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Evaluation of Genetic Diversity among Naga Chili (*Capsicum chinense* Jacq.) Samples Available in Bangladesh

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Key words: PCR, RAPD, Molecular characterization, Dendrogram, Polymorphisms

Abstract

Naga chili possesses a unique aroma and pungency level. Due to its diversified fruit morphology and uses, it has a special acceptance among consumers. Present experiments were designed to evaluate the genetic divergence of morphologically diverged twelve samples collected from three divisions (Barishal, Khulna and Sylhet) of Bangladesh employing the PCR-based RAPD technique. Analysis by seven decamer primers resulted in 33 RAPD loci ranging from 300 to 5000 bp with 84.84% polymorphism. Indicating a high polymorphism existed among those samples. The distance matrix and UPGMA dendrogram showed a considerable level of genetic divergence from 0.0589 to 0.8318 in those samples. This result indicates that the samples collected from the different division are genetically diverged. This outcome can play a vital role in future molecular analysis of Naga chili.

Introduction

Capsicum chinense Jacq. is familiar with varied local names, for example, Naga Chili, Naga Morich (Nagaland), Omorok (Manipur), Bhut Jolokia (Assam), Kobra Chili (Sri Lanka), Habanero Pepper (America), Bombai Morich (Bangladesh) throughout the world (Verma et al. 2013). It is an evergreen, erect, short-lived, bushy, fast-growing perennial herb under the Solanaceae family (Ince et al. 2009). The presence of a comparatively higher amount of capsaicin makes Naga chili the hottest chili with a record 1, 001, 304 SHU (Scoville Heat Unit) in the world (Verma et al. 2013). This capsaicin has tremendous properties of analgesic, antiarthritic, anticancer and antioxidant (Prasad et al. 2005) and other medicinal uses.

In recent times, because of its multipurpose uses and nutritional and medicinal value, Naga chili has gained unprecedented importance in the world market, especially

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in Bangladesh (Kumar et al. 2022). Although the environmental condition of Bangladesh is favorable for Naga chili production, the average yield in Bangladesh is comparatively lower than other countries like the UK (51.88 t/ha), Sweden (54.35 t/ha), Austria (56.70 t/ha) and Israel (64.20 t/ha). The probable reasons behind this are farmers' unawareness and the lack of systemic and scientific knowledge (FAO 2000). In the Bangladeshi local market, morphologically diverse Naga chilies are available; however, they are not categorized into varieties. On the contrary, the exponentially increased use of Naga chilies has led to the development of a novel variety with specific attributes to meet customers' demands.

For the development of a new variety, molecular characterization of that particular crop is the prerequisite. As highly diverged fruits of the species imply that sole morphological attributes are not always adequate for the characterization and identification (Olszewska et al. 2017). Characterization using DNA-based marker techniques is an impressive tool for studying genetic information and variability (Bered et al. 2005, Sweety et al. 2017). Among them, the PCR-based RAPD markers are widely used because of their significant advantages such as their simplicity, rapid, requirement of low quantity of DNA, inexpensive, and ability to produce polymorphisms (Islam et al. 2015, Williams et al. 1990). In addition, this genetic knowledge assists in comparing different samples and choosing competent parents for hybridization (Sony et al. 2012). Around the world, different Naga chili accessions were analyzed using the RAPD technique. Nevertheless, molecular characterization of Naga chili was hardly carried out in Bangladesh. The prime goal of this study is to analyze the genetic diversity and polymorphisms among morphologically different Naga chili samples collected from different regions of Bangladesh.

Materials and Methods

In these experiments, fruit samples were collected from local markets of different localities of the Barishal, Khulna and Sylhet divisions of Bangladesh. Fruit diversities (shape, color and size) were the major criteria for collection. The collected fruits were categorized based on fruit morphology and sources of collection (Table 1). Those fruits were named after their source of collection and acronyms were given according to their source. After sun drying, the seeds were collected from the fruits. Then, the seeds were sown on a seedbed in the net house of the University of Barishal. Leaves from the 28-day-old plants were used for genomic DNA isolation.

With slight modification, the CTAB method of Attitalla (2011) was applied to conduct the genomic DNA isolation from freshly harvested leaves of four-week plants. Here, 2 ml of extraction buffer (10% CTAB, 5M NaCl, 0.5M EDTA, 1M Tris-Base, β-mercaptoethanol) was used for the 300 mg leaves sample. The quantity and quality of isolated DNA were measured by Nanodrop Spectrophotometer analysis and 1% agarose gel electrophoresis respectively.

Table 1. List of plant materials used in this present study.

Samples Title	Source	Fruit morphology			
		Shape	Top end	Bottom end	Color at the ripening stage
BP_1	Boithaghata, Pirojpur	Campanulate	Tomb	Blunt	Red
BP_2	Boithaghata, Pirojpur	Campanulate	Flat	Blunt	Red
BP_3	Boithaghata, Pirojpur	Elongated	Tomb	Pointed	Red
BP_4	Boithaghata, Pirojpur	Elongated	Flat	Blunt	Deep red
SS_1	Sreemangal, Sylhet	Elongated	Tomb	Pointed	Red
SP_2	Sadar, Potuakhali	Campanulate	Flat	Blunt	Orange
KJ_1	Kathalia, Jhalokathi	Elongated	Tomb	Pointed	Deep Red
SP_1	Sadar, Potuakhali	Elongated	Tomb	Pointed	Orange
SK_1	Sadar, Khulna	Elongated	Tomb	Blunt	Red
SK_2	Sadar, Khulna	Campanulate	Flat	Blunt	Orange
CB_1	Charmonai, Barishal	Elongated	Flat	Pointed	Red
CS_1	Companyganj, Sylhet	Campanulate	Flat	Pointed	Deep red

Twenty-five µl of PCR reaction mixtures [2.5 µl 10× *Taq* DNA buffer, 100 µM dNTPs, 1 µM primer, 1U *Taq* polymerase (Thermo-scientific) and 50 ng genomic DNA] were prepared by maintaining the appropriate temperature. Then, the PCR reaction was performed in a Thermocycler by maintaining one cycle of 94°C for 4 min., 35 cycles of 94°C for 45 sec., 32°C or 34°C for 30 sec., 72°C for 3 min., and finally one cycle at 72°C for 7 minutes. The reactions were held at 4°C after completing the amplification. The PCR products were analyzed through 1.5% agarose gel electrophoresis containing ethidium bromide. The gel run was performed in 1X TAE buffer at 90 volts and 300 mA for 40 minutes. A 1kb plus DNA ladder was also electrophoresed with PCR-amplified products, and gel photographs were taken using the Gel Documentation system.

From the gel photographs, the size of the amplified product was estimated by comparing it with known size fragments of 1 kb plus ladder. The data were arranged in an excel sheet as a binary matrix where '1' indicates the presence of a band and '0' for the absence of bands. With these data, genetic distance and UPGMA dendrogram were constructed among those samples using the computer program "NTSYSpc V.2.10".

Results and Discussion

In this study, the genetic dissimilarities and polymorphisms among those twelve Naga chili samples were assayed by RAPD primers because RAPD markers have been proven effective for analyzing genetic distances and mapping in chili (Bhadragoudar and Patil 2011). Initially, eleven decamer primers were screened but seven primers amplified

reproducible products. These seven primers were used to conduct this experiment. The other primers cannot form reproducible banding patterns, considered a general property of the RAPD technique (Pervaiz et al. 2010). The amplified RAPD profile formulated with the primers across the selected samples is visualized in Fig. 1.

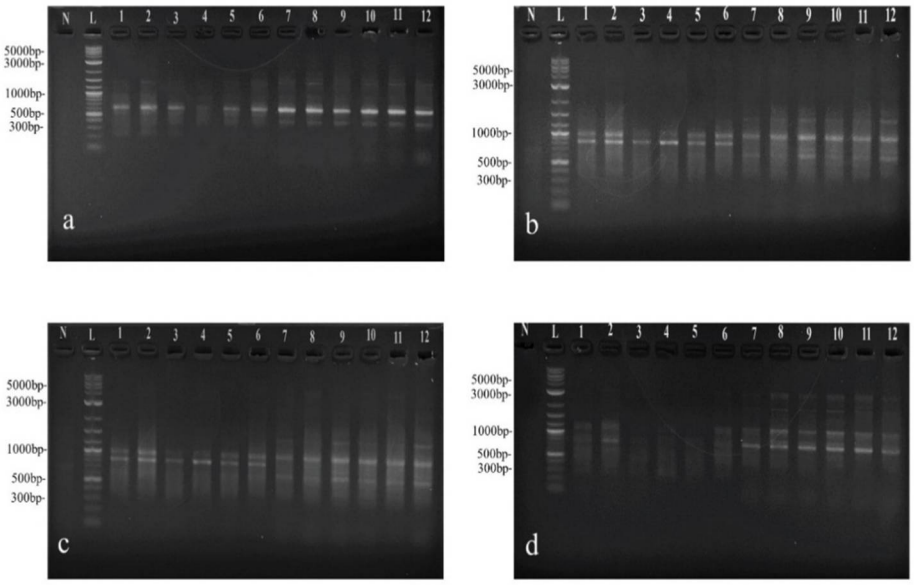

Fig. 1. RAPD profile obtained from 12 Naga chili samples with (a) primer OPA01, (b) primer OPA03, (c) primer OPA04, (d) primer OPA05; Lane L-Ladder, Lane N- Negative (water control), lane 1-BP_1, lane 2 -BP_2, lane 3 -BP_3, lane 4 - BP_4, lane 5 - SS_1, lane 6 - SP_2, lane 7 - KJ_1, lane 8 - SP_1, lane 9 -KS_1, lane 10 - KS_2, lane 11 - CB_1, lane 12 - CS_1.

A considerable level of polymorphism was identified among the samples by the utilization of seven primers. A total of 33 RAPD loci sizes ranging from 300 to 5000 bp were scored (Table 2). Different primers produced varied numbers of RAPD fragments and ranged from 3 (OPB02) to 7 (OPA05) bands with an average of 4.71 per primer. The range of average number of RAPD loci per primer varies from plant to plant for instance, 6 in *Vernonia amygdalina* (Aikpokpodion et al. 2018), 29.2 in tomato (Tabassum et al. 2013), etc. The maximum polymorphism (100%) was observed in OPA02, OPB02, and OPB04 primers whereas the lowest polymorphisms (71.43%) were recorded in OPA05 primer. The average polymorphisms were 84.84% by the utilization of all seven primers (28 polymorphic bands from a total of 33 bands). According to the earlier report, RAPD markers were proven significant in detecting polymorphisms in different crops as 91.24% in onion (Kutty et al. 2006), 60.09% in mustard (Ahmad et al. 2007), 62.5% in *Coccinia grandis* L. (Hossain et al. 2016), 61% in canola (Moghaieb et al. 2014) and 84.44% in cabbage (Saxena et al. 2011).

Table 2. Analysis of reproducible amplified loci with different RAPD primers within the Naga chili samples.

Primer code	Sequences (5′-3′)	Total number of RAPD loci	Number of polymorphic loci	Proportion of polymorphic loci (%)
OPA01	CAG GCC CTT C	4	3	75
OPA02	TGC CGA GCT G	4	4	100
OPA03	AGT CAG CCA C	5	4	80
OPA04	AAT CGG GCT G	6	5	83.33
OPA05	AGG GGT CTT G	7	5	71.43
OPB02	TGA TCC CTG G	3	3	100
OPB04	GGA CTG GAG T	4	4	100

Here, the genetic distances between and among the 12 Naga chili samples collected from different regions of Bangladesh were measured based on the proportion of RAPD fragments. To measure the genetic variability, Nei's distance matrix (Nei 1972) was utilized. This matrix revealed a considerable level of genetic variability among those 12 samples of Naga chili which varies from 0.0589 to 0.8318 (Table 3). Hazarika and Neog (2014) found genetic variability ranges from 0.08 to 0.43 among 30 Naga chili accessions collected from three North-East Indian states. The highest genetic distance (0.8318) was observed between BP_4 and CB_1; and BP_4 and CS_1 samples whereas the lowest genetic distance (0.0589) was found between SP_2 and BP_3 samples. At the same time, relatively higher genetic variability (0.8047) was measured between BP_4 and SP_1, BP_4 and SK_1, BP_4 and SK_2, BP_1 and CB_1 Naga chili samples. This result exhibited that the samples collected from

Table 3. Summary of Nei's (1972) genetic distances of 12 Naga chili samples.

	BP_1	BP_2	BP_3	BP_4	SS_1	SP_2	KJ_1	SP_1	SK_1	SK_2	CB_1	CS_1
BP_1	0											
BP_2	0.1897	0										
BP_3	0.1924	0.2027	0									
BP_4	0.0912	0.3662	0.0668	0								
SS_1	0.2428	0.5431	0.0668	0.1788	0							
SP_2	0.1171	0.2064	0.0589	0.2386	0.3565	0						
KJ_1	0.5019	0.3811	0.4469	0.5304	0.6853	0.3739	0					
SP_1	0.6506	0.3436	0.6020	0.8047	0.6853	0.5022	0.1949	0				
SK_1	0.6220	0.3993	0.6020	0.8047	0.6609	0.4778	0.2350	0.1298	0			
SK_2	0.6220	0.3436	0.5675	0.8047	0.6853	0.5022	0.1342	0.1542	0.0757	0		
CB_1	0.8047	0.4469	0.6020	0.8318	0.7085	0.5254	0.1575	0.1233	0.1530	0.1233	0	
CS_1	0.8047	0.5107	0.6343	0.8318	0.6853	0.5022	0.1949	0.1542	0.0757	0.1542	0.1774	0

two different divisions show higher genetic distances whereas, the lowest genetic distance was recorded between two different localities of the same division, Barishal. It might be possible because of the geographical distribution of these samples (Aikpokpodion et al. 2018). According to an earlier report, the RAPD method was considered a crucial vehicle for finding out the genetic distance among different samples of other crops, for example, rice 0.0373 to 0.5983 (Mitra et al. 2017).

The dendrogram was constructed using Nei's (1972) distance matrix and the UPGMA method. All twelve samples were segregated into two major clusters (Fig. 2). Depending on similarity coefficient these 2 major clusters were stratified into four different sub-clusters. The dendrogram analyses clearly distinguished all twelve samples from each other as genetically closed samples were situated in the same cluster, whereas genetically divergent samples were located in different clusters. Here, two major clusters (C1 and C2) were formed. Both major clusters, C1 and C2, were divided into sub-clusters. Sub-cluster 1 & 4 were presented in major cluster 1 and sub-cluster 2 and 3 were situated in major cluster 2. BP_1, BP_4, BP_3, SP_2, SS_1, BP_2 samples were present in the major clusters 1 (C1) and rest samples (KJ_1, SP_1, CB_1, SK_1, SK_2, CS_1) were situated in major cluster 2 (C2). BP_3, SP_2, BP_1, and BP_4 samples were distributed in sub-cluster 1, which indicated relatively low genetic divergence as all these four samples were collected from the same division, Barishal. On the other hand, sub-cluster 2 consisted of SK_1, SK_2 and CS_1 samples. In this cluster, the coefficient of distance ranged from 0.11-0.19, indicating high relatedness among the samples. Here, SK_1 and SK_2 were from the same location but CS_1 was from another location. The probable reason is the migration of fruits in different markets. SP_1, CB_1, and KJ_1 samples were grouped under sub-cluster 3. The remaining samples, i.e., SS_1 and BP_2 are included in sub-cluster 4. The last sub-cluster indicated the high genetic dissimilarity as SS_1 and BP_2 were collected from two different districts of Sylhet and Barishal respectively.

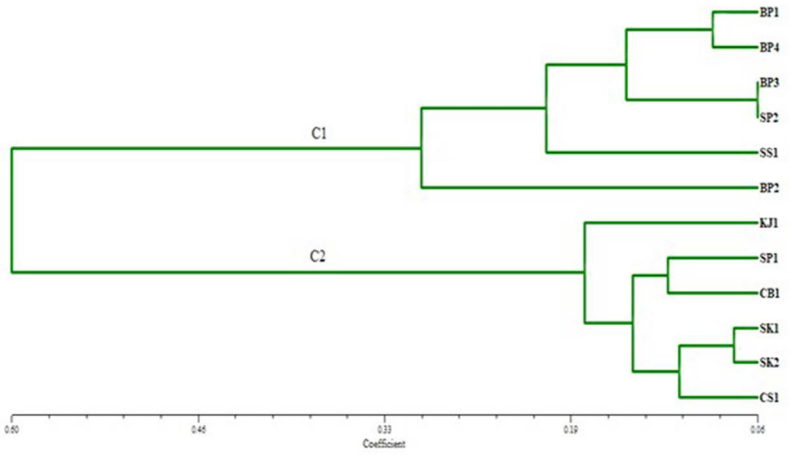

Fig. 2. UPGMA dendrogram constructed based on Nei's (1972) genetic distance summarizing the data on differentiation among 12 Naga chili samples by RAPD analysis.

The identification of genetic variability is crucial for future crop improvement (Ravi et al. 2003). In this study, the detection of polymorphism and genetic variation has been carried out among the twelve Naga chili samples using RAPD markers. Cluster and matrix represent that samples with the same source might have genetic similarities with some exceptions. Moreover, this study uncovered that samples in the different clusters possess genetic dissimilarities. The outcome of this present investigation is a probability of constructing a genetic map that will lead to the application of marker-assisted selection tools in the genetic improvement of Naga chili.

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