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Rich Genetic Diversity of Capsicum Species in Northeast India, as a Potential Source for Chilli Crop Improvement

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ABSTRACT

Northeastern India with its diverse topography ranging from plains of Assam and Tripura to mountainous regions of Sikkim and Arunachal Pradesh possesses great diversity of flora and fauna, including varieties of Capsicum species. Although, Northeastern India is rich in genetic diversity of *C. species*, information about biotic and abiotic stresses and the source of resistance, breeding approaches to improve the chilli landraces of NE India, ethno-pharmacological applications, capsaicin/capsaicinoid content and other useful compounds of many landraces are still largely unexplored. This review article highlights about the various research work carried out so far in chillies of NE India, related to genetic diversity study and tissue culture techniques, etc.

Keywords: Capsicum species, Northeast India, Genetic diversity, Landraces, Capsaicin

Abbreviations: NE India: Northeast India; C. species: Capsicum species; CAU: Central Agricultural University

INTRODUCTION

Chillies belong to the genus Capsicum of the Solanaceae family. Capsicum is derived from the Greek word 'kapsimo', meaning 'to bite' [1]. There are 25-30 species of Capsicum of which 5 species, i.e., *C. annum* L., *C. frutescens* mill, *C. chinense*, *C. baccatum* L. and *C. pubescens* have been domesticated and cultivated [2]. Capsicum species are diploids with most of them having 24 chromosome (n=x=12), but species with genome size of 2n=2x=32 [3] and 2n=2x=48 [4] also have been reported. It is a self-pollinated dicot plant. However, occurrence of cross pollination leads to the formation of variants within the species. The primary centre of origin of chilli is thought to be Mexico (American tropics) with secondary centers in Guatemala and Bulgaria [5]. Chilli is believed to have been introduced to India by Portuguese explorers [1] and to NE India by Christian missionaries [6]. The NE India has a rich natural heterogeneity due to its distinctive geographical location which has been identified as one of the twelve "Genetic Epicentres" for the evolution of world flora. In NE India, several Capsicum landraces are traditionally grown, which are distinguished based on their morphological appearance **(Figure 1)**, flavor and pungency levels. These are referred to by a number of vernacular names, e.g. Naga King chilli (Bhut Jolokia/Bih Jolokia/Naga Jolokia, Lota Bhut, Dhan Jolokia, Krishna Jolokia, Mem Jolokia, Raja mircha, U-morok), In Sikkim, they are known as Dalle Khursani (round chillies), Thadey Khursani (erect fruit), Thalo Khursani (vegetable type), Jeerey Khursani (thin fruit), Lamchey Khursani (medium size-less pungent) and so on.

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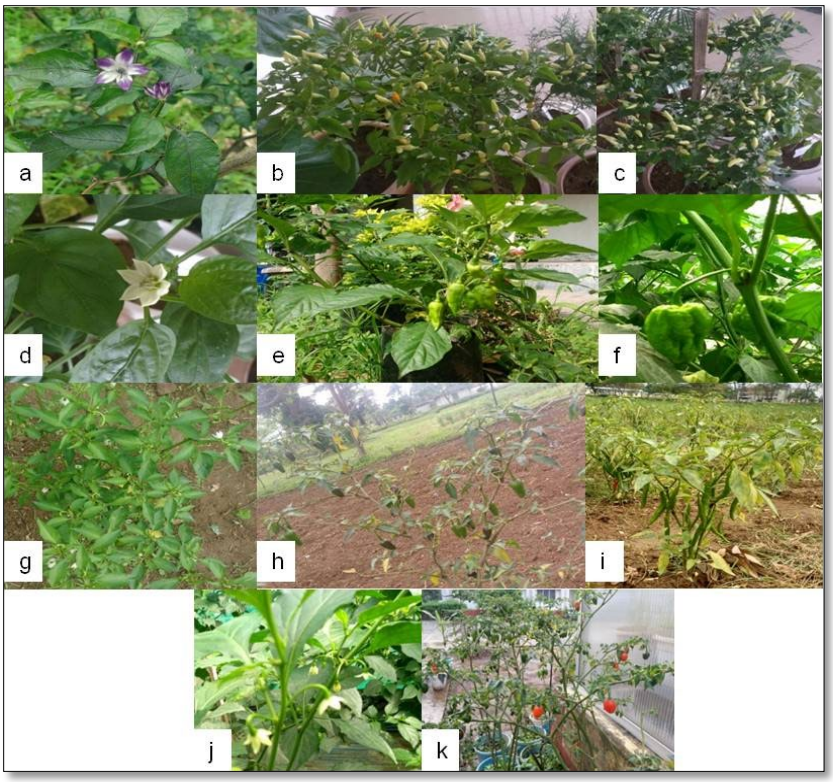

Figure 1. Flowering and fruit morphology of *Capsicum frutescence* (a, b and c), *C. chinense*/King chilli (d, e and f), *C. annum* (g, h and i) and Dalle khursani (*C. annum*) (j and k).

Capsicum is used as a spice in a variety of cuisine all over the world as a basic ingredient. It is also used as a colorant and flavorant to make the foods more attractive and tasty. The archaeological data suggested or proved that the human civilization has been cultivating Capsicum since the past 7000 years to be used in food recipes for hotness and coloration [7]. Capsicum not only gives attractive color and flavor to the foods but also provides vitamin C, vitamin A, vitamin B complex, vitamin E and minerals like Molybdenum, Manganese, Folate, Potassium and Thiamine. Chilli contains seven times more vitamin C than orange. Beta carotenoids, Vitamin C and vitamin A in chillies are antioxidants that destroy free radicals [8]. Pungency (hotness) of the chilli pepper is due to the presence of capsaicin. It is an active component of chili peppers. Capsaicin is the main capsaicinoid in chilli peppers, followed by dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin [9]. Capsaicin present in chilli pepper is used as a counter irritant in Lumbago (pain in the muscles and joints of the lower back), Neuralgia (pain in the distribution of a nerve or nerves), Rheumatic disorders (chronic, often intermittent pain affecting the joints and/or connective tissue) and non-allergic Rhinitis (inflammation of the inner part of the nose that is not caused by an allergy). It has a tonic and carminative action. In combination with Cinchona, it is used for the treatment of intermittent and lethargic affliction, tympanitis (pain due to inflammation of the middle ear as well as oozing blisters on the eardrum) and paralysis [2]. The plants have also been used as indigenous medicine for asthma, arthritis, dropsy, colic, diarrhea, toothache and muscle cramp [10].

AREA AND PRODUCTION OF CHILLI IN NE STATES OF INDIA

India is the largest producer, consumer and exporter of chillies in the world [11]. In India, per capita consumption is highest for chillies, among the spices produced. It occupies around 30 % of the area shared among major spice crops of the country [12]. Though chillies are grown all over India, NE states contribute 51.72% of its annual production while having only 8% area under chilli cultivation (Spice Statistics, Spice Board, 2004). This region comprising the states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura is located between 22-29.3° N and 89.7-97.8° E. Among the NE states, Assam has the maximum area under chilli cultivation, and it seems Nagaland is at top among the NE states in terms of production **(Table 1)**. NE region of India is an excellent place to find the diverse range of chilli genotypes, because there are numerous heterogeneous landraces of chilli available in this region and these landraces serve as a reservoir of genetic variability for chilli breeders [13].

Table 1. Area and Production of chillies (green) in north-eastern states of India in the year 2015-2016.

S. No.	Name of the State	Year: 2015-2016	
		Area (000' ha)	Production (000' t)
01	Assam	21.31	19.12
02	Nagaland	5.40	40.08
03	Mizoram	9.14	9.33
04	Tripura	2.15	19.80
05	Manipur	0.20	1.51
06	Sikkim	0.09	0.32
07	Arunachal Pradesh	1.2*	1.6**
08	Meghalaya	1.8*	1.1**

*** indicates the data of the year 2005-2006*
Source: Horticulture at a Glance, 2017. Government of India, Ministry of Agriculture and Farmers Welfare, Horticulture Statistics Division

REPORTS ON ASSESSMENT OF GENETIC DIVERSITY IN CAPSICUM SPECIES OF NE INDIA

For strategic germplasm collection, maintenance, conservation and utilization, sound knowledge of diversity in any plant species is an important requirement [14]. Numerous researches have been conducted on genetic diversity of Capsicum using morphological as well as molecular markers. However, there has been very little research on genetic diversity of Capsicum accessions collected from NE India.

Mohammadi and Prasanna [14] assessed genetic diversity using morphological characters of 52 genotypes of Capsicum which were collected from different regions of NE India. Wide variations were observed in various morphological characters studied which suggested the diverse nature of germplasm collected. The study on genetic relationship of nineteen genotypes belonging to *C. annuum*, *C. frutescence*, *C. chinense* and Bhut Jolokia showed that Bhut Jolokia shared more markers from *C. chinense* than with C. frutescence. The average genetic similarity of Bhut Jolokia and *C. chinense* was 0.79 which is closer to the mean genetic diversity within *C. chinense* (0.82) than that within *C. frutescens* (0.85). Based on the presence of both *C. frutescens* and *C. chinense* specific markers in Bhut Jolokia, the study concluded it as an interspecific hybrid between the two species [15]. This study also identified some of the species specific markers which will be useful in species identification as well as for marker assisted breeding of chilli crops. Evaluation of genetic diversity of Naga Jolokia with morphological characters revealed a considerable amount of genetic variability with respect to fruit shape and color in this germplasm [16]. Twenty five chilli genotypes which were from NE region of India, evaluated for phytochemical composition and antioxidant potential showed variations for capsaicin, oleoresin, phenolics, carotenoids and other antioxidants contents [17]. Genetic differences between Bhut Jolokia, *C. frutescens* and *C. chinense* was revealed when six genotypes of Bhut Jolokia with eight known Capsicum species were characterized by using three nuclear genes, namely; ITS 1, ITS 2 and 5.8 S. However, the study failed to discriminate between *C. frutescens* and *C. chinense* [7]. Cluster analysis of genetic diversity of seven Capsicum landraces of *C. annuum*, *C. frutescence* and *C. chinense* using 10 RAPD showed two major clusters where *C. frutescens* and *C. chinense* genotypes grouped in one cluster apart from the *C. annuum* genotypes. The average genetic diversity was more in *C. frutescens* and *C. chinense* genotypes than in *C. annuum* [18]. The genetic diversity analysis of 53 chilli landraces of NE India using pun 1 locus revealed 79 SNPs and 3 indels in pun1 gene and when SSR markers and morphological traits were used to study diversity, they detected 3-9 alleles for each SSR locus with an average of 5.36 alleles/locus. Cluster analysis based on morphological and SSR markers separated the accessions with campanulate fruits type from those with erect fruits type [19]. When SSR and random amplified microsatellite polymorphism (RAMPO) markers were used to evaluate the genetic diversity of germplasm collected from nine countries which included 4 species of Capsicum, improved lines and 5 landraces from NE India, the dendrogram based on both the markers showed two major clusters and was able to separate *C. annuum* genotypes from others. The study revealed the presence of higher genetic diversity in other *Capsicum* spp. than *C. annuum* genotypes [20]. Genetic diversity based on twelve quantitative characters of 30 chilli (*Capsicum annuum* L.) genotypes collected from different regions of India, majority from Arunachal Pradesh, showed considerable variability among the genotypes for the character studied and some genotypes were identified as

better parents for an efficient hybridization programme of chilli [21]. Protein profiling of 30 genotypes of chilli collected from NE India showed considerable variation in banding pattern of total protein which ranged from 7-19 numbers of bands [22]. Five different ecotypes of Bhut Jolokia collected from different locations of NE India showed significant difference in terms of phenolic content combined with ascorbic acid in the fruits [23]. Diversity analysis by estimating the bioactive compounds present in 72 chilli landraces of NE India showed significant difference in total phenolic and flavonoid content. The result also suggested that *Capsicum frutescens* L. is a potent source of natural antioxidants [13]. Significant intra and inter-specific variations for fruit morphological traits, fruiting habits and 65 fruit metabolites were observed in the collected Capsicum germplasm belonging to three Capsicum species, i.e., *C. chinense* (Bhut jolokia, 63 accessions), *C. frutescens* (17 accessions) and *C. annuum* (56 accessions). Phenotypic diversity of 72 landraces of chilli consisting predominantly of *C. frutescence* collected from different regions of NE India using 29 qualitative traits and 14 quantitative traits revealed the vast intra-specific diversity of *C. frutescence* landraces of NE India [24]. SDS-PAGE analysis of 31 chilli genotypes collected from different regions of NE India showed considerable variation in number of protein bands ranging from 8-18 and a total of 70 protein bands as per Rm values were identified by silver staining [25]. Study on about 30 Bhut Jolokia genotypes to determine the distinctiveness of Bhut Jolokia with the germplasm using SSR markers revealed 100% polymorphism in 9 out of 10 markers [26].

OTHER RESEARCH ACTIVITIES ON CHILLIES OF NE INDIA

Although NE India is rich in genetic diversity of chilli crops, there has been very little research conducted in chilli crops belonging to NE India regions. Therefore, there is a high probability of discovering a number of genes/alleles contributing to resistance against abiotic and biotic stresses, some chemicals/compounds that can be used as medicine and other secondary metabolites that are of high value. Below are some of the researches conducted on chillies of NE India for various purposes. Capsaicin and Capsaicinoid have been estimated from different chilli cultivars of NE India [27-30]. Growing environment, sowing time and crop geometry influenced the synthesis of capsaicin or capsaicinoids in Chilli pepper [31,32]. Comparative expression analysis (through qRT-PCR) of candidate genes involved in capsaicin/capsaicinoid biosynthesis pathway showed many fold higher expression of majority of the genes in *C. chinense* compared to *C. frutescens* and *C. annuum* suggesting that the possible reason for extremely high pungency might be due to the higher level of candidate gene(s) expression [33]. Many components of chilli fruits are known to have medicinal values. The ethno-pharmacological applications of king chilli and its derivatives is well described [34-36]. Susceptibility of the chilli genotypes to a number of abiotic and biotic stresses has restricted their potential yield. Abiotic stresses which significantly reduced the yield and quality of peppers include extreme of temperature, moisture, light, nutrients and pH among others. Biotic factors include susceptibility of chilli plants to various fungi, bacteria, viruses, nematodes and various insects. Among the biotic factors, the one that poses a great threat to cultivated chilli is viral infection [37]. All these problems can be overcome by identifying novel sources of resistance [38-40], heterosis breeding [41] and proper identification and management of causal organism [42-46]. Number of characteristics like very low germination percentage, recalcitrant nature, genotype dependent, etc. associated with the Capsicum creates problems in improvement of chilli plants by conventional breeding techniques as compared to other solanaceous crops [2]. Hence, the plant biotechniques which include plant tissue culture and genetic engineering are gradually becoming a functional aspect of classical breeding programs and boost the improvement in Capsicum crop. Many researchers [4,23,28,32,47-57] reported the efficient method for *in vitro* regeneration/micro-propagation of chillies belonging to NE India, that will help in further improvement of the chilli crop through biotechnological approaches that involve tissue culture.

CONCLUSION

The NE India has a rich natural heterogeneity due to its distinctive geographical location where several landraces of crops including Capsicum species are traditionally grown. These landraces of chillies have various traditional uses including uses in different ethnic cuisines and ethno-pharmacological use [58]. Although many studies have been done in chilli landraces of NE India including diversity/evolution, *in vitro* regeneration, scientific cultivation, diseases and their management, the potentiality of chilli landraces of NE India as the source of biotic and abiotic stress resistance, medicinal properties, etc. are still largely unexplored. Chilli cultivation in NE India also faces the problems like lack of warehouse/godown for proper storage, poor transport facility for harvested product and lack of market information to farmers for efficient and timely production of chilli on their farms. There is a need to conduct more researches on NE India chilli to develop varieties that are better yielding, tolerant to various stresses, etc. Further, awareness about market situation, cultivation techniques, proper storage and transportation will make chilli cultivation more remunerative for chilli farmers in NE India [59-61].

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