. Clear blue colored supernatant was read against blank solution in a UV-visible spectrophotometer at 650 nm. The concentration of capsaicin in the sample was calculated from the slope of the standard curve. The result was expressed as '%' of capsaicin content of the sample.

$$\text{Capsaicin content}(\%) = \frac{\text{mg} \times 100 \times 100}{1000 \times 1000 \times 1 \times 2}$$

Pigment Estimation (α and β- carotenoids)

α and β- carotenoids analysis was done by following the method given by Nagata *et al.* (2008) [9]. Fruit sample (1 g) was extracted in 80% acetone and mixture was centrifuged at 10,000 rpm for 10 minutes. The supernatant was read against blank solution of 80% acetone in a UV-visible spectrophotometer at 443, 475, 492 and 505 nm. The amount of α and β- carotene was worked out by using the following formula:

α- Carotene (mg/L) = 0.984 A_{443} + 3.091 A_{475} – 2.758 A_{492} – 0.299

β- Carotene (mg/L) = -1.292 A_{443} + 3.698 A_{492} + 0.131

Where,

A_{443} = absorbance at 443 nm

A_{475} = absorbance at 475 nm

A_{492} = absorbance at 492 nm

Statistical An alysis

The experiment was laid out in Randomized Block Design (RBD) with three replications. Statistical analysis of the data was carried out by using OPSTAT [10] and for significant results mean was done using LSD at 5%.

Result and Discussion

Significant variation among the sixteen King chilli genotype were observed for dry weight of fruit per plant (Fig. 1). The maximum dry fruit yield per plant was recorded in CHFKC-12 (0.039 kg/plant) followed by CHFKC-5 (0.038 kg/plant) and CHFKC-13 (0.038 kg/plant) while the minimum was recorded in CHFKC-3 (0.011 kg/plant) with population mean of 0.028 kg/plant (Fig. 1). Similarly Bhunya *et al.* (2015) [11] reported 0.094 kg/plant dry fruit weight.

Ascorbic acid content varied significantly among the different genotypes (Fig. 2). The genotype CHFKC-1 (301.11 mg/100 g) had the highest ascorbic content which

was statistically at par with CHFKC-15 (292.00 mg/100g) and CHFKC-5 (244.00 mg/100g) (Fig.2). Minimum value was recorded in genotype CHFKC-7 (92.07 mg/100g) with population mean of 176.81 mg/100g. Similarly Orobiyi *et al*. (2015) [12] reported 84.64 - 192.64 mg/100g ascorbic acid content in the fresh fruits of chilli pepper. With conformity with the present finding Teodoro *et al*. (2013) [13] also reported the vitamin C contents in the range of 54.1 to 129.8 mg/100 g in Habanero pepper accessions (*Capsicum chinense*). Kantar *et al.* (2016) [14] found 146 mg/g ascorbic acid in *Bhut Jholokia*. Campos *et al*. (2013) [15] reported ascorbic acid content in the seven studied *Habanero* genotypes which ranged from 187.24 to 281.73 mg/100 g in fruit sample.

The capsaicin content of king chilli fruit showed wide variation among genotypes. The genotype CHFKC-6 had the highest capsaicin content (3.65 %) followed by CHFKC- 10 (3.14) and CHFKC-14 (3.01) (Fig. 3). While, minimum value was recorded in CHFKC- 16 (0.75) with population mean of 1.86. Similarly Baruah *et al*. (2014) [16] reported 2.54 % capsicinoid content in *Bhut Jholokia*. With conformity with the present finding Sanatombi *et al.* (2008) [17] reported 2.06 % capsaicin content in Naga chilli. The concentration of Capsaicin and dihydrocapsaicin in Bhut Jolokia was 5.36% [18]. Concentrations of capsaicin varied among cultivars from 0.38 mg g^{-1} dry weight in Ancho sample to 4.76 mg g^{-1} dry weight in Serrano [19]. A capsaicin content of 2.45% was reported by Sarwa *et al*. (2013) [20] in *Bhut Jolokia*.

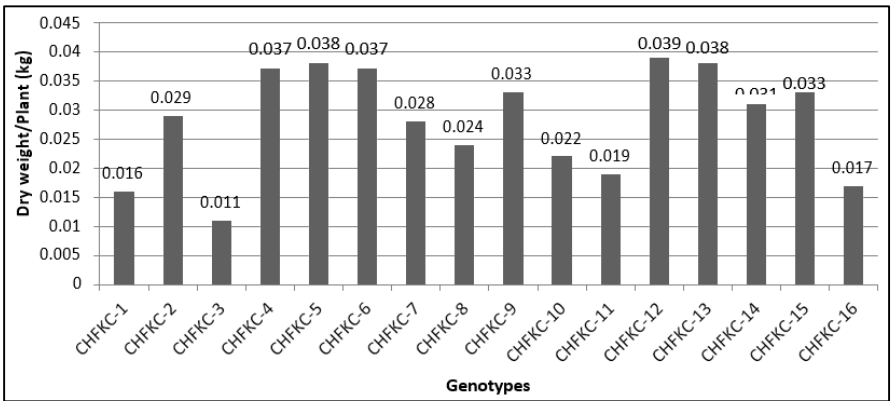

Fig 1: Dry weight per plant of sixteen King Chilli genotypes (LSD at 5% is 0.008)

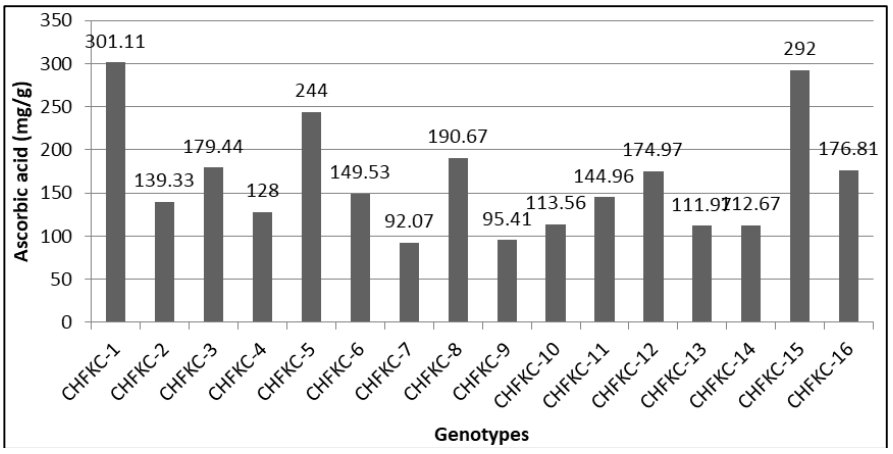

Fig 2: Ascorbic acid content of sixteen different genotypes (LSD at 5% is 46.44)

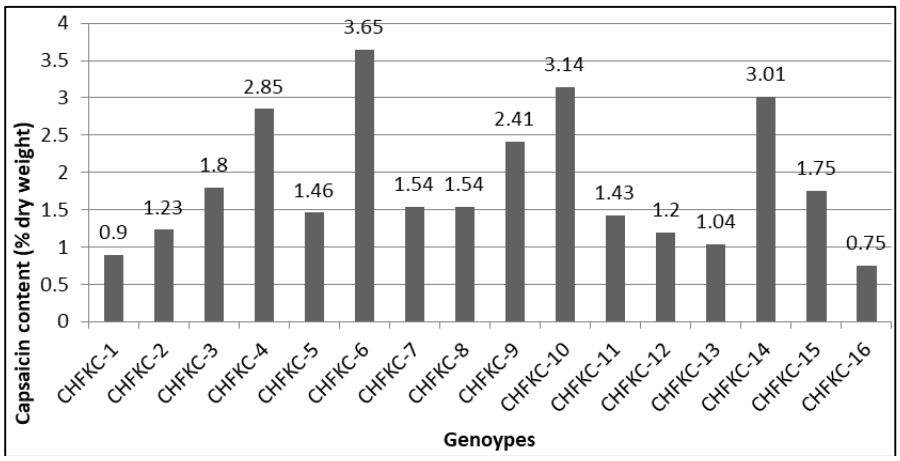

Fig 3: Figure showing capsaicin content in the different genotypes (Least Significant Difference at 5% is 0.44)

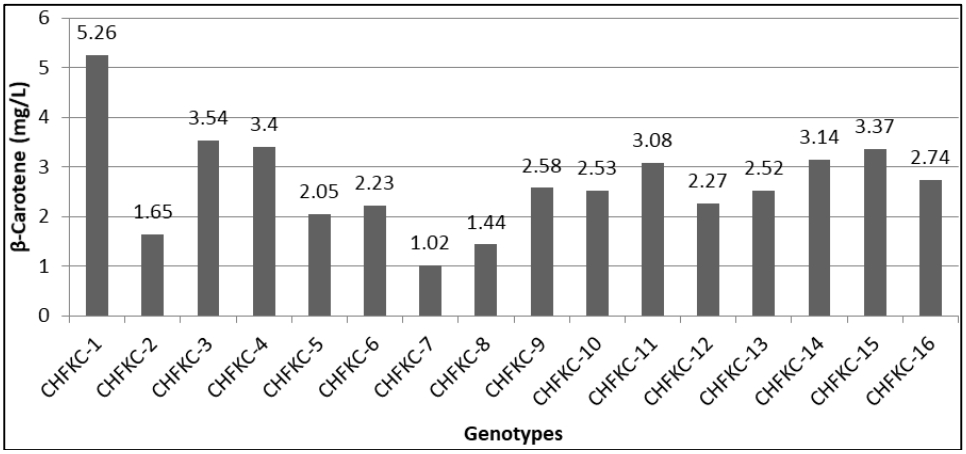

Fig 4: β-Carotene content of different King Chilli Genotypes **(**LSD at 5% is 0.31)

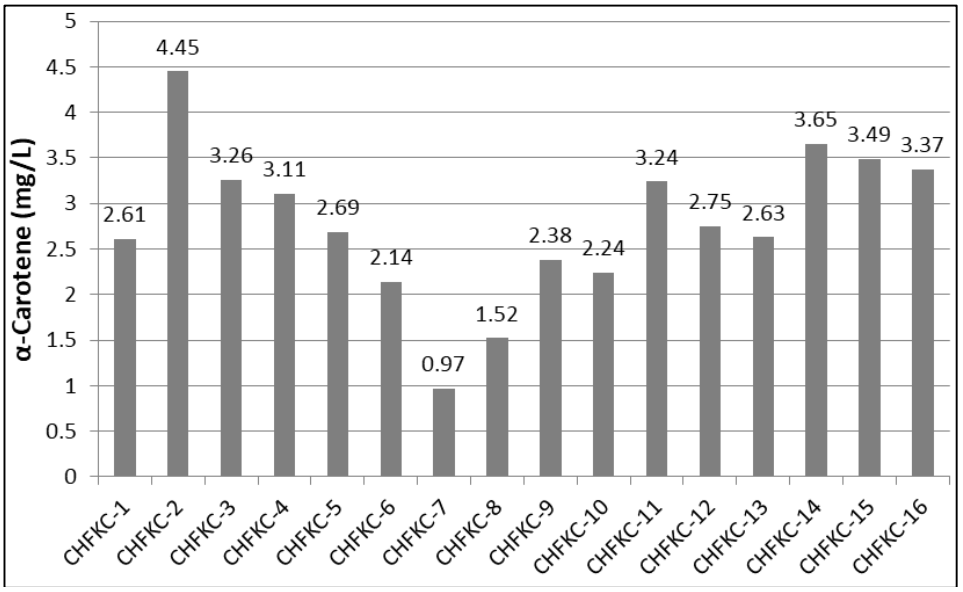

Fig 5: α-Carotene content of different King Chilli Genotypes **(**LSD at 5% is 0.39)

The β-Carotene content varied significantly between the genotypes (Fig.4). The observations recorded showed that the maximum β-carotene content was found in genotype CHFKC-1 (5.26 mg/L) followed by CHFKC-3, CHFKC-4, CHFKC-15 and CHFKC-14 whereas the minimum amount was recorded in genotype CHFKC-7 (1.02 mg/L) with a population mean of 2.68 mg/L (Fig.4). Carotenoids are known to play an important role in preventing oxidative damage [15] and are rich in King Chilli. Kantar *et al.* (2016) [14] reported vitamin-A (9000 IU) in *Bhut Jolokia*. Campos *et al.* (2013) [15] reported carotenoids content in ranged of 1.00 to 1.26 mg/100 g in Habanero (*Capsicum chinense*) genotypes.

The α-Carotene content varies significantly among the different genotypes (Fig.5). The genotype CHFKC-2 contains the highest amount of α-Carotene content which was significantly superior to others followed by CHFKC-14, CHFKC-15, CHFKC-16 and CHFKC-3. The lowest amount was observed in CHFKC-7.

Conclusion

The king chilli genotypes varied significantly for all the studied characters. The genotype CHFKC-6 has the highest content of capsaicin content and it can be utilized for pharmaceutical use. While the genotype CHFKC-1 was good for ascorbic acid content and β-Carotene. CHFKC-15 has average ascorbic acid, α-Carotene and β-Carotene content.

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