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Short Communication

Investigation of intraspecific diversity in *Capsicum chinense* using morphological and molecular markers

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Abstract

Sixty accessions of *Capsicum chinense* were investigated to reveal the genetic diversity based on morphological traits along with ISSR based molecular markers. Genetic distances for morphological traits and ISSR markers respectively ranged from 1.41 to 8.60 and 0.05 to 0.49. Fourteen ISSR markers showed mean of 53.84% polymorphism. The Mantel test revealed low correlation (r=0.34) between the genetic distances based on morphological and molecular markers. All the accessions of *Capsicum chinense* found genetically diverse which can be used for future crop improvement programme.

Key words: *Capsicum chinense,* genetic diversity, ISSR, morphological traits

Capsicum chinense Jacq. is known as 'Bhut jolokia' in Assam. The main pungency principle of *C. chinense* 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 [1]. Traditionally, *Capsicum* species are identified based on important morphological traits like number of branches per plant, plant height, number of fruits per plant, days to maturity flower morphology, including flower color, calyx constriction and the number of flowers per axil and fruit morphology, including fruit shape and size, colour etc [2]. But for accurate species identification and diversity study morphological markers coupled with molecular markers is important. Comparison to morphological markers, molecular markers have the more reliability for precise determination of genetic relatedness. Several different molecular markers have been used to assess genetic diversity in the genus *Capsicum* [3-6]. But no studies have been reported so far to reveal the correlation between the genetic distances based on morphological markers and ISSR based molecular markers in *C. chinense*. The aim of the present work was to study the genetic diversity as well as correlation between the morphological and genetic distances in *C. chinense*.

Sixty accessions of *C. chinense* were collected from different localities of Upper Brahmaputra valley, Assam with enough geographical representation. Seeds of *C. chinense* accessions were sown in raised seed beds and the seedling were transplanted in the experimental plots at departmental gene banks of Dibrugarh University in Randomized Block Design. Morphological data were collected following the morphological descriptors, established by the International Plant Genetic Resources Institute (IPGRI) for the genus *Capsicum* [7]. Total genomic DNA from the fresh young, tender leaves was extracted by following the CTAB procedure [8]. The quality and quantity of DNA was determined by comparing band intensity of DNA samples with the standard series of λ-DNA. Thirty five UBC-ISSR primers obtained from GCC Biotech (India) were initially screened. After screening, fourteen ISSR primers which generated reproducible and distinct bands were selected for the assay of genetic diversity.

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The polymerase chain reaction (PCR) was performed with an initial step of 5 min at 94^{o}C. Then the PCR reaction was followed by 36 cycles of 30 second at 94^{o}C for denaturation, 30 second at 45^{o}-60^{o}C (depending on the used primer) for annealing and 72^{o}C for Extension for 1minute. After completion of all the cycles a final 5 minute of extension step at 72^{o}C was programmed (Eppendorf, Germany). The polymerase chain reaction mixture containing 20ng/μl of template DNA, 10X PCR Buffer, 10mM of dNTPs, 2.5mM $MgCl_2$, 20μM ISSR Primer and Taq DNA polymerase (5units/μl). The final volume was made 25μl by adding de-ionized water. PCR amplified products were separated by 2% agarose gel electrophoresis in 1x TBE buffer at 80v for 80min. Gels were documented under UV light using a gel documentation system (Alpha Innotech, Alpha Imager, USA) with 100bp DNA ladder as marker.

Polymorphism percentage was calculated based on the banding pattern [9] and scored for presence and absence of the ISSR bands. The data were entered in to a binary matrix as discrete variables (1) for the presence of the amplification product or band and (0) for the absence of the band. A Dendrogram was constructed based on Jaccard's coefficient using the DARwin (version 5) software [10, 11]. The mantel matrix correspondence test [12] was used to compare the distance matrices obtained from morphological and molecular markers

Table 1. Details of ISSR primers used for the genetic characterization of 60 accessions of *C. chinense*

Primer	Nucleo-tide	No. of bands		Polymor-phism (%)
		Total	Poly-morphic	
UBC-812	$(GA)_8A$	4	2	50.00
UBC-814	$(CT)_8A$	3	2	66.67
UBC 818	$(CA)_8G$	5	3	60.00
UBC 830	$(TG)_8G$	7	4	57.14
UBC 836	(AG)YA	7	3	42.86
UBC 838	(TA)RC	6	3	50.00
UBC-840	$(GA)_8YT$	6	1	16.67
UBC 841	$(GA)_8YC$	4	1	25.00
UBC-842	$(GA)_8YG$	4	0	0.00
UBC-843	$(CT)_8RA$	6	5	83.33
UBC 848	$(CA)_8RG$	6	4	66.67
UBC 850	$(GT)_8YC$	8	7	87.50
UBC 852	$(TC)_8RA$	7	5	71.42
UBC 860	$(TG)_8RA$	5	2	40.00
	Total	78	42	
	Average	5.57	3.00	53.84

[Y=C/T; R=A/G]

The ISSR primers produced 78 score able bands out of which 42 bands were found to be polymorphic which constitute 53.84% polymorphism (Table 1). The present study showed that CA repeat sequences primers having primer codes UBC-818 and UBC-848 showed polymorphism rate of 60% and 66.67%, respectively. Similarly, GT repeat primer having primer code UBC-850 showed highest polymorphism rate *i.e.* 87.50%, with maximum eight distinct bands (Fig. 1). Lee (2004) [13] found CA/GT microsatellite as the most abundant and less polymorphic motif in comparison to other motif of pepper. So, ISSR primers

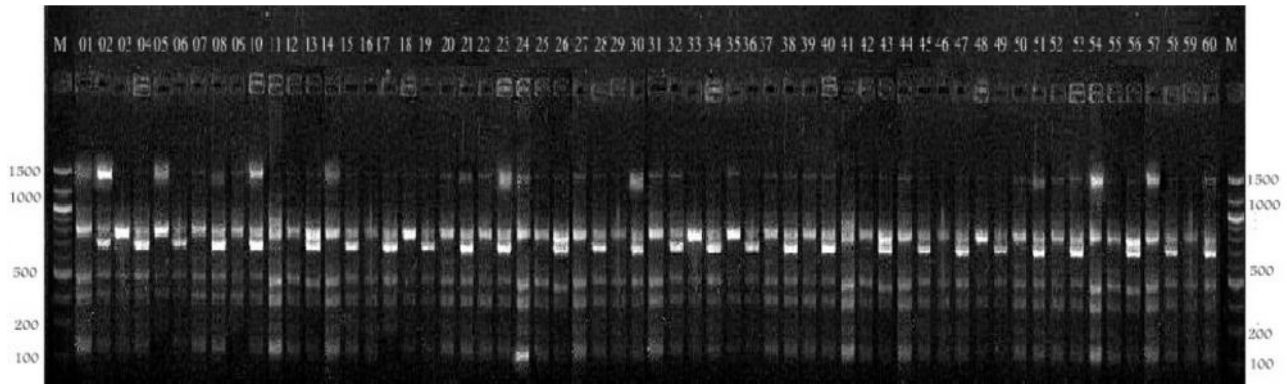

Fig. 1. Genetic diversity analyzed among the 60 accessions of *C. chinense* using ISSR primer (UBC-850), M for 100bp DNA ladder

targeted to these motif regions may be useful for inter repeat polymorphism study. The genetic distances were ranged from 0.05 to 0.49 and showing three different clusters (Fig. 2). The polymorphism percentage and genetic distances are higher among interspecific varieties in comparison to intrapecific varieties of *Capsicum*. In the earlier studies 47% of polymorphism found within *C. annum* accessions and 89% among different species of *Capsicum* [14]. So, depending upon pollination pattern, polymorphism rate may vary within and among the species.

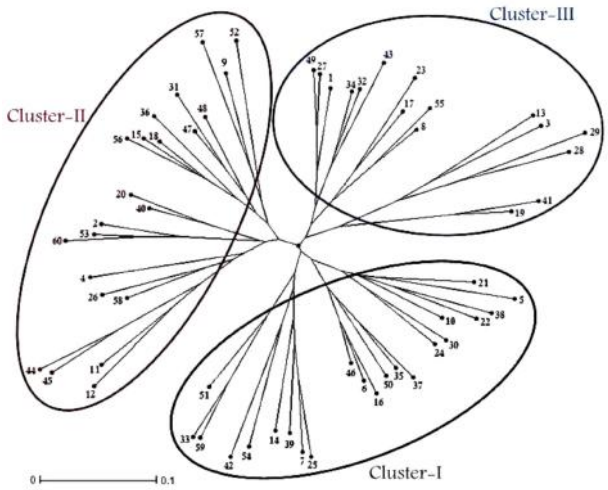

Fig. 2. Dendrogram of sixty *C. chinense* accessions showing 3 major clusters

In the present investigation distinct frequency variation was observed in morphological traits. Based on the morphological variation the dissimilarity values obtained from 1.41 to 8.60 and categorized all the sixty accessions into three main clusters. Dendrogram obtained from morphological as well as molecular markers showed no association between the clusters formed with geographic location from where the accessions were collected. Generally, geographical separation is considered as an important parameter to collect germplasm for diversity study, but the geographical separation is not always predict the genetic differences very clearly [15]. The lack of association may be due to the exchange of plant materials across the regions as *Capsicum chinense* is traditionally very popular, important and frequently used food crop.

However, morphological distances revealed low correlation with the genetic distances (r=0.34). The relationship between molecular markers and phenotypic traits could be significant if the markers are linked to selected loci [16]. The variation detected by molecular markers is non-adaptive so, no subject to either natural or artificial selection; on the other hand, the phenotypic characters are subject to both natural and artificial selection, aside from their high environmental dependence [17, 18]. *C. chinense* is highly influenced by environmental factors. Therefore, it would be prudent to collect accessions from different environmental conditions and geographic regions so as to get clear association of morphological and molecular markers data.

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