Original Research Article Website : www.ijbasr.org ISSN : 2349-1965
International Journal of Basic & Applied Science Research
[2014; 1 (1) pp 72-79]

EVALUATION OF GENETIC DIVERSITY IN BHUT JOLOKIA (*CAPSICUM CHINENSE* JACQ) ACCESSIONS USING ISSR MARKER

Hazarika R. and Neog B.
Department of Life Sciences, Dibrugarh University, Assam-786004, India

ABSTRACT :

The genetic diversity of thirty accessions of Bhut jolokia collected from three states of North-East India were studied for genetic diversity using Inter Simple Sequence Repeat (ISSR) marker. Genetic diversity analysis showed 54.5% of polymorphism with dissimilarity ranged from 0.08 to 0.43. Based on the banding pattern, the cluster analysis was done which distributed all the accessions in three distinct clusters. Present investigation revealed wide ranges of genetic variation and ISSR marker found to be useful tool for the diversity studies.

KEY WORDS : *Bhut Jolokia, Capsicum, Genetic diversity, Inter Simple Sequence Repeat (ISSR)*

INTRODUCTION:

The genus *Capsicum*, belongs to the family Solanaceae, has significant economic importance in worldwide due to their unique pungency and flavour. The agro-climatic conditions of North - East India are favourable for the cultivation of *Capsicum chinense* Jacq., (GBWR, 2006). It is known by various names in different regions such as 'Bhut/Bhot jolokia' or 'Bih jolokia' in Assam, 'Umorok' in Manipur and 'Ghost pepper' by the western media. Traditionally, *Capsicum* species are identified and characterized based on important morphological traits like number of branches per plant, plant height, number of fruits per plant, days to maturity, flower morphology and fruit morphology (Fekadu, *et al.*, 2008). However, in comparison to morphological markers, the molecular markers revealed genetic differences with more details without the interference caused by environmental effects (Arif, *et al.*, 2010; Leal, *et al.*, 2010 and Oliveira, *et al.*, 2010). Several studies have been conducted using molecular markers like RFLP, AFLP, ISSR and RAPD to assess the level of variation among *Capsicum* species (Prince, *et al.*, 1995; Paran, *et al.*, 1998; Rodriguez, *et al.*, 1999 and Ruanet, *et al.*, 2005). Generally, RAPD and ISSR technique has been widely used due to their simplicity, cost effectiveness and power to detect differences even among closely related

Corresponding Author :
Bijoy Neog
E-mail: bejoy_draj@yahoo.co.in
Date of Acceptance : 14.04.2014
Date of Publication : 20.04.2014

individuals (Jain, *et al.,* 1994; Hoey, *et al.,* 1996; Liu, *et al.,* 1999 and Kumar, *et al.,* 2001) but now a day's ISSR marker is widely used for genetic diversity analysis due to its high reproducibility than RAPD (Nagaoka, *et al.,* 1997; Ajibade, *et al.,* 2007; Galvan, *et al.,* 2003). Capsaicinoid the pungency principle of *Capsicum* is also affected by genetic make-up of the cultivar, weather conditions, growing conditions and fruit age (Aloni, *et al.,* 1999; Blum, *et al.,* 2003 and Stewart, *et al.,* 2005). Capsaicinoids is used in different pharmaceutical applications due to their analgesic, anti-arthritic, anticancer and antioxidant properties (Szolcsanyi, 2003; Prasad, *et al.,* 2005 and Mori, *et al.,* 2006). Populations with low genetic diversity at risk of genetic erosion, it is almost need to adapt measures to diversify the crop. So in the present investigation attempt was made to characterize genetic diversity in *Capsicum chinense* accessions using ISSR molecular marker from three states of North-East.

MATERIALS AND METHODS:

Plant materials and DNA Extraction

Thirty accessions of Bhut jolokia collected from three states of North - East region, *i.e.* Assam, Arunachal Pradesh and Manipur with enough geographical representation through an extensive survey between 2010 and 2012. Seeds of Bhut jolokia accessions were sown in raised seed beds. When seedlings had attained 10 to 15 cm of height (after approximately 60 days of sowing), transplanted in experimental plots in the main field in randomized block design with three replications for each accession. Spacing was 100cm between rows and 50cm between plants. The accession numbers with place of collection are given in Table 3. Morphological data were collected from six representative plants per accession based on the morphological descriptors, established by the International Plant Genetic Resources Institute (IPGRI) for the genus *Capsicum* (IPGRI, 1995). Total genomic DNA from the fresh young sterile leaves was extracted by following the CTAB Procedure (Doyle and Doyle, 1990). The concentration and quality of DNA was checked by spectrophotometer and agarose gel electrophoresis using 0.80% gel comparing with a standard series of DNA.

Genetic diversity Study:

Genetic diversity was studied using ISSR molecular marker.

ISSR amplification:

Five ISSR primers obtained from GCC Biotech (India) Pvt. Ltd. were used for ISSR based thermo profiling (Table -1) with a thermal cycler (Eppendorf, Germany) containing 25µl reaction mixture (Table - 2). Separation of PCR amplified products by 2% agarose gel electrophoresis in 1x TBE buffer at 80v for 2 hrs (Fig1). Gels were documented under UV light using a gel documentation system (Alpha InfoTech, Alpha Imager, USA).

Table 1. Thermo Profile for PCR

Sl. No	Step	Temperature (°C)	Duration	Number of cycles
01	Initial denaturation	94	5 min	1
02	Denaturation	94	30 sec	36
03	Annealing	42	30 sec	
04	Extension	72	1 min	
05	Final extension	72	5 min	1
06	Dump	4	For ever	-

Table 2. ISSR Reaction Mixture

Template DNA (20ng/µl)	5.0 µl
10X Buffer	2.5 µl
25mM $Mgcl_2$	1.0 µl
10mM dNTPs	0.5 µl
20µM ISSR Primer	1.0 µl
de-ionized Water	14.9 µl
Taq DNA polymerase (5units/µl)	0.1 µl
Total Volume	**25 µl**

Data analysis:

Based on the banding pattern, polymorphism percentage was calculated with five different primers (Blair, *et al.,* 1999). Genotypes were 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 and this matrix were subjected to further analysis. A Dendrogram was constructed based on Jaccard's coefficient with unweighted pair Neighbor - Joining method using the DARwin (version 5) software (Perrier, *et al.,* 2003 and Perrier and Jacquemoud-Collet, *et al.,* 2006).

RESULTS AND DISCUSSION:

Genetic diversity:

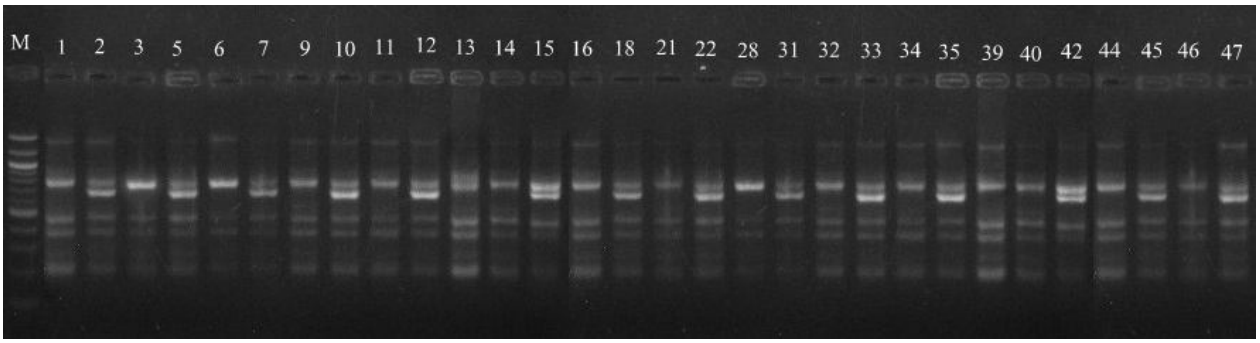
Genetic diversity and genetic relationship among the accessions of *Capsicum chinense* was analyzed using five different ISSR molecular markers. Out of total 33 bands, 18 bands were polymorphic with polymorphism percentage of 54.5% (Table - 4). The binary data from the polymorphic primers were used for computing Jaccard's coefficient which was found 0.08 to 0.43. Dendrogram obtained from ISSR showed three different clusters (Fig 2). Similarly, several researchers also found high degree of polymorphism among *Capsicum* germplasm through ISSR markers (Wang, *et al.,* 1998; Patel, *et al.,* 2011) and 47% of diversity found among *C. annuum* accessions and 89% among different species of *Capsicum* in previous studies (Thul, *et al.,* 2012). In our present study, higher polymorphism rate was found in UBC - 812, UBC - 18, UBC - 843 primers and the lower polymorphism rates were found in UBC - 840 and UBC - 842 primers. Similarly, in *Brassica* no amplification was found by these two primers (Gupta, *et al.,* 2004) and UBC - 840 primer showed less polymorphism in rice bean accessions (Muthusamy, *et al.,* 2008).

Table - 3 : Accession numbers with places of collection for 30 accessions of *C. chinense*

Sl. No	Accession Number	Place of collection	State
01	AC-001	Biswanath Chariali	Assam
02	AC-002	Nahorkotia	Assam
03	AC-003	Bishnupur	Manipur
04	AC-005	Imphal	Manipur
05	AC-006	Biswanath Chariali	Assam
06	AC-007	Baihata Chariali	Assam
07	AC-009	Dhemaji	Assam
08	AC-010	Dibrugarh University Campus	Assam
09	AC-011	Jokai (Dibrugarh)	Assam
10	AC-012	Namsai	Arunachal Pradesh
11	AC-013	Sonapur	Assam
12	AC-014	Dibrugarh	Assam
13	AC-015	Hajo	Assam
14	AC-016	Jorhat	Assam
15	AC-018	Jorhat	Assam
16	AC-021	Ledo	Assam
17	AC-022	Ledo	Assam
18	AC-028	Jagun	Assam
19	AC-031	Borgolai	Assam
20	AC-032	Borgolai	Assam
21	AC-033	Jagun	Assam
22	AC-034	Tirap-Gate-I	Assam
23	AC-035	Tirap-Gate-II	Assam
24	AC-039	Borgolai	Assam
25	AC-040	Khowang	Assam
26	AC-042	Dighola Gaon, Dibrugarh	Assam
27	AC-044	Borgolai	Assam
28	AC-045	Tirap District	Arunachal Pradesh
29	AC-046	Tirap District	Arunachal Pradesh
30	AC-047	Lohit District	Arunachal Pradesh

Table - 4 : Details of ISSR primers used for the genetic characterization of 30 accessions of *C. chinense*

Sl no	Primer	Nucleotide sequence (5`-3`)	No. of Bands			Polymorphism (%)
			Total	Monomorphic	Polymorphic	
1.	UBC-812	$(GA)_8A$	5	2	3	60.0
2.	UBC-818	$(CA)_8G$	8	2	6	75.0
3.	UBC-840	$(GA)_8YT$	7	5	2	28.5
4.	UBC-842	$(GA)_8YG$	5	5	0	0.0
5.	UBC-843	$(CT)_8RA$	8	1	7	87.5
Total			**33**	**15**	**18**	
Average			**6.6**	**3**	**3.6**	**54.5**



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

Fig1. Genetic diversity analysed among the 30 accessions of C. chinense using ISSR primer (UBC-843). Lane No. in gel corresponds to the No. of accessions listed in Table. 3 and M for 100bp DNA ladder.

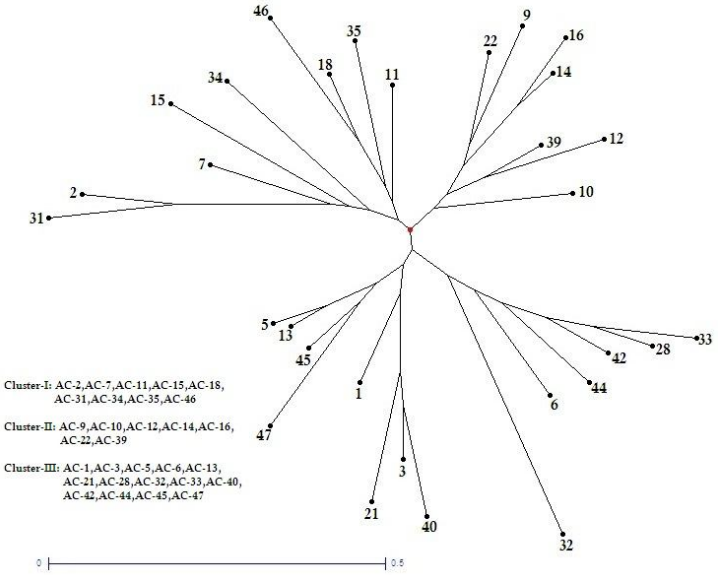


Fig2. Dendrogram using the software DARwin (version5) based on ISSR marker data

The present study showed that ISSR analysis is quick and reliable procedure for diversity analysis with sufficient polymorphism within the experimental population. The obtained clusters were not in accordance with the geographical location. The lack of association between geographic location and grouping may be due to wide dispersal and facultative cross pollination nature of *C. chinense* leading to the genetically similar background.

ACKNOWLEDGEMENT:

The authors are thankful to the Department of Biotechnology (DBT), Govt. of India, for the financial support to carry out the research work.

REFERENCES:

1. Ajibade S. R., Weeden N. F. and Michite S., Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna. Euphytica,* 2007; 111: 47 - 55.
2. Aloni B., Pressman E. and Karni L., The effect of fruit load, defoliation and night temperature on the morphology of pepper flowers and on fruit shape. *Ann. Bot.,* 1999; 83: 529-534.
3. Arif I. A., Bakir M. A., Khan H. A., Al Farhan A. H., Al Homaidan A. A., Bahkali A. H., Al Sadoon M. and Shobrak M. A., A brief review of molecular techniques to assess plant diversity. *Int. J. Mol. Sci.,* 2010; 11: 2079 - 2096.
4. Blair M. W., Panaud O. and Mc Couch S. R., Inter simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and finger printing in rice (*Oriza sativa* L.). *Theor. Appl. Genet.,* 1999; 98: 780 - 792.
5. Blum E., Mazourek M., O'Connell M., Curry J., Thorup T., Liu K., Jahn M. and Paran I., Molecular mapping of capsaicinoid biosynthesis genes and quantitative trait loci analysis for capsaicinoid content in *Capsicum. Theor. Appl. Gen.,* 2003; 108: 79 - 86.
6. Doyle J. J. and Doyle J. L., A rapid total DNA preparation procedure for fresh plant tissue. *Focus,* 1990; 12: 13 - 15.
7. Fekadu M., Lemma D., Singh H., Chemeda F. and Ronald S., Genetic components and heritability of yield and yield related traits in hot pepper. *Research Journal of Agriculture and Biological Science,* 2008; 4: 803 - 809.
8. Galvan M. Z., Bornet B., Balatti P. A. and Branchard M., Inter simple sequence repeat (ISSR) marker as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). *Euphytica,* 2003; 132: 297-301.
9. GBWR, (Guinness Book of World Records) Hottest Spice. 2006, www.guinnessworldrecords.com.
10. Gupta K., Prem D. and Singh Negi M., ISSRs: An efficient tool to characterize inter-specific F1 hybrids of *Brassica* species. Proc. IV Intern. *Crop science congress,* 2004 (www. cropscience. org. au.).

11. Hoey B. K., Crowe K. R., Jones V. M. and Polans N. O., A phylogenetic analysis of Pisum based on morphological characters, and allozyme and RAPD markers. *Theor. Appl. Genet.,* 1996; 92: 92 - 100.

12. International Plant Genetic Resources Institute (IPGRI). Descriptors for *Capsicum* (*Capsicum* spp.). International Plant Genetic Resources Institute (IPGRI), Rome, 1995.

13. Jain A., Bhatia S., Banga S. S., Prakash S. and Lakshmikuran M., Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationships to heterosis. *Theor. Appl. Genet.,* 1994; 88: 116-122.

14. Kumar D. L., Kathirvel M., Rao G. V. and Nagaraju J., DNA profiling of disputed chilli samples (*Capsicum annum*) using ISSR-PCR and FISSR-PCR marker assays. *Forensic Science International,* 2001; 116: 63 - 68.

15. Leal A. A., Mangolin C. A., do Amaral A. T. Jr, Gonçalves L. S., Scapim C. A., Mott A. S., Eloi I. B., Cordoves V. and Da Silva M. F., Efficiency of RAPD versus SSR markers for determining genetic diversity among popcorn lines, *Genet. Mol. Res.* 2010; 9: 9 - 18.

16. Liu Z. Q., Pei Y. and Pu Z. J., Relationship between hybrid performance and genetic diversity based on RAPD markers in wheat, *Triticum aestivum* L. *Plant Breeding,* 1999; 118: 119 - 296.

17. Mori A., Lehmann S., O'Kelly J., Kumagai T., Desmond J. C., Pervan M., McBride W. H., Kizaki M. and Koeffler H. P., Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. *Cancer Research,* 2006; 66: 3222 - 3229.

18. Muthusamy S., Kanagarajan S., and Ponnusamy S., Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (*Vigna umbellata*) landraces. *Electronic Journal of Biotechnology,* 2008; 11: 32 - 41.

19. Nagaoka T. and Ogihara Y., Applicability of inter simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theoretical and Applied Genetics,* 1997; 94: 597 - 602.

20. Oliveira E. C., Amaral Junior A. T., Gonçalves L. S., Pena G. F., Freitas Junior S. P., Ribeiro R. M., Pereira M. G., Optimizing the efficiency of the touchdown technique for detecting inter-simple sequence repeat markers in corn (*Zea mays*). *Genet. Mol. Res.*, 2010; 9: 835 - 842.

21. Paran I., Aftergoot E. and Shifriss C., Variation in *Capsicum annuum* revealed by RAPD and AFLP markers. *Euphytica,* 1998; 99: 167 - 173

22. Patel A. S., Sasidharan N., Ashish G. V. and Vinay K., Genetic relation in *Capsicum annuum* L. cultivars through microsatellite markers: SSR

and ISSR. Electr. *J. Plant Breed.*, 2011; 2: 67 -76.

23. Perrier X., Flori A. and Bonnot F., Data analysis methods. In. Genetic diversity of cultivated tropical plants. (ed. Hamon, P., Seguin, M., Perrier, X. ,Glaszmann, J. C.). *Enfield, Science Publishers. Montpellier:* 2003; 43 - 76.
24. Perrier X., Jacquemoud-Collet J.P. 2006. DARwin software http://darwin.cirad.fr
25. Prasad N. B. C., Shrivastava R. and Ravishankar G. A., Capsaicin: A promising multifaceted drug from *Capsicum* species. *Evidence based integrative medicine,* 2005; 2: 147 - 166.
26. Prince J. P., Lackney V. K., Angeles C., Blauth J. R. and Kyle M. M., A survey of DNA polymorphism within the genus *Capsicum* and the fingerprinting of pepper cultivar. *Genome,* 1995; 36: 404 - 417.
27. Rodriguez J. M., Berke T., Engle L. and Nienhuis J., Variation among and within *Capsicum* species revealed by RAPD markers. *Theor Appl Genet,* 1999; 99: 147 - 156.
28. Ruanet V. V., Kochieva E. Z. and Ryzhova N. N., The use of a self-organizing feature map for the treatment of the results of RAPD and ISSR analyses in studies on the genomic polymorphism in the genus Capsicum L., *Russian Journal of Genetics,* 2005; 41: 202 - 210.
29. Stewart C. J., Byoung C. K., Liu K., Mazourek M., Moore S. L., Yoo E. Y., Byung D. K., Paran I. and Jahn M. M., The Pun1 gene for pungency in pepper encodes a putative acyltransferase. *Plant J.,* 2005; 42: 675 - 688.
30. Szolcsanyi J., Future perspectives of capsaicin research. In. The Genus *Capsicum* (ed. A.K. De). *Taylor and Francis, London:* 2003; 248 - 269.
31. Thul S. T., Darokar M. P., Shasany A. K. and Khanuja S. P., Molecular profiling for genetic variability in *Capsicum* species based on ISSR and RAPD markers. *Molecular biotechnology,* 2012; 51: 137 - 147.
32. Wang J. J. and Fan M. J., Comparison on microsateliite DNA and RAPD markers for germplasm identification of *Capsicum annuum* L., *J. Agric. Res. China,* 1998; 47: 267 - 282.

***