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# Genetic Diversity Analysis in Chilli (*Capsicum annuum* L.) Found in Manipur Using RAPD Markers

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**A B S T R A C T**

The present study was undertaken to understand the genetic diversity of Chilli found in Manipur using RAPD primers. Twenty (20) primers were screened, out of which 9 primers gave polymorphic bands which were used for further analysis. The polymorphic information content (PIC) values ranged from 0.2 (OPB-15) to 0.45 (OPW-04) with an average of 0.35. The resolving power was found highest in OPW04 with value of 7.44 and lowest in OPA11 (2.10). Overall, it was found that OPW04 is a good primer which produced 100% polymorphism with high discriminating power. Cluster analysis using Un-weighted Pair Group Method with Arithmetic Averages (UPGMA) grouped all the chilli samples into three major clusters at a distance coefficient of 0.50, major cluster 1 consist of 4 cultivars (CAU 1, CAU 4, CAU 9, CAU 10). The 2nd major clusters consist of 6 cultivars- (CAU 2, CAU 5, CAU 7, CAU 6, CAU 8, CAU 3. The 3rd major cluster consist of only 1 cultivar (CAU 11).The results showed that RAPD can be effectively used to analysed genetic diversity of chilli found in Manipur.

## Introduction

Chilli (capsicum sp.) belongs to solanaceae family, originated in Mexico, with secondary centres in Guatemala and Bulgaria (Salvador, 2002). Columbus introduced chilli in India in 15th century (Basu and Krishna, 2003). Now, India is the world's largest producer, consumer and exporter of chillies. India is the largest producer of chillies with a total production of 26,804,941 tonnes (FAOSTAT, 2015). Several interspecific hybrids/derivatives of chilli originated in North-east, India (Daliwal, 2014). Chilli cultivars in India are famous for its pungency (hotness) and colour. Bhut jolokia, cultivated in Assam is the world's hottest chilli pepper (Bosland and Baral, 2007).

Characterization using morphological markers have been successfully used in different plants (Cong-Ying Yuan *et al.*, 2015; Fang *et al.*, 2017; Warnakula *et al.*, 2017) but due to environmental factors, it suffer certain

limitations. So, the molecular markers proven to be more effective than morphological and biochemical markers. RAPD are useful markers as it does not need of prior knowledge about the genome (Williams *et al.,* 1990). RAPD markers have been successfully used in genetic diversity studies of chilli (Makari *et al.,* 2009; Peeraullee *et al.,* 2013; Mullianathan *et al.,* 2014; Ferniah *et al.,* 2018; Lalduhzuala *et al.,* 2018).

## Materials and Methods

### Plant Material

In the present study, seeds from 11 (eleven) chilli cultivars were procured which was maintained at germplasm of CAU (Central agricultural university) Imphal. Details of chillis given in Table 1. The seeds were thoroughly rinsed with distilled water, allowed to germinate using paper towel method and transplanted to small pot after 10 days.

### Genomic DNA

The genomic DNA was extracted from leaf tissues of 20 days old young chilli plant using modified CTAB method (Doyle and Doyle, 1990). The leaves were grounded in 600uL extraction buffer (1M Tris HCl, pH 8.0; 5M NaCl; 0.5M EDTA; 10% SDS) and the mixture was treated with 5µL of Proteinase K (20mg/mL) for 1h at 36°C. To this cell lysate, 500µL of CTAB buffer (10% CTAB; 1M Tris HCl pH 8.0; 0.5M EDTA; 5M NaCl; 5% PVP was added and incubated for 2h at 65°C. The suspension was extracted thrice with equal volume of phenol: chloroform: isoamyl alcohol (24: 25:1). The DNA was precipitated using isopropanol, 3M sodium acetate and 70% ethanol, 10µL RNase (10µg/mL) was added to each 50 µL DNA and incubated for 30 m at 37°C. DNA quantification was carried out using UV spectrophotometer (Cary 60 UV-Vis, Agilent Technology) by measuring the DNA concentration at 260 nm and 280 nm. Purity of DNA was determined by calculating the ratios of absorbance at ratios 260 nm to that of 280 nm. The isolated DNA were run on 0.8% agarose gel electrophoresis to check the quality of DNA along with DNA ladder and photographed using gel documentation system (Biorad universal hood, USA).

### RAPD-PCR amplification

A total of 20 decamer primers were initially screened, out of which 9 primers showed clear and polymorphic bands. RAPD-PCR was carried out in 25 µL containing 50 ng of genomic DNA, 2.5 mM $MgCl_2$, 0.5 mM dNTP mix, 2.5 mM buffer, 1UTaq polymerase and 10 pmol of each primer with the cycling condition at 94°C for 4m, followed by 40 cycles of 94°C for 1m, 36°C for 45s and 72°C for 2m and final extension of 72°C for 7m. Amplified PCR products were separated by electrophoresis along with 100bp DNA ladder on a 1.8 % agarose gel prepared in 1X TAE buffer.

### Scoring and PCR analysis

Photographed Gel was scored as a binary matrix, where 1 represent band present and 0 represent band absent and dendrogram for cluster analysis using UPGMA (Unweighted Pair group Method with Arithmetic average) and genetic similarity matrix using NTSYS - pc version 2.02 (Rohlf, 1998) software were generated. Polymorphism information content ($PIC_i$) of a band was calculated according to Anderson *et al.*, (1993) as follow:

$$PIC_i = 1 - \sum_j f_{ij}^2$$

Where $f_{ij}$ is the frequency of the $j^{th}$ pattern of the $i^{th}$ band.

Resolving power (Rp) was calculated according to Prevost and Wilkinson, 1999 formula

$$RP = \sum_{i=1}^{n} IB_i$$

Where Informativeness of a band ($BI_i$) was calculated as:

$BI_i$ = 1- (2 X | 0.5 – *p* |) where *p* is the proportion of all accessions containing the band.

## Results and Discussion

A total of 20 RAPD primers were initially screened for their amplification, out of which only 9 primers (OPA11, OPB-01, OPC03, OPB11, OPW04, OPB-15, OPC03, OPL05 and OPD13) produced a clear and reproducible banding pattern in all the samples. A total of 76 bands were produced from 9 RAPD primers, out of which 60 were polymorphic (78.9%). The average band per primer was 8.4. The PIC values range from 0.28 (OPB-15) to 0.45(OPW04). The percent polymorphism ranged from 70% (OPCOB) to 100% (OPW04). The resolving power was found highest in OPW04 with value of 7.44 and lowest in OPA11 (2.10). Overall, it was found that OPW04 is a good primer which produced 100% polymorphism with high discriminating power (Table 2). The amplification using RAPD primer (OPB 11) is shown in Figure 1.

The similarity data generated by NTSYS-pc version 2.02 (Rohlf, 1998) using simple matching matrix showed the highest similarity value (0.85) found between cultivars (CAU 5 – CAU 7), (CAU 6 –CAU 7) and (CAU 9 – CAU 10). And the lowest value of 0.5, found in cultivars (CAU 1 –CAU 11) (Table 3).

At a distance coefficient of 0.55, the dendrogram divided 11 cultivars in three major cluster (Fig. 2). Major cluster 1 consist of 4 cultivars (CAU 1, CAU 4, CAU 9, CAU 10). The 2nd major cluster consist of 6 cultivars- (CAU 2, CAU 5, CAU 7, CAU 6, CAU 8, CAU 3. The 3rd major cluster consist of only 1 cultivar (CAU 11).

The dendrogram, is again divied into 5 major groups at a distance coefficient of 0. 30. Group I consist of 4 cultivars – (CAU 1, CAU 4, CAU 9 and CAU 10). Group II consist of 1 cultivar (CAU 2), Group III consist of 4 cultivars (CAU 5, CAU 7, CAU 6, CAU 8), Group IV consist of only 1 cultivar (CAU 3) and the last Group V consist of cultivar CAU 11.

**Fig.1** RAPD gel profile of 11 chilli cultivars using OPB-11 primer

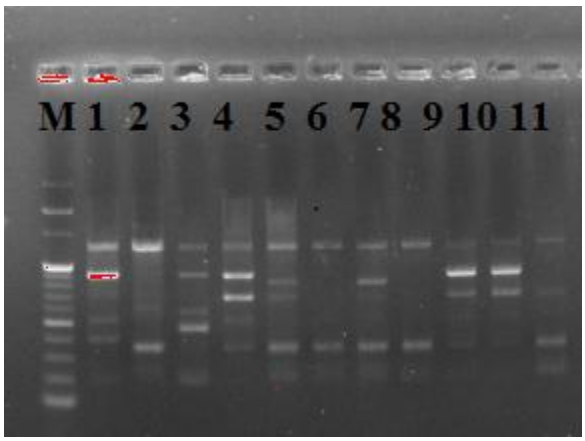

**Fig.2** Dendrogram obtained from 11 chilli cultivars using RAPD data

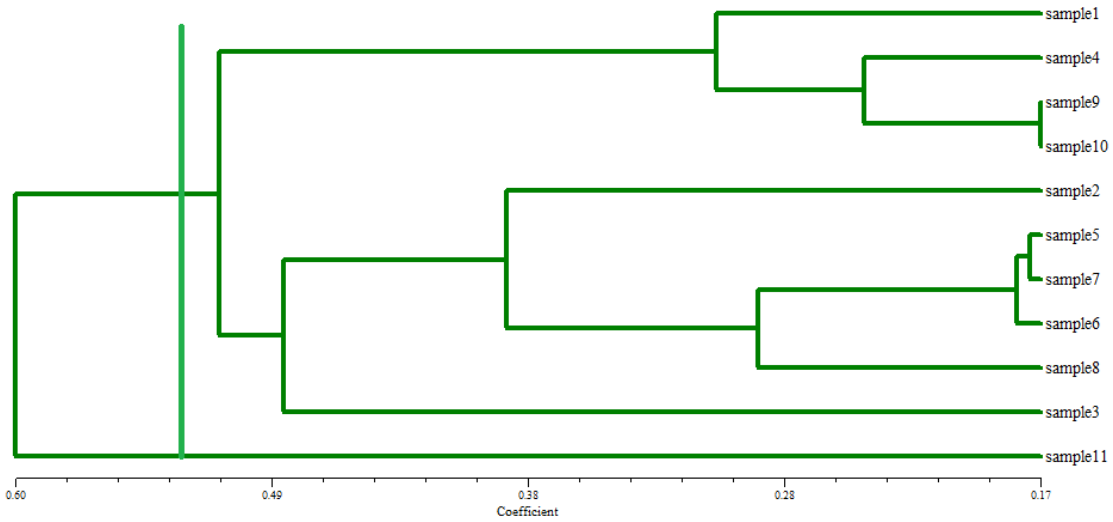


**Table.1** Details of 11 chilli samples used in present studies

| Sl.no. | Vourcher no. | Cultivars | Source |
|---|---|---|---|
| 1. | CAU-1 | Crispy long chilli | Central agricultural university germplasm, Imphal |
| 2. | CAU-2 | Morok Asangbi | Central agricultural university germplasm, Imphal |
| 3. | CAU-3 | Colour xanadu | Central agricultural university germplasm, Imphal |
| 4. | CAU-4 | Meitei morok asabi | Central agricultural university germplasm, Imphal |
| 5. | CAU-5 | Yensang morok | Central agricultural university germplasm, Imphal |
| 6. | CAU-6 | Morok macha | Central agricultural university germplasm, Imphal |
| 7. | CAU-7 | Churachandpur morok | Central agricultural university germplasm, Imphal |
| 8. | CAU-8 | Fireball | Central agricultural university germplasm, Imphal |
| 9. | CAU-9 | Meitei morok | Central agricultural university germplasm, Imphal |
| 10. | CAU-10 | Meitei morok macha | Central agricultural university germplasm, Imphal |
| 11. | CAU-11 | Xanadu | Central agricultural university germplasm, Imphal |

**Table.2** Details of RAPD –PCR amplification obtained from 11 chilli cultivars

| Sl.no. | Primer name | Primer sequence (5' – 3') | Total band | PMB | MMB | %PM | PIC | Resolving power |
|---|---|---|---|---|---|---|---|---|
| 1. | OPA11 | CAA TCG CCG T | 10 | 9 | 1 | 90 | 0.31 | 2.10 |
| 2. | OPB-01 | GTTTCGCTCC | 7 | 6 | 1 | 85.7 | 0.4 | 4.52 |
| 3. | OPC03 | GGGGGTCTTT | 9 | 5 | 4 | 55.5 | 0.36 | 6.22 |
| 4. | OPB11 | GTAGACCCGT | 9 | 7 | 2 | 77.7 | 0.4 | 6.9 |
| 5. | OPW04 | CAGAAGCGGA | 7 | 7 | 0 | 100 | 0.45 | 7.44 |
| 6 | OPB-15 | GGAGGGTGTT | 5 | 4 | 1 | 80 | 0.28 | 4.36 |
| 7 | OPC03 | GGGGGTCTTT | 10 | 7 | 3 | 70 | 0.31 | 5.48 |
| 8 | OPL05 | ACGCAGGCAC | 10 | 8 | 2 | 80 | 0.34 | 3.86 |
| 9 | OPD13 | GGGGTGACGA | 9 | 7 | 2 | 77.7 | 0.32 | 3.7 |
| | | | 76 | 60 | | 78.9 | | |

PMB – Polymorphic band; %PM – Percentage of polymorphic band; PIC- Polymorphic information content; MMB-Monomorphic band; Rp – Resolving power.

**Table.3** Simple matching (SM) matrix using RAPD data from 11 chilli cultivars

| | CAU-1 | CAU-2 | CAU-3 | CAU-4 | CAU-5 | CAU-6 | CAU-7 | CAU-8 | CAU-9 | CAU-10 | CAU-11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CAU-1 | 1.00 | | | | | | | | | | |
| CAU-2 | 0.65 | 1.00 | | | | | | | | | |
| CAU-3 | 0.52 | 0.63 | 1.00 | | | | | | | | |
| CAU-4 | 0.80 | 0.69 | 0.58 | 1.00 | | | | | | | |
| CAU-5 | 0.62 | 0.68 | 0.55 | 0.61 | 1.00 | | | | | | |
| CAU-6 | 0.55 | 0.77 | 0.60 | 0.63 | 0.83 | 1.00 | | | | | |
| CAU-7 | 0.61 | 0.76 | 0.61 | 0.64 | 0.85 | 0.85 | 1.00 | | | | |
| CAU-8 | 0.51 | 0.62 | 0.58 | 0.60 | 0.75 | 0.82 | 0.71 | 1.00 | | | |
| CAU-9 | 0.71 | 0.61 | 0.52 | 0.77 | 0.62 | 0.62 | 0.58 | 0.65 | 1.00 | | |
| CAU-10 | 0.73 | 0.67 | 0.58 | 0.81 | 0.58 | 0.63 | 0.67 | 0.60 | 0.85 | 1.00 | |
| CAU-11 | 0.50 | 0.61 | 0.50 | 0.58 | 0.67 | 0.64 | 0.63 | 0.73 | 0.57 | 0.54 | 1.00 |

From this dendogram, we can conclude that, among 11 cultivars, the cultivar which is closely related is cultivars (CAU 9 and CAU 10) and the most diverse one is CAU 3 and CAU 11.

This finding supports molecular genetic diversity analysis using RAPD primers correlates with the morphological finding of Atom *et al.*, (2017).

In conclusion, the RAPD primers used in this study are still efficient marker for successful identification and diversity studies of chilli.

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