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Intra- and interspecific genetic diversity exploration in chilli (*Capsicum* spp) using morphological and randomly applied polymorphic DNA markers

R BORGOHAIN[1], J DEVI[2] and R N SARMA[3]

Assam Agricultural University, Jorhat, Assam 785 013

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ABSTRACT

An experiment was conducted to study intra- and inter-specific genetic diversity during the winter season of 2001 and 2002 in 33 chilli (*Capsicum* spp) accessions of North-Eastern India belonging to 3 species using morphological characteristics and randomly amplified polymorphic DNA (RAPD) markers. Forty *Capsicum* descriptor-based characters were used for morphology based hierarchical cluster analysis, which delineated 2 major clusters. One of the cluster included all but 1 *C. annuum* L. accession, while the other had all the *C. chinense* Jacq. and *C. frutescens* L. accessions. However, accessions belonging to later 2 species were separated at sub-cluster level. For RAPD analysis, 20 random oligonucleotide primers were used and only 15 showed amplification. A total of 101 bands with a mean of 6.73 bands/primer were amplified in the test accessions out of which 84 were polymorphic. The fragment size ranged from 325 to 1 436 bp. The RAPD markers detected 83.17% polymorphism among the accessions. Two distinct clusters were delineated here too with all the *C. frutescens* accessions in one and *C. annuum* and *C. chinense* accessions in another. However, the *C. chinense* accessions were separated from *C. annuum* at sub-cluster level. Thus, the accession belonging to 3 species were differentiated by this technique and the clusters corresponded to previous morphology based classification of the species. Cultivar specific RAPD markers for eleven accessions were also detected in the study.

Key words : Chilli, *Capsicum* spp, Hierarchical clustering, RAPD, Poplymorphism

The genetic diversity and evolution within the genus *Capsicum* had been investigated using chromosome morphology (Pickersgill 1977), electrophoresis of soluble protein (Anu and Peter 2003), isozyme (Yamamoto *et al.* 2005) and molecular markers (Toquica *et al.* 2003). In each case, the cytological and molecular evidences, generally, confirmed previous species identification based on floral morphology and interfertility. Though some studies suggested low level of intra and inter specific DNA polymorphism in chilli (Toquica *et al.* 2003, Kochieva 2003, Votava *et al.* 2005), other studies however established the presence of high level of polymorphism (Prince *et al.* 1995, Lanteri *et al.* 2003). In the North East India, mainly 3 species of chilli, viz *C. annuum* L., *C. frutescens* L. and *C. chinense* Jacq. are grown and a myriad array of morphological variations are observed within each species. However, there is no systematic information available yet on the nature and extent of genetic diversity in these accessions. The present study was, therefore, undertaken to characterize the genetic diversity in 33 chilli accessions belonging to three species using morphological and RAPD markers.

[1,2,3]Associate Professor, Department of Plant Breeding and Genetics

MATERIALS AND METHODS

The materials comprised 33 chilli accessions collected from different parts of north-eastern India. Of these 21 accessions belonged to *C. annuum*, 6 to *C. chinense* and 6 to *C. frutescens*. The experiment was conducted at Jorhat, Assam during the winter season of 2000–2001and 2001–2002. Evaluation of the materials was done in 5 m × 2 m plots and seedlings were transplanted in 50 cm × 40 cm spacing.

For morphological based hierarchical cluster analysis 40 characters comprising 6 seedling, 11 vegetative, 20 inflorescence and fruit and 3 seed characters were used from the *Capsicum* descriptors published by International Plant Genetic Resources Institute (IPGRI). Of these, 9 were considered useful primarily in separating species while other characters included attributes that were helpful in morphological characterization within species. The scores or weightage as recommended for each of the qualitative character in the capsicum descriptors and actual numerical data for the quantitative characters were used for morpho-logical study.

For performing hierarchical cluster analysis from morphological data, Euclidean distance coefficient among the accessions were first calculated from the standardized (Z-score standardization) data, The cluster dendrogram was then drawn from the Euclidean distance matrix by unweighted pair

group method on arithmetic average(UPGMA).The clustering analysis was performed using computer software.

Out of 33 accessions, 24 were taken for RAPD analysis which included 16 from *C. annuum* and 4 each from *C. frutescens*. Total genomic DNA was extracted from leaf tissue of 25 days old plants following method of Dellaporta *et al.*(1983) and diluted to 10ng/µl for polymerase chain reaction(PCR) amplification.

Polymerase chain reaction was performed following Skorch *et al.* (1992) using 20 random decamer primers with slight modifications. Amplification was performed in a reaction volume of 15 µl containing 30 ng DNA template, 800 mM each of DNTPs, 0.2 mM of RAPD primers, 1 unit Taq DNA polymerase, 1.6 mM $Mgcl_2$ and 1 × PCR buffer. Amplification was performed in Gene Amp^R PCR system. Amplification condition for the first 2 cycles were 2 min at 94°C for denaturation, 1 min at 42°C for annealing and 2 min at 72°C for elongation. For the subsequent 38 cycles, denaturation was set for 1 min, annealing for 30 sec and elongation for 1 min. These 40 cycles were followed by a 4 min hold at 72°C. Amplified products were separated by electrophoresis in 1.5% agarose gel using Tris Borate EDTA (TBE) buffer at 50 V. The bands were scored on the basis of presence (1) or absence (0) without considering the intensity of the bands. Genetic relationship between the accessions were calculated using Nei's unbiased estimates of standard genetic distance and were represented by a dendrogram using unweighted pair group method based on arithmetic average (UPGMA) clustering algorithm (Nei 1987). The calculations were carried out using the software package Tools For Population Genetic Analyses (TFPGA) programmed by Miller (1997).

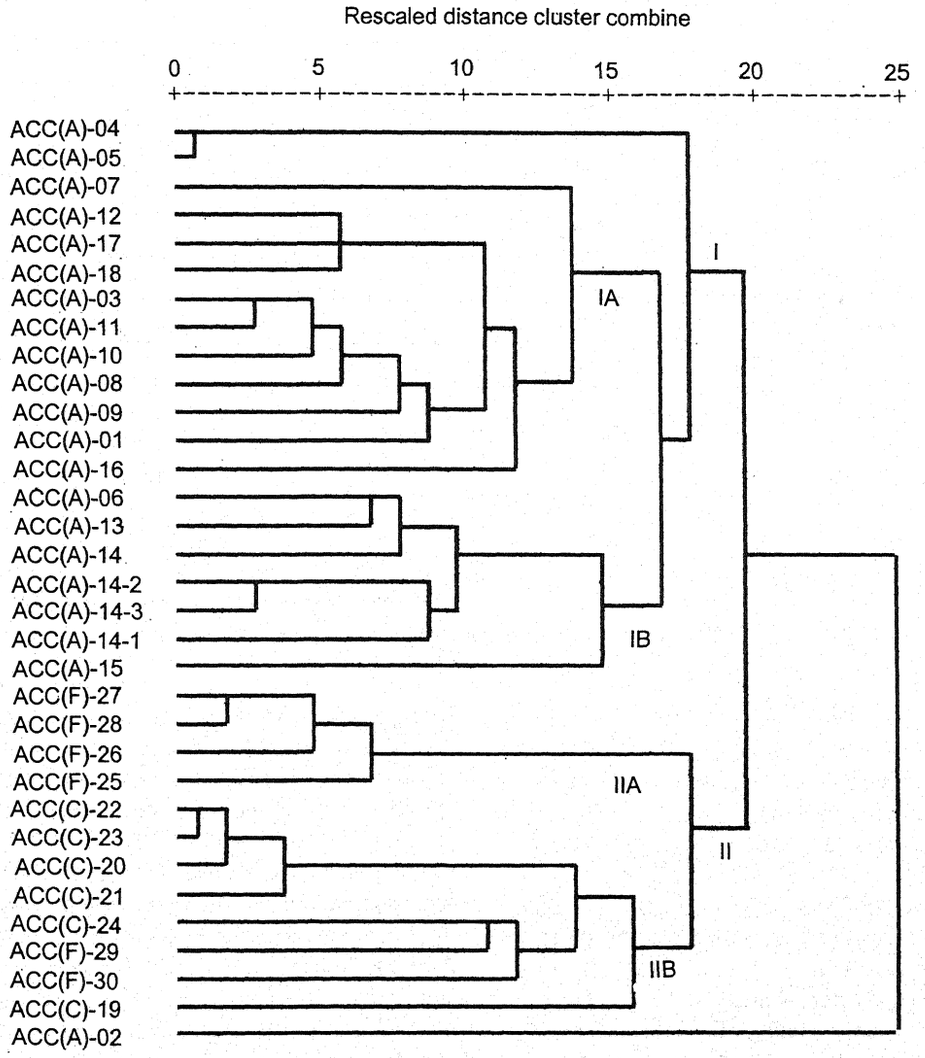

Fig 1 Dendrogram of 33 chilli cultivars based on average linkage cluster analysis using 40 morphological characters

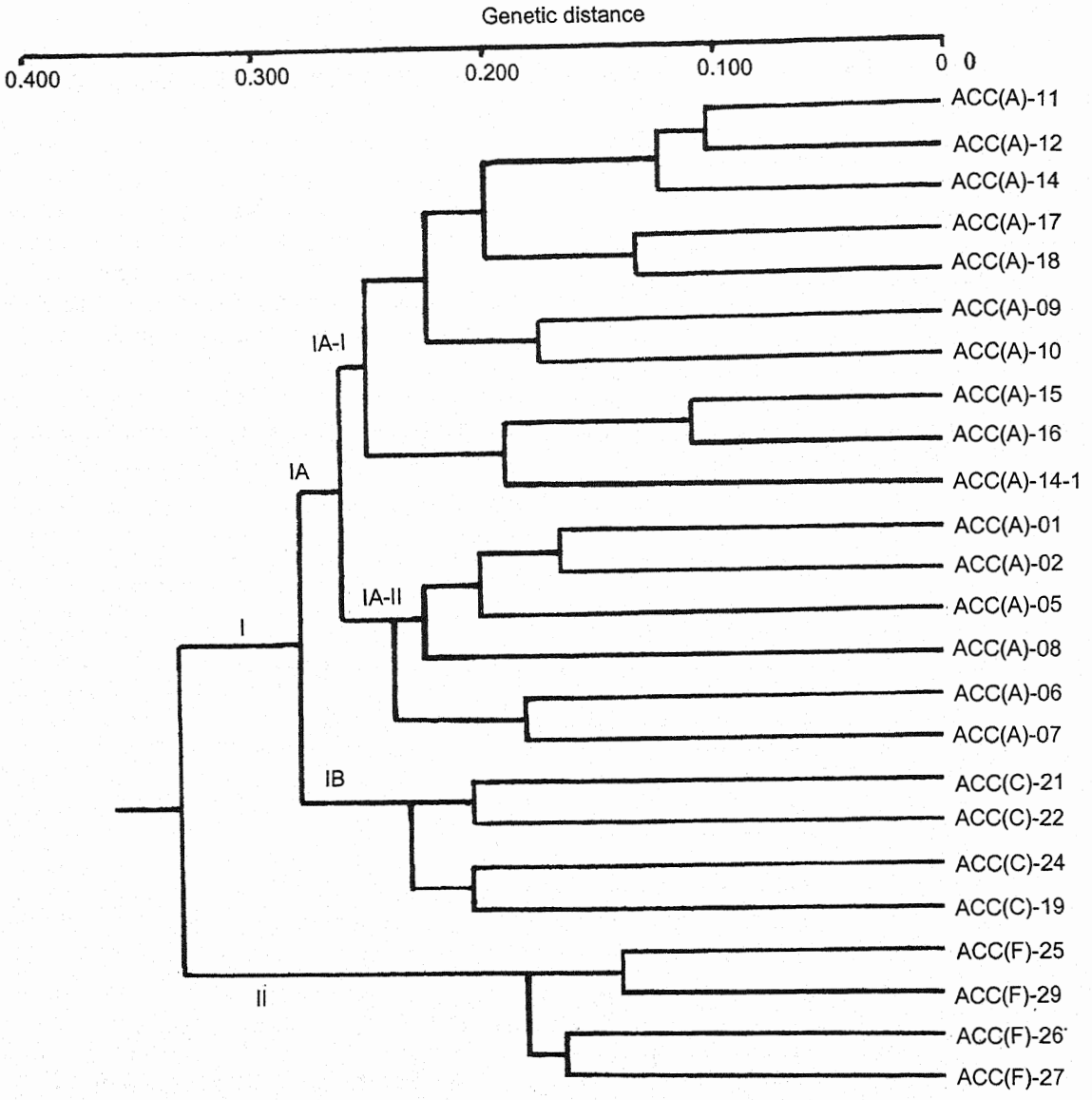

Fig 2 Dendrogram of 24 chilli accessions based on UPGMA cluster analysis using RAPD markers

RESULTS AND DISCUSSION

Hierarchical cluster analysis on morphological data delineated 2 major clusters (Fig 1), cluster I included all but one *C. annuum* accession, while cluster II included all the *C. frutescens* and *C. chinense* accessions. However, 3 distinct major clusters recognizing accessions of each species were expected, but *C. frutescens* and *C. chinense* accessions were separated only at the sub cluster level. Moreover, 3 of the *C. frutescens* accessions, viz ACC (F)-29 and ACC(F)-30, were grouped with the sub cluster formed by the *C. chinense* accessions. This discrepancy could be due to the fact that *C. annuum* , *C. frutescens* and *C. chinense* are closely related species (Pickersgill 1991)and morphological features between *C. frutescens* and *C. chinense* are strikingly similar. Thus, the present cluster analysis based on morphological characters could not distinguished between the accessions of these 2 species at the major cluster level. However, differences in specific characters between the 2 species lead to separate grouping at the sub cluster level.

The hierarchical cluster analysis, however, successfully separated majority of the accessions in to species level. It was also observed that except a few accessions [ACC(A)-04; ACC(F)-27,28 and ACC(C)-22,23], within group dissimilarity among the accessions of the 3 species were quite high, indicating a lot of morphological diversity. Despite this morphological heterogeneity, most of the accessions of the 3 species formed their own distinctive groups before fusing with the groups representing other species. Thus, the basic

Table 1 Per cent polymorphism revealed by RAPD analysis

Primer	Total number of bands	Monomorphic bands	Polymorphic of band	Per cent polymorphism
OPC-02	8	2	6	75.00
OPC-06	8	2	6	75.00
OPC-08	7	3	4	57.14
OPC-10	7	2	5	71.43
OPC-18	6	1	5	83.33
OPF-01	9	1	8	88.88
OPF-05	8	2	6	75.00
OPF-06	10	1	9	90.00
OPF-07	11	0	11	100.00
OPF-12	5	1	4	80.00
OPM-05	3	0	3	100.00
OPM-06	4	0	4	100.00
OPM-07	4	1	3	75.00
OPM-09	6	0	6	100.00
OPM-01	5	1	4	80.00
Total	101	17	84	83.17

Table 2 Cultivar specific markers detected by RAPD assay

Markers	Accessions distinguished
OPC-02 $_{(1230)}$	ACC(C)-24, ACC(F)-27
OPC-02 $_{(900)}$	ACC(F)-27
OPC-06 $_{(800)}$	ACC(F)-26
OPC-06 $_{(700)}$	ACC(F)-29
OPC-18 $_{(460)}$	ACC(F)-29
OPC-18 $_{(400)}$	ACC(A)-16
OPF-01 $_{(695)}$	ACC(A)-05
OPF-05 $_{(1180)}$	ACC(A)-05
OPF-06 $_{(800)}$	ACC(A)-16
OPF-09 $_{(890)}$	ACC(A)-10, ACC(C)-19
OPM-07 $_{(540)}$	ACC(F)-27
OPM-09 $_{(1000)}$	ACC(A)-07, ACC(A)-12, ACC(A)-14

Values in subscript represent approximate size of the fragments in bp

distinction among the 3 species had not been obscured in the analysis. The exclusion of one of the *C. annuum* accession [ACC (A)-02] from any of the two major cluster was, apparently, due to the fact that it was a sweet pepper accession, as a result, it exhibited differences in some characters, especially fruit characters, compared to other accessions.

Out of 20 decamer random primers used for amplification of DNA sequences from 24 accessions of chilli, 5 did not show any amplification or polymorphism and were excluded from the analysis. The remaining 15 primers amplified a total of 101 bands out of which 84 were polymorphic (Table 1) and 3 to 11 bands/primer were produced with a mean of 6.73 (Choe *et al.* 1998). The size of the fragments ranged from 325–1 436 bp (Rodriguez *et al.* 1999, Votava and Bosland 2001).

A high level of polymorphism (83.17%) was detected among the 24 accessions as compared to only 11.9 and 16.5% polymorphism observed by Toquica *et al.* (2003) and Kochieva and Ryzhova (2003) respectively. This may be due to their studies included either selected breeding lines or closely related commercial varieties which naturally represented only a small portion of diversity in chilli. On the other hand, high level of polymorphism in the present study seemed plausible on the basis of the assumption that diverse accessions were introduced in the North-East India over a long period of time. The subsequent genetic drift owing to small initial population size coupled with changed environment and farmer selection might have led to the variations in coding and noncoding regions.

Based on genetic distance, the accessions exhibited 2 distinct clusters (Fig 2) with all 4 *C. frutescens* accessions in one; and the *C. annuum* and *C. chinense* accessions in another. However, within the second major cluster the *C. chinense* accessions were grouped into a prominent subcluster separated from the *C. annuum* accessions. The inclusion of *C. chinense* in a major cluster along with the *C. annuum* accessions instead of a completely independent cluster is attributable to the close genetic relationship between the three species. Moreover, occasional cross fertilization could also occur between any two of the species and interspecific hybrid origin of *C. chinense* would make them even closer to one another. Because of the close genetic relationship among the 3 species, earlier studies based on allozymic variation (Jensen *et al.* 1979) and RFLP analysis (Prince *et al.* 1995) also placed the three species in an overlapping group.

The present grouping of the accession of the three species based on RAPD analysis supported the identification of these species based on floral morphology and interfertility originally suggested by Smith and Heiser (1951) and Legg and Lippert (1966). Moreover, the grouping of the accessions in to 3 species on the basis of morphological characters (Fig 1) was confirmed by RAPD analysis. Thus, there was a general consistency between the pattern of morphological and DNA variation at species level. However, relationship among the accessions under each species was variable in RAPD based grouping from that of morphology based one. This discrepancy was obvious because morphological variations presumably arose from the effect of only a few loci but DNA markers are distributed throughout the genome embracing coding and noncoding regions and DNA markers like RAPD may not always represent the loci expressing particular morphological character.

The RAPD marker system also detected cultivar specific markers for eleven accessions (Table 2), viz 6 of *C. annuum*, 2 of *C. chinense* and 3 of *C. frutescens*. The species diagnostic marker which is defined with a frequency of ≤ 0.50 among the accessions of a particular species but is absent in accessions of all other species (Rodriguez *et al.* 1999) could not be identified in this study. To detect species specific diagnostic marker, a more rigorous search for polymorphism using more number of primers would be necessary.

REFERENCES

Anu A and Peter K V. 2003. Analysis of seed protein of 29 lines of *Capsicum annuum* L. by polyacrylamide gel electrophoresis. *Genetic Resources and Crop Evolution* **50**(3) : 239–43.

Choe Y W, Park D Y and Suh K H. 1989. Pepper (*Capsicum annum* L.) cultivar identification and seed purity test by RAPDs and phosphoglucomutase (PGM) isozyme. *RAD Journal of Horticultural Science* **40**(1) : 15–23.

Dellaporta S L, Wood J and Hicks J B. 1983. A plant DNA mini preparation, Version II. *Plant Molecular Biology Reporter* **1** : 19–21.

Jensen R J, McLeod M J, Eshbaugh W H and Guttman S J. 1979. Numerical taxonomic analysis of allozyme variation in capsicum(Solanaceae). *Taxon* **28**(2) : 315–27.

Kochieva E Z and Ryzhova N N. 2003. Molecular AFPL analysis of genotypes of pepper (*Capsicum annuum*) cultivars. *Russian Journal of Genetics* **39**(12) : 1 345–8.

Lanteri S, Acquadro A, Quagliotti L and Portis E. 2003. RAPD and AFLP assessment of genetic variation in land race of pepper (*Capsicum annuum* L.) grown in North-West Italy. *Genetic Resources and Crop Evolution* **50** : 723–35.

Legg P D and Lippert L F. 1966. Estimates of genetic and environmental variability in a cross between two strains of pepper (*C. annuum*). *Proceedings of American Society of Horticultural Sciences* **89** : 443–8.

Miller M P. 1997. Tools for population genetic analyses(TFPGA) 1.3: A windows Program for analysis of allozyme and molecular population genetic data. *Computer software distributed through net (http://www.herb.bio.nau.edu/miller/index.html.).*

Nei M. 1987. *Molecular Evolutionary Genetics*, pp 149–207. Colombia University Press, New York, USA.

Pickersgill B.1997. Chromosomes and evolution in *Capsicum*. (*in*) *Capsicum*, pp 77. Pochard E (Ed). INRA Montfavet-Avignon, French.

Pickersgill B. 1991. Cytogenetics and evolution of *Capsicum*. (*in*) *Chromosome Engineering in plants, Part-B*, pp 139–60. Tsuchiya T and Gupta P K, Elsevics Science Publishers.

Prince J P, Lackney V K, Angeles C, Blauth J R and Kyle M M.1995. A survey of DNA polymorphism within the genus capsicum and fingerprinting of pepper cultivars. *Genome* **38**(2) : 224–31.

Rodriguez J M, Berke T, Engle L and Neinhuis J. 1999. Variation among and within capsicum species revealed by RAPD markers. *Theoretical and Applied Genetics* **99** : 147–56.

Skorch P W, Tivang J and Nienhuis J. 1992. Analysis of genetic relationship using RAPD marker data. (*in*) *CSSA-ASHS-AGS. Joint Plant Breeding Symposium Series*.Minneapolis, Crop Science Society of America, Madison, WI, USA, pp 26–3.

Smith P G and Heiser C B. 1951. Taxonomic and Genetic studies on cultivated pepper *Capsicum annuum* L. And *C. frutescens* L. *American Journal of Botany* **38** : 362–8.

Toquica S P, Rodriguez F, Martinez E, Duque M C and Tohme J. 2003. Molecular characterization by AFLPs of capsicum germplasm from the Amazon department of Colombia. *Genetic resources and crop evolution* **50** : 639–47.

Votava E J and Bosland P W. 2001. Genetic diversity of *Capsicum pubescens* revealed via RAPD analysis. *Capsicum and eggplant Newsletter* **20** : 60–3.

Votava E J Baral J B and Bosland A P. 2005. Genetic diversity of chile (*Capsicum annuum* var *annuum* L.) landraces from Northern New Msexico, Colorado and Mexico. *Economic Botany* **59**(1) : 8–7.

Yamamoto S and Nawata E. 2005. *Capsicum frutescens* L in South East and East Asia and its dispersal routes into Japan. *Economic botany* **59**(1) : 18–28.