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Characterization of Eleven Bhut Jolokia (*Capsicum chinense* Jacq.) Genotypes for Morphological Traits and Biochemical Characters

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Abstract The present experiment was conducted on eleven Bhut jolokia (*Capsicum chinense* Jacq.) genotypes. Observations were recorded for morphologic traits based on 11 morphological characters and biochemical characters. Results revealed that among the genotypes only four characters leaf color, fruit surface, fruit shape at pedicel attachment and fruit color at ripe stage showed polymorphism. All the genotypes showed significant variation with regard to the biochemical characters except moisture content. Genotype G_1 (Titabar, Jorhat) showed the highest content for potassium, iron, capsaicin, moisture and DPPH scavenging activity. It was observed that the crude protein, beta-carotene and oleoresin content were highest in G_3 (Khensa, Mokokchung) compared to the other genotypes. G_6 (Diphu, Karbianlong) gave the highest value for ascorbic acid and phosphorus content compared to the other genotypes under study.

Keywords Ascorbic acid, Bhut jolokia, Capsaicin, Characterization, Oleoresin.

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Introduction

Bhut jolokia (*Capsicum chinense* Jacq.) has received the attention of scientific community throughout the world due to its extremely high pungency and unique aroma. It has been cultivated in a traditional manner since time immemorial and there exists a considerable amount of genetic variability among the landraces [1]. It was acknowledged as the hottest chilli (Guinness World Records, 2006), however as of November 20, 2013, Carolina Reaper (a cultivar of *Capsicum chinense* species) was announced as the hottest pepper in the world with a Scoville heat unit of 2,200,000 SHU. The Bhut jolokia has now been placed at the seventh position among the world's hottest chilli. It has been found by various researchers that the fresh fruits of peppers are good source of vitamin C, carotenoids and antioxidants. Peppers have been found to show protective against cholesterol and obesity [2]. Consumption of 100 g FW of peppers provide 100-200% vitamin C of the RDA (recommended daily administration). Many biochemical and pharmacological properties which includes antioxidants, antiallergenic and anti-carcinogenic activities are found in peppers. Bhut jolokia plant is location specific,hence, the plants of the same genotypes grown under different environmental condition vary from one another in various aspects which proves to be a boon to bring about improvement for efficient breeding works. In the present investigation an attempt has been made to collect various genotypes from different North Eastern parts of India and analyzed for its morphologic traits and biochemical characters.

Materials and Methods

The present experiment was conducted during the year 2013 and 2014 in the Experimental Fram, Department of Horticulture, Assam Agricultural University, Jorhat on 11 Bhut jolokia (*Capsicum chinense* Jacq.) genotypes for its morphologic traits and biochemical character. The experimental site was situated at $26^0 47'$N latitude and $94^0 12'$E longitude at an elevation of 86.8 m above mean sea level with uniform topography. The design of the experiment was laid out in randomized block design with three replications. Seeds of the selected fruits from healthy fruits were extracted and washed in clean water and kept under shade for drying. A spacing of 1 m ×1 m was maintained in all 33 plots with 25 number of plants/plot. In the nursery, the soil used was treated with 0.1% captan solution and covered with polyethylene sheet for 72 hours. The experimental field was thoroughly ploughed followed by harrowing. Thereafter a total of 825 plants were planted in all the 33 plots. Pits of 30 cm^3 were dug and filled with top soil along with 1kg FYM one month before transplanting. Seedlings having 6-8 leaves were selected for transplanting. The roots of each selected seedlings were treated with Bavistin @2g/L for 15 minutes before transplanting. The genotypes were randomly distributed within the plots of each block. Neem oil was sprayed at 10 days interval to act as deterrent for insects like aphids, irrigation was done manually. At monthly interval the plants were supplied with 250 g/pit of well decomposed farmyard manure and 150 g/pit vermicompost. Five plants were randomly selected in each plot of the various genotypes for observation.

Results and Discussion

Morphological traits

Study on the morphological traits was conducted with the help of morphological descriptors as the ones indicated by the International Plant Genetic Resources Institute for the genus *Capsicum,* IPGRI (1995) which helps to easily describe the morphological features of a genotype and thus helps exchange of information about new accessions. The morphologic characterization, evaluation of the genetic diversity and the documentation of a gene bank are essential to maintain an active basis for the exoloration of the genetic variability in breeding programs. All the eleven genotypes exhibited dark green stem color (Table 1). However, variation in leaf color was observed where majority of the genotypes showed dark green color, genotypes viz. G_1, G_5, G_7 and G_{11} exhibited light green color indicating variation among the entries. The leaf shape in all the entries recorded lanceolate shape. The corolla color, anther color and flower position were similar in all the genotypes which were creamy white, pale blue and intermediate respectively. The pendant orientation of flower bud at emergence was observed in all the genotypes. However, based on the description of the IPGRI, divergence was observed among the genotypes in the mature ripe fruits in terms of fruit

Table 1. Characterization of genotypes based on morphological traits.

Genotype	Stem color	Leaf color	Leaf shape	Corolla color	Anther color	Flower position
G_1 (Assam)	Dark green	Light green	Lanceolate	Creamy white	Pale blue	Intermediate
G_2 (Nagaland)	Dark green	Dark green	Lanceolate	Creamy white	Pale blue	Intermediate
G_3 (Nagaland)	Dark green	Dark green	Lanceolate	Creamy white	Pale blue	Intermediate
G_4 (Manipur)	Dark green	Dark green	Lanceolate	Creamy white	Pale blue	Intermediate
G_5 (Assam)	Dark green	Light green	Lanceolate	Creamy white	Pale blue	Intermediate
G_6 (Manipur)	Dark green	Dark green	Lanceolate	Creamy white	Pale blue	Intermediate
G_7 (Nagaland)	Dark green	Ligh green	Lanceolate	Creamy white	Pale blue	Intermediate
G_8 (Arunachal Pradesh	Dark green	Dark green	Lanceolate	Creamy white	Pale blue	Intermediate
G_9 (Assam)	Dark green	Dark green	Lanceolate	Creamy white	Pale blue	Intermediate
G_{10} (Nagaland)	Dark green	Dark green	Lanceolate	Creamy white	Pale blue	Intermediate
G_{11} (Arunachal Pradesh)	Dark green	Light green	Lanceolate	Creamy white	Pale blue	Intermediate

Table 2. Characterization of genotypes based on morphological traits.

Genotype	Flower bud orientation at bud emergence	Fruit surface	Fruit shape at pedicel attachment	Fruit color at maturity (ripe)	Seed color
G_1 (Assam)	Pendant	Highly wrinkled	Cordate	Red	Tan brown
G_2 (Nagaland)	Pendant	Highly wrinkled	Truncate	Red	Tan brown
G_3 (Nagaland)	Pendant	Semi wrinkled	Truncate	Red	Tan brown
G_4 (Manipur)	Pendant	Semi wrinkled	Truncate	Light red	Tan brown
G_5 (Assam)	Pendant	Wrinkled	Obtuse	Light red	Tan brown
G_6 (Manipur)	Pendant	Wrinkled	Obtuse	Red	Tan brown
G_7 (Nagaland)	Pendant	Wrinkled	Obtuse	Chocolate brown	Tan brown
G_8 (Arunachal Pradesh)	Pendant	Smooth	Truncate	Light red	Tan brown
G_9 (Assam)	Pendant	Wrinkled	Truncate	Light red	Tan brown
G_{10} (Nagaland)	Pendant	Wrinkled	Truncate	Red	Tan brown
G_{11} (Arunachal Pradesh)	Pendant	Wrinkled	Obtuse	Light red	Tan brown

surface, fruit shape and fruit color. The predominant type of fruit surface recorded in the present investigation was wrinkled type which was observed in G_5, G_6, G_7, G_9, G_{10} and G_{11}. On the other hand genotypes G_1 and G_2 showed highly wrinkled fruit syrface and semi wrinkled fruit surface was recorded in G_3 and G_4. Smooth type of fruit surface was recorded only in G_8. The fruit shape at pedicel attachment was found to be variable (Table 2). Six genotypes G_2, G_3, G_4, G_8, G_9 and G_{10} showed truncate type of fruit shape, four genotypes exhibited obtuse type of fruit shape viz, G_5, G_6, G_7 and G_{11}. While G_1 was the only genotype that showed cordate type of fruit shape at pedicel attachment. The fruit color at maturity was also found to be variable. Five genotypes each in red (G_1, G_2, G_3, G_6 and G_{10}) and light red (G_4, G_5, G_8, G_9 and G_{11}) were exhibited while G_7 recorded chocolate brown color fruit at maturity. The seed color, on the other hand was found to be tan brown in all the genotypes. In the present investigation it was revealed that there was no variation among the genotypes in respect to stem color, leaf shape, corolla color, flower position, flower bud orientation at bud emergence and seed color. The inability to show variable characters in these aspects might be attributed to the limited entry of genotypes. The extend of variability for leaf color and fruit color may be attributed to several pigment composition and concentrations including chlorophylls, carotenoids and flavonoids [3]. The genetic variability evidenced in the diversity of fruit colors and shapes was reported to be inherent to the species *C. chinense* [4].

Biochemical analysis

Significantly the highest phosphorus content was recorded in G_6 (479.33 mg/100g) while the lowest content of phosphorus was found to be associated with G_5 (327.61 mg/100g) (Table 3). The highest potassium content (2066.55 mg/100g) was recorded in genotype G_1 significantly the lowest amount of potassium content (1163.28 mg/100g) was recorded in G_4. The considerable high levels in phosphorus and potassium content could be possibly due to genetical difference among the genotypes in *Capsicum annum* and *Capsicum frutescens.* The data on iron content (Table 3) indicated that there was significant variation among various genotypes ranging between 67.56 mg/100g to 58.61 mg/100g. Significantly the maximum content of iron (67.56 mg/100g) was recorded in G_1 followed by G_3 (63.55 mg/100g). The minimum content of iron (58.61 mg/100g) was exhibited by G_5. The differences in iron content may be attributed to differential iron absorption by the genotypes.

The highest oleoresin content was observed in G_3 (14.90%) On the other hand the lowest content for oleoresin was noted in G_5 (10.01%) (Table 3), the reason for highest oleoresin content may be that the content of capsaicin, carotenoid are higher in this genotype, so oleoresin yield is also higher [5]. Chilli has acquired a great importance because of the presence of oleoresin, which permits better distribution of color and flavor in foods.

Table 3. Phosphorus, potassium and iron content in Bhut jolokia (mg/100g, pooled data of two years).

Genotypes	Phosphorus (mg/100g)	Potassium (mg/100g)	Iron (mg/100g)	Oleoresin (%)	Capsaicin (%)	Protein (mg/100g)
G_1 (Assam)	381.07	2066.55	67.56	12.53	1.83	2028.00
G_2 (Nagaland)	356.55	1866.22	62.62	13.89	2.19	1414.00
G_3 (Nagaland)	431.38	1930.14	63.55	14.80	2.34	2125.33
G_4 (Manipur)	376.25	1163.28	61.99	12.16	2.23	1883.33
G_5 (Assam)	327.61	1649.38	58.61	10.01	2.20	1960.00
G_6 (Manipur)	479.33	1899.44	62.93	11.47	2.06	1997.33
G_7 (Nagaland)	376.55	1733.07	60.98	10.14	1.71	1959.66
G_8 (Arunachal Pradesh)	376.11	1844.07	59.85	11.20	1.81	1780.00
G_9 (Assam)	406.67	1272.05	60.83	12.30	1.84	1997.67
G_{10} (Nagaland)	357.88	1388.77	61.68	10.33	1.96	1853.67
G_{11} (Arunachal Pradesh)	385.33	1567.78	61.05	12.35	1.47	1756.33
Mean	386.79	1670.98	61.97	11.92	1.97	1886.84

	SEd (±)	CD(0.05)	SEd (±)	CD (0.05)	SEd (±)	CD(0.05)	SEd (±)	CD (0.05)	SEd (±)	CD (0.05)	SEd (±)	CD(0.05)
Year (Y)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Genotype (G)	8.83	17.83	105.43	212.97	0.28	0.58	0.42	0.85	0.19	0.38	16.65	33.64
YXG	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Significantly G_3 was found to record the highest capsaicin content (2.34%), while the lowest content of capsaicin (1.47%) among the genotypes was observed in G_{11} (Table 3). The active principle in pepper responsible for its pungency is capsaicin. The differences in the capsaicin content may be due to the variation in the accumulation of capsaicinoid related to the process of cell clongation [6] have showed that variation in capsaicin content in some chilli genotypes.

Significantly the highest crude protein content of 2125 mg/100g was found to be associated with G_3 and the lowest crude protein content of 1414.00mg/100g was found to be associated with G_2 (Table 3). Proteins are the most abundant macromolecules in cells and they are extremely versatile in function. The significant increase in protein content might be due to enzymes involved in synthesis of various substances in chilli fruit. While the decline of *de novo* synthesis of various enzymes with maturity might be the reason for the low content of protein in the chillies [5].

The highest value for β Carotene (141.86 mg/100g) was recorded in genotype G_3 while the lowest value of 48.76 mg/100g was found to be associated with G_4 (Table 4). Along with fruit development chloroplast is transformed into chromoplast and at the same time carotenoid synthesis begins. Hence the variation in carotenoid synthesis among the genotypes might have caused the variation in them.

Significantly the highest ascorbic acid content of (462.28 mg/100g) was recorded in G_6 while the lowest ascorbic acid content of 354.67 mg/100g was found to be associated with G_5 (Table 4). The variation in ascorbic acid content among the genotypes might be attributed to their inherent genetic makeup. The exact mechanism of ascorbic acid accumulation at maturity is not fully understood, however the accumulation may likely be associated with senescence or by ascorbic acid transportation from leaves to the fruit during ripening. Ascorbic acid is one of the powerful antioxidant agents that has a number of health promoting functions [7] has showed that varietal variations in ascorbic acid content in chilli.

The highest crude fiber content of 44.73% was recorded in G_2 however the lowest fiber content of 18.41% was found to be recorded in G_8 (Table 4). The variation in per cent crude fiber content in the different genotypes might be due to their variable genetic makeup.

Table 4. β- Carotene (mg/100g), Ascorbic acid (mg/100g), Fiber (%), Moisture (%) and DPPH scavenging activity (%) in Bhut jolokia (pooled data of two years).

Genotypes	β-Carotene (mg/100g)		Ascorbic acid (mg/100g)		Fiber (%)		Moisture (%)		DPPH scavenging activity (%)	
G_1(Assam)	124.03		461.18		44.70		89.55		83.62	
G_2 (Nagaland)	105.21		454.16		44.73		87.81		66.46	
G_3 (Nagaland)	141.86		413.00		41.67		89.23		60.26	
G_4 (Manipur)	48.76		403.78		27.40		86.50		63.16	
G_5 (Assam)	120.17		354.67		41.55		87.35		56.23	
G_6 (Manipur)	140.02		462.28		30.75		88.54		73.04	
G_7 (Nagaland)	98.10		421.00		29.45		86.64		45.82	
G_8 (Arunachal Pradesh)	123.54		436.32		18.41		86.56		68.26	
G_9 (Assam)	65.73		447.83		29.30		87.40		53.19	
G_{10} (Nagaland)	120.34		451.00		21.83		83.54		49.04	
G_{11} (Arunachal Pradesh)	76.71		407.67		21.75		86.17		49.05	
Mean	105.86		428.91		31.96		87.21		60.65	
	SE(±)	CD (0.05)	SE (±)	CD (0.05)	SE (±)	CD (0.05)	SE (±)	CD (0.05)	SE (±)	CD (0.05)
Year (Y)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Genotype (G)	0.79	1.59	2.05	4.15	2.39	4.82	2.03	4.10	0.34	0.69
YXG	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

The highest moisture content of 89.55% was found to be associated with genotype G_1 which was found to be at par with all other genotypes except G_{10} (83.54%) (Table 4). The least variation in moisture content of the genotypes might be attributed to similar environmental/growing condition. In present investigation it was observed that the fruit volume indicated a corresponding trend where it revealed that more the volume of fruits higher was the moisture content. Further significant difference in moisture content of various genotypes is possibly due to genetical differences.

Significantly the highest DPPH scavenging activity of 82.62% was found to be recorded under G_1 while the lowest of 49.04% was recorded under G_{10} which was at par with G_{11} (49.05) (Table 4). The differences in DPPH scavenging activity might be attributed to the individual genetic makeup of the different genotypes. The highest DPPH scavenging activity recorded in G_1 might be attributes to the high value of ascorbic acid and capsaicin content (Table 4).

Among the various morphological characters, leaf color, fruit surface, fruit shape at pedicel attachment and fruit color (ripe) could distinguish the genotypes. From the study of biochemical parameters, it was observed that genotypes G_1 showed the highest content for potassium, iron, capsaicin, moisture and DPPH scavenging activity while the protein, beta carotene and oleoresin contents were highest in G_3 compared to the other genotypes. The genotype G_6 gave the highest value for ascorbic acid and phosphorus content compared to the other genotypes under study. This investigation opens up avenues for furtherance of research in both agronomical and biochemical field. Future studies may also be made to reduce the duration of the crop by some agronomical or hormonal manipulation while analysis with sophisticated methods and more efficient techniques like High Performance Liquid Chromatography (HPLC) should be taken up for the biochemical analysis.

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