# RAPD Marker Assisted Study on Genetic Diversity of Indigenous Chilli (*Capsicum* sp.) Landraces of Nagaland, India

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**Article History**

Manuscript No. 328
Received in in 19th June, 2012
Received in revised form in 6th December, 2012
Accepted in final form 5th March, 2013

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***Keywords***

NCL, RAPD markers, similarity matrix, dendrogram, genetic diversity

**Abstract**

Random Amplified Polymorphic DNA (RAPD) analysis was carried out with sixteen promising chilli landraces comprising of three *Capsicum chinense*, five *Capsicum frutescens* and eight *Capsicum annuum* to study the inter and intra-specific genetic diversity. The RAPD analysis produced a total of 114 bands, out of which 109 bands were polymorphic indicating high level of polymorphism (95.6%). The average PIC value of the primers was 0.75 indicating that the markers were able to distinguish a high degree of variation. Jacquard's similarity matrix constructed using Software NTSYS-pc, version 1.7. Rohlf (1992) showed a similarity coefficient ranging from 2.682-8.378, indicating high degree of variability among the chilli landraces evaluated. Dendrogram generated from Jaccard's similarity coefficient matrix using UPGMA clustering method showed two major clusters. Cluster I include three *Capsicum chinense* and cluster II included both *Capsicum annuum* and *Capsicum frutescens* landraces. Cluster II was further sub-divided into two sub-clusters, Sub-cluster II A included five *Capsicum frutescens* landraces and sub-cluster II B included eight *Capsicum annuum* landraces. The grouping of the three species viz. *Capsicum chinense, Capsicum frutescens* and *Capsicum annuum* into three separate clusters at species level by the molecular analysis corresponds and support the morphology based grouping of the three chilli species by Smith and Heiser (1951). The RAPD marker system detected two unique bands detected by primer OPA-01 for chilli landrace NCL-05 (*Capsicum frutescens*) and Primer OPA-02 detected one band, unique to all chilli landraces belonging to *Capsicum chinense*.

## 1. Introduction

Genetic markers are of great value in breeding programme and research. Genetic diversity in a crop species is basic to the improvement of the species and can be estimated at the molecular level. Among the different types of DNA markers used for genetic analysis, RAPD analysis is fast as these markers are easily generated by PCR (Williams et al., 1990). RAPD is not only the fastest, simplest and less costly, but a single primer can identify multiple loci in a single reaction (Karp et al., 1997).Factors such as speed, efficiency and amenability to automation, make RAPD a useful method for effective germplasm management with respect to estimating diversity, monitoring genetic erosion and removing duplicates from germplasm collection (Virk et al., 1995). Genetic diversity study using molecular markers in *Solanaceae* has been reported by many workers (Singh et al., 2006; Borgohain et al., 2005; Votava and Bosland, 2001; Prince et al., 1995).

Nagaland possesses wide array of varietal types of chilli landraces including those growing in wild. Till date no systematic effort has been made to study the genetic diversity and to characterize and classify the indigenous chilli landraces of Nagaland. The present study was therefore undertaken to investigate the diversity at molecular level using RAPD markers. Characterization of germplasm at molecular level would be of great help to horticulturist and breeders in breeding and selecting outstanding varieties, with desirable characters.

## 2. Materials and Methods

Sixteen economically important chilli landraces of Nagaland comprising of three *Capsicum chinense*, five *Capsicum*

*frutescens* and eight *Capsicum annuum* were selected for RAPD analysis. The investigation was carried out in the research farms of Department of Genetics and Plant Breeding (25º45' 43" N 93º52'285" E), School of Agricultural Sciences and Rural Development (SASRD), Medziphema Campus, Nagaland University, Nagaland, India, during the *rabi* season of 2005-06. The farm is situated about 310 meters above mean sea level and receives annual rainfall of 2000 mm.

Accession number was assigned to each of them. The abbreviated accession code 'NCL' stands for Nagaland Chilli Landrace. The different chilli landraces along with their accession number, vernacular name, and place of collection are presented in Table 1. Twenty random oligonucleoitideprimers were used in the present investigation (Table 2).

Total genomic DNA was isolated from 30-35 days old seedlings of the sixteen chilli landraces using the mini protocol for purification of total DNA from plant tissue. For optimization of RAPD protocol, PCR reaction was performed for several times with different DNA concentrations and different PCR programmes. The final PCR programme followed for amplification of the isolated DNA was 94 ºC-4 min, another 45 cycles of 94 ºC-1min, 35 ºC-1 min, 72 ºC-2 min, 72 ºC-5 min, 4 ºC-infinity . The reaction mixture for PCR and their concentrations are as follows 2x Master Mix-12.5 µ l, Primer-01.0 µ l, DNA Sample-01.0 µl, Nuclease free water-10.5 µ l. DNA amplification was carried out in Applied Biosystems Gene Amp® PCR system 9700.The amplified products were then subjected to agarose gel electrophoresis in 1 X TAE Buffer at 50 V. After the run, the gel was observed in a gel documentation system (Alpha Imager® HP) and the photograph was captured by a digital camera attached to the system. For each individual primer, the PCR products were sequentially designated from slow to fast migrating bands on the basis of approximate base pairs as determined from the marker DNA. Data were scored as discrete variables, using 1 to indicate presence and 0 to indicate absence of the band. Jaccard's similarity coefficient matrix was generated from RAPD data and dendrogram was generated by UPGMA using Software NTSYS-pc, version 1.7 (Rohlf, 1992).

Table 1: List of chilli landraces with vernacular names and places of collection

| Sl. No | Accession No | Vernacular name | Place of collection |
|---|---|---|---|
| 1 | NCL - 01(C) | Malchatom(Ku) | Athibung |
| 2 | NCL -02(C) | Malchapom(Ku) | Peren |
| 3 | NCL -03(C) | Kedi-chusi (Ang) | Medziphema |
| 4 | NCL -04(F) | Machii-nnuko (Lo) | Medziphema |
| 5 | NCL -05(F) | Sangpangmerutsu II (Ch) | Changki |
| 6 | NCL -06(F) | Mekheri Machi (Lo) | Dimapur |
| 7 | NCL -07(F) | Malcha(Ku) | Jalukie |
| 8 | NCL -08(F) | Kuki-merutsu (Ch) | Medziphema |
| 9 | NCL-09(A) | Chakro-Chusi-I(Ang) | Medziphema |
| 10 | NCL-10(A) | Chakro-Chusi-II(Ang) | Chumukedima |
| 11 | NCL-13(A) | Khonoma-Chusi (Ang) | Khonoma |
| 12 | NCL-18(A) | Kyong-Macjhi(Lo) | Wokha |
| 13 | NCL-19(A) | Aor-mersu (Ao) | Mokokchung |
| 14 | NCL-20(A) | Machi-enyoko I (Lo) | Dimapur |
| 15 | NCL-27(A) | Machi-enyoko II (Lo) | Medziphema |
| 16 | NCL-28(A) | Machi-enyoko III (Lo) | Dimapur |

(A=annuum; F=frutescens; C=chinense) (Ang = Angami; Ao=Ao; Ku= Kuki; Lo=Lotha; Rg= Rengma; Ch= Changki);

### 3. Results and Discussion

Sixteen out of the twenty primers produced scorableamplicons. Four primers *viz.* OPA-4, OPA-6, OPA-17 and OPA-19 which did not produce any scorable amplification were dropped and the remaining primers were used for further analysis. The RAPD analysis using sixteen random oligonucleotide primers produced a total of 114 bands. The number of bands for each primer varied from five (OPA-03, 07 and OPA-11) to twelve (OPA-13). OPA-13 produced the maximum number of bands (12). On an average 7.12 bands per primers were produced. Out of the 114 bands 109 bands were polymorphic indicating a very high degree of polymorphism. Studies have shown the presence of high level of polymorphism in chilli (Lanteri et al., 2003; Prince et al., 1995). The total percent polymorphism detected by the RAPD analysis was 96.05% (Table 2). Similarity, high level of polymorphism 83.17% and 87% was also detected by Borgohain et al., (2005) and Votava and Bosland (2001) respectively in *Capsicum* sp. The high level of polymorphism observed in the chilli landraces of Nagaland may be due to cross pollination and the fact that diverse chilli cultivars were introduced into the state over a long period of time. The number of polymorphic bands (markers) per primer varied from five (OPA-01, 03, 07, 08, & OPA-11) to ten (OPA-13) with a mean of 6.68. Primers OPA-02, OPA-03, OPA-05, OPA-07, OPA-10, OPA-11, OPA-12, OPA-14, OPA-15, OPA-16, OPA-18 and OPA-20 were 100% polymorphic, while for primers OPA-01, OPA-08, OPA-09, and OPA-13 above 80% polymorphism were detected.

Average PIC value of the primers was 0.75 indicating that the markers were able to distinguish a high degree of variation.

Polymorphism content (PIC) value of each marker (primer) was calculated using the formula: $1 - \sum x^2$ of allele frequencies at each locus and each band was taken as one locus. All the molecular works were carried out in the department of Genetics and Plant Breeding Division, Indian Council of Agricultural Research (ICAR) for North-East Region, Umiam, Shillong, Meghalaya.

Table 2: Details of RAPD Analysis and its value and percentage

| Details of oligonucleotides primers used in RAPD analysis | | | | Percent polymorphism revealed by individual primers in RAPD analysis. | | | | | PIC value of primers used in RAPD analysis: | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sl. No. | Accession No | Sequence | GC % | Primers | Total number of bands | Monomorphic bands | Polymorphic bands | Percent Polymorphism | Primers | Locus amplified | PIC value |
| 1 | OPA-01 | CAGGCCCTTC | 70 | OPA-01 | 06 | 1 | 05 | 83 | OPA-01 | 6 | 0.76 |
| 2 | OPA-02 | TGCCGAGCTG | 70 | OPA-02 | 08 | 0 | 08 | 100 | OPA-02 | 8 | 0.76 |
| 3 | OPA-03 | AGTCAGCCAC | 60 | OPA-03 | 05 | 0 | 05 | 100 | OPA-03 | 5 | 0.58 |
| 4 | OPA-04 | AATCGGGCTG | 60 | | | | | | | | |
| 5 | OPA-05 | AGGGGTCTTG | 60 | OPA-05 | 08 | 0 | 08 | 100 | OPA-05 | 8 | 0.82 |
| 6 | OPA-06 | GGTCCCTGAC | 70 | | | | | | | | |
| 7 | OPA-07 | GAAACGGGTG | 60 | OPA-07 | 05 | 0 | 05 | 100 | OPA-07 | 5 | 0.79 |
| 8 | OPA-08 | GTGACGTAGG | 60 | OPA-08 | 06 | 01 | 05 | 83 | OPA-08 | 6 | 0.58 |
| 9 | OPA-09 | GGGTAACGCC | 70 | OPA-09 | 08 | 01 | 07 | 87.5 | OPA-09 | 8 | 0.77 |
| 10 | OPA-10 | GTGATCGCAG | 60 | OPA-10 | 08 | 0 | 08 | 100 | OPA-10 | 8 | 0.80 |
| 11 | OPA-11 | CAATCGCCGT | 60 | OPA-11 | 05 | 0 | 05 | 100 | OPA-11 | 5 | 0.65 |
| 12 | OPA-12 | TCGGCGATAG | 60 | OPA-12 | 06 | 0 | 06 | 100 | OPA-12 | 6 | 0.73 |
| 13 | OPA-13 | CAGCACCCAC | 70 | OPA-13 | 12 | 02 | 10 | 83.3 | OPA-13 | 12 | 0.48 |
| 14 | OPA-14 | TCTGTGCTGG | 60 | OPA-14 | 07 | 0 | 07 | 100 | OPA-14 | 7 | 0.89 |
| 15 | OPA-15 | TTCCGAACCC | 60 | OPA-15 | 07 | 0 | 07 | 100 | OPA-15 | 7 | 0.91 |
| 16 | OPA-16 | AGCCAGCGAA | 60 | OPA-16 | 08 | 0 | 08 | 100 | OPA-16 | 8 | 0.88 |
| 17 | OPA-17 | GACCGCTTGT | 60 | | | | | | | | |
| 18 | OPA-18 | AGGTGACCGT | 60 | OPA-18 | 07 | 0 | 07 | 100 | OPA-18 | 7 | 0.72 |
| 19 | OPA-19 | CAAACGTCGG | 60 | | | | | | | | |
| 20 | OPA-20 | GTTGCGATCC | 60 | OPA-20 | 08 | 0 | 08 | 100 | OPA-20 | 8 | 0.91 |
| | | | | | | | Average | 96.05 | | | 0.75 |

PIC value was highest for OPA-15 & OPA-20 followed by OPA-14 and OPA-16. Thus these markers would be very useful for genetic diversity studies. On the other hand PIC value was lowest in OPA13. PIC values were also quite low for OPA-08, OPA-03 and OPA-11.

Jaccard's similarity coefficient matrix (Table 3) constructed based on the RAPD data, revealed similarity coefficient ranging from 2.682-8.378, indicating high degree of variability among the chilli landraces evaluated. The genetic relationship among the landraces were displayed in the form of dendrogram (Figure 1) generated based on Jaccard's similarity coefficient matrix using UPGMA clustering method. Two major clusters were revealed by the dendrogram. Cluster I include three *Capsicum chinense* and cluster II included landraces from both *Capsicum annuum* and *Capsicum frutescens*. Cluster II was further sub-divided into two sub-clusters, sub-cluster II A and sub-cluster II B. Sub-cluster II A included five chilli landraces belonging to *Capsicum frutescens* and sub-cluster II B included eight *Capsicum annuum*.

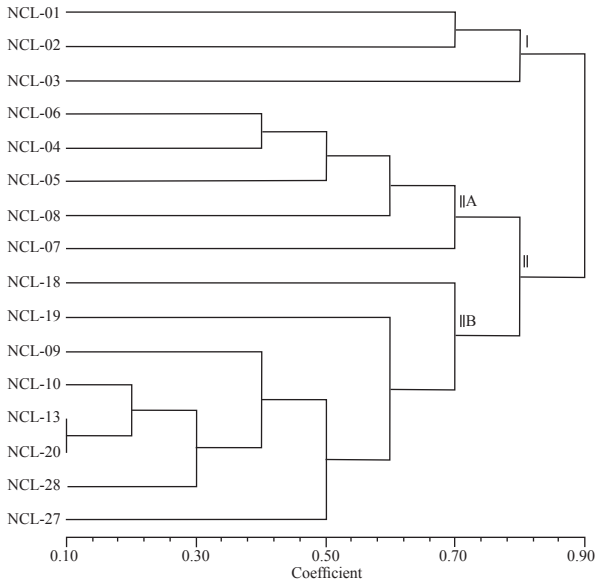


Figure 1: Dendrogram of 16 chilli landraces constructed from the Jaccard's genetic similarity matrix based on RAPD data.

Table 3: Jaccard's similarity coefficient matrix of 16 chilli landraces derived from RAPD data

| Sl. No. | Landraces | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | NCL-01(C) | 1.000 | | | | | | | | | | | | | | | |
| 2. | NCL-02(C) | 7.272 | 1.000 | | | | | | | | | | | | | | |
| 3. | NCL-03(C) | 5.901 | 5.645 | 1.000 | | | | | | | | | | | | | |
| 4. | NCL-06(F) | 3.815 | 3.766 | 3.544 | 1.000 | | | | | | | | | | | | |
| 5. | NCL-04(F) | 4.027 | 4.166 | 4.189 | 7.611 | 1.000 | | | | | | | | | | | |
| 6. | NCL-05(F) | 3.500 | 4.285 | 4.050 | 7.083 | 7.352 | 1.000 | | | | | | | | | | |
| 7. | NCL-07(F) | 4.533 | 4.145 | 5.066 | 5.641 | 6.351 | 6.075 | 1.000 | | | | | | | | | |
| 8. | NCL-08(F) | 2.976 | 3.214 | 3.827 | 6.486 | 7.101 | 6.282 | 5.609 | 1.000 | | | | | | | | |
| 9. | NCL-18(A) | 3.600 | 0.506 | 3.250 | 3.255 | 3.670 | 4.000 | 4.457 | 3.793 | 1.000 | | | | | | | |
| 10. | NCL-19(A) | 3.194 | 3.472 | 2.894 | 3.012 | 2.682 | 3.294 | 2.954 | 2.808 | 4.657 | 1.000 | | | | | | |
| 11. | NCL-09(A) | 3.846 | 3.797 | 3.333 | 3.678 | 3.975 | 3.707 | 3.820 | 3.977 | 4.556 | 5.571 | 1.000 | | | | | |
| 12. | NCL-10(A) | 4.050 | 3.780 | 3.522 | 3.863 | 3.975 | 4.000 | 4.111 | 4.111 | 5.657 | 6.197 | 7.083 | 1.000 | | | | |
| 13. | NCL-13(A) | 3.875 | 3.780 | 3.536 | 4.418 | 4.252 | 4.157 | 4.555 | 4.545 | 5.128 | 6.056 | 7.027 | 8.260 | 1.000 | | | |
| 14. | NCL-20(A) | 3.764 | 3.522 | 3.720 | 4.772 | 4.651 | 4.673 | 4.725 | 5.056 | 5.180 | 5.584 | 5.975 | 7.532 | 8.378 | 1.000 | | |
| 15. | NCL-27(A) | 3.661 | 3.561 | 3.026 | 4.666 | 4.078 | 4.024 | 3.975 | 3.975 | 4.594 | 4.454 | 6.086 | 5.833 | 6.714 | 6.266 | 1.000 | |
| 16. | NCL-28(A) | 4.285 | 3.600 | 3.246 | 4.375 | 3.855 | 3.604 | 4.285 | 4.096 | 5.970 | 5.147 | 5.972 | 7.462 | 7.605 | 7.162 | 6.060 | 1.000 |

(A=annuum, F=frutescens, C=chinense). A= NCL-01 (C); B= NCL-02 (C); C= NCL-03 (C); D= NCL-06 (F); E= NCL-04 (F); F=NCL-05 (F); G= NCL-07 (F); H= NCL-08 (F); I= NCL-18 (A); J= NCL-19 (A); K= NCL-09 (A); L= NCL-10 (A); M= NCL-13 (A); N= NCL-20 (A); O= NCL-27 (A); P= NCL-28 (A);

Genotypes in the cluster of *Capsicum chinense* were quite variable and their similarity was only 30-40%. Genotypes in the cluster of *Capsicum frutescens* were also highly variable and their similarity ranged from 30-60%. Similar variation was also seen in the genotypes of cluster of *Capsicum annuum*. However, two genotypes NCL-13(*C. annuum*) and NCL-20(*C. annuum*) were almost similar. The present grouping of the three species based on RAPD analysis supports the identification of species based on floral morphology and inter-fertility originally suggested by Smith and Heiser (1951) and Lippert (1966).

Further it was observed that collections originating from various parts of the state did not form well distinct groups and were interspersed with each other, indicating no association between RAPD pattern and geographical origin of accession. Overall a very high degree of variation was observed among the genotypes and the molecular analysis was able to distinguish the accessions. The RAPD marker system detected one unique band at 908.12 bp with primer OPA-01, for chilli landrace NCL-05 (*Capsicum frutescens*). Primer OPA-02 detected one unique band at 2045.41bp for all the three *Capsicum chinense* landraces (NCL-01, NCL-02 and NCL-03) (Table 4). These unique bands can be sequenced and used as SCAR (Sequence Characterized Amplified Region) markers specific to the particular chilli landraces and used as fingerprints for their identification. Screening with more RAPD markers are expected to generate specific fingerprints for all the landraces.

Table 4: Distinct finger prints generated by RAPD assay.

| Sl. no. | Primer | Band size (Bp) | Landraces distinguished |
|---|---|---|---|
| 1 | OPA-01 | 908.12 (approx.) | NCL-05 (Capsicum frutescens). |
| 2 | OPA-02 | 2045.41 (approx.) | NCL-01 (Capsicum chinense). NCL-02 (Capsicum chinense). NCL-03 (Capsicum chinense). |

## 4. Conclusion

The chilli germplasm of Nagaland has a high degree of genetic diversity as revealed by the molecular marker system (95.6% polymorphism). Despite the high level of intra-specific variability, the basic distinction among the three species is clear, indicating species distinctness. Selection for divergent parent basing on these characters will be useful for heterosis breeding in chilli. There is no association between RAPD pattern and geographical origin of the chilli accessions, as collections originating from various parts of the state did not form well distinct groups and were interspersed with each other.

## 5. References

Borgohain, R., Devi, J., Sarma, R.N., 2005. Intra and inter-

specific genetic diversity exploration in chilli using morphological and randomly applied polymorphic markers. IndianJournal of Agricultural Sciences 75, 582-5.

Karp, A., Edwards, K., Bruford, M., Vosman, B., Morgante, M., Seberg, O., Kremer, A., Boursoy, P., Arctander,P., Tautz, D., Hewitt, G., 1997. Newer molecular technologies for biodiversity evaluation: opportunities and challenges. Nature Biotechnology 15, 625-628.

Lanteri, S., Acquadro, A., Quagliotti. L., Portis, E., 2003. RAPD and AFLP analysis of genotypes of pepper (*Capsicum annuum*) cultivars. Russian Journal of Genetics 39, 1345-1348

Rohlf, F.J., 1992. NTSYS-pc Numerical Taxonomy and Multivariate Analysis System, Version 1.70. Applied Biostatistics New York.

Prince, J.P., Lackney, V.K., Angeles, C., Blauth, J.R., Kyle,M.M., 1995. A survey of DNA polymorphism within the genus capsicum and the fingerprinting of pepper cultivars. Genome 224-231.

Singh, A.K., Major Singh, Singh, A.K., Rakesh Singh, Sanjev Kumar, Kallo, G., 2006. Genetic diversity within the genus *solanum* (Solanaceae) as revealed by RAPD markers. Current Science 90, 711-716.

Smith, P.G., Heiser, C.B., 1951. Taxonomic and genetic studies on the cultivated pepper.*Capsicum annuum* L. and *C. frutescens* L. American Journal of Botany 38, 362-368.

Virk, P.S., Ford-Lloyed, B.V., Jackson, T.M., New bury, H.J., 1995. Use of RAPD for the study of diversity within plant germplasm collection. Heredity 74, 170-179

Votava, E.J., Bosland, P.W., 2001. Genetic diversity of *Capsicum pubescens* revealed *via.*RAPD analysis. Capsicum and Egg plant Newsletter 20, 60-63.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski. J.A., Tannery, S.V., 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic acids Research 18, 6531-6535.