



## Isolation and Identification of Molecular Markers for Fingerprinting of Chilli Hybrids and its Parental Lines

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### ABSTRACT

Chilli (*Capsicum annum*) is the predominant sp., which is cultivated in both hot and sweet papers. The maintenance of the genetic purity of chilli plant is a matter of great concern for the breeders. For genetic purity analysis, between true hybrids and off-types, breeders find out morphological differences between them, but this technique is cannot be recognized easily and also costly, tedious to score, and environmentally sensitive. Alternatively, molecular markers based genetic purity analysis can be employed. The molecular marker-based technique was thus used to overcome the conventional method drawbacks. The main objective of the study is to identify informative molecular markers (ISSR and RAPD) capable of distinguishing Chilli hybrids and their parental lines and their utilization in seed purity assessment. Five parental lines of Chilli (*i.e* CH10, CH12, CH530, CH709, CH734) were used for the production of 3 hybrids. Total 30 ISSR and 8 RAPD primers were selected for the study of 5 parental lines, among them 2ISSR and 1 RAPD primers produced unique fingerprinting across the hybrids. The ISSR marker UBC815 amplified alleles specific to different parental lines(CH10 & CH12) for hybrids (ACH112), The ISSR marker UBC 827, amplified alleles specific to different parental lines(CH709 & CH12) for hybrids (ACH179). Likewise, RAPD primer B20 for hybrid ACH 753 and their parental lines(CH734 & CH530). Thus, the above study showed that the aid of molecular markers are more reliable, highly efficient, and reproducible for assessing fingerprinting of Chilli commercial hybrid seeds with more accuracy.



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### INTRODUCTION

Chilli belongs to the Solanaceae family consists of the self-pollinating flower, bearing diploid genome (2n) of modest size chromosome number 24 (Hat-tab *et al.*, 2017). Chilli is commercially traded all over the world because of its pungent taste and as a delicious taste; it serves to the various cuisines as well as due to its organoleptic and functional food properties (Weber and May, 1989). The cultivated sp. of chilli is *Capsicum frutescens*, *Capsicum annum*, *Capsicum baccatum*. *Capsicum chinense* and

*Capsicum pubescent* (Kumar et al., 2001). In 2018, more than 300 thousand metric tonnes of chili pepper were produced in India. Telangana was the largest producer of chili across India in 2018. India is the world's largest chilli producer and exporter. The demand for this crop is increasing because of its beneficial properties and good market demands for the high yielding crop, which has led its production to an extreme level with the help of hybrid plants. Various hybrid plant productions had led to the less variation in the morphological genetic difference among these plants, so diversification and purification among the different breeding lines are important to protect the breeder's right.

The degree of variation may remarkably decrease in crosses between lines of *Capsicum annum*. There are several molecular markers such as Inter simple sequence repeats-(PCR), Simple Sequence Repeats (SSRs)/Microsatellite, Random Amplified Polymorphic DNA (RAPD), and Amplified Fragment Length Polymorphism (AFLP) are used for different field like forensic science, agricultural science and for human health to determine the gene responsible for genetic diseases and assumption for mutative relationship among *sp.* (Broun and Tanksley, 1996). Simple sequence repeats or microsatellites are ubiquitous in eukaryotic genomes (Zietkiewicz et al., 1994). The first DNA marker applied for the analysis of the plant is RFLP (Beckmann and Soller, 1983). Simple sequences are made up of stretches of continuous repeated short nucleotide motifs (Tautz, 1989). Primers are allowed to anneal in multiple loci on the DNA when PCR is performed in low temperatures (Vos et al., 1995). There is PCR based molecular marker, which is used as a large molecular tool for different gene analysis. It is applied in DNA Fingerprinting for identification in between *sp.*, and protection of plant variety rights, different hybrid diversity, and phylogenetic analysis, and also for the genome mapping and gene tagging (Williams et al., 1990). ISSR and RAPD are a reliable and quick procedure for diversity analysis with sufficient polymorphism within the population. ISSR capable of reading the interspecific sequences, making it more specific to differentiate between closely related individuals and gives a more polymorphic difference between the lines. Analysis of gene by RAPD marker is very effective for determining many crops, including potato, fruits, flowers, and *Capsicum*. RAPD markers are efficient and reliable for cultivar to the identification and hybrid purity test in *Capsicum annum* (Ilbi, 2003). ISSRs have the advantage over other markers like RAPDs, AFLPs, and more definite SSRs that are reproducible due to their better stringency (Liu et al., 2007).

The aim of this study is to determine the genetic purity in the given hybrid and parental lines of chilli using PCR based ISSR and RAPD marker.

## MATERIALS AND METHODS

### Plant material

Leaf samples of commercial hybrid (ACH 112, ACH 179, and ACH 753) and their parental lines (Table 1) used in this study were collected from Aditya Seeds PVT. LTD. Raipur. Fresh and young leaves from all chilli plants were acquired for DNA extraction.

**Table 1: Name of chilli hybrids and their parental lines used in this study**

Hybrid	Female line	Male line
ACH 112	CH 10	CH 12
ACH 179	CH 12	CH 709
ACH 753	CH734	CH 530

### Chemicals

All Chemicals were used in this study were molecular biology grade. And the PCR buffer and enzyme used is of New England bio labs chemical company. All glassware and plastic wares and buffers were prepared in Mille Q water and was disinfected through autoclaving at 15lbs for 15 min before it is going to be used.

### DNA extraction

The genomic DNA was separated by a modified CTAB method. Which was then quantified by analyzing that on 1 % agarose gel using diluted uncut lambda DNA as a standard. Then all this genomic DNA sample was diluted to a final concentration of 40ng/ $\mu$ l in 1X TE buffer (10 mM TRIS- HCL, 1mM EDTA, pH 8.0). DNA samples were stored at  $-20^{\circ}\text{C}$  for more use.

### RAPD & ISSR-PCR amplification and agarose gel electrophoresis analysis

For the polymorphic identification marker among the parental line and their hybrid, 30 ISSR-PCR primers and 8 RAPD primers were used. PCR reaction was performed using genomic DNA template (40ng), 1X buffer, high fidelity DNA polymerase (1U), MgCl<sub>2</sub>(1.5mM), 2.5mM dNTPs (2.5mM), BSA (10mg/ml) and forward and reverse primer 6pmol each. The amplification cycle was initial 5 minutes denaturation; after that 35 cycles of denaturation at 94°C for 20 sec, annealing was conducted for 30 sec and extension 72°C for 1.30 min. Final extension 72°C for 7 min. The PCR amplified product was analysed on 1% agarose gel along with DNA molecular weight marker.

## RESULTS AND DISCUSSION

High molecular weight DNA, which was extracted from leaves of chilli, are found to be pure and free of RNA. DNA was diluted as a concentration of 40ng/ $\mu$ l, which was then amplified by using ISSR and RAPD marker for polymorphism. All the primers had good amplification. Out of 30 ISSR primers & 8 RAPD markers UBC 815, UBC 827 & B20 (Table 2) shows the polymorphism in parental line of ACH112,ACH179 & ACH 753 respectively. These were analyzed on 2% agarose gel and showed polymorphism in parents. All 3 markers were collectively amplified in a specific fingerprint for all hybrids and, therefore, were effective for distinguishing them from one another. The polymorphisms observed between the parents are used as markers for hybrid identification.

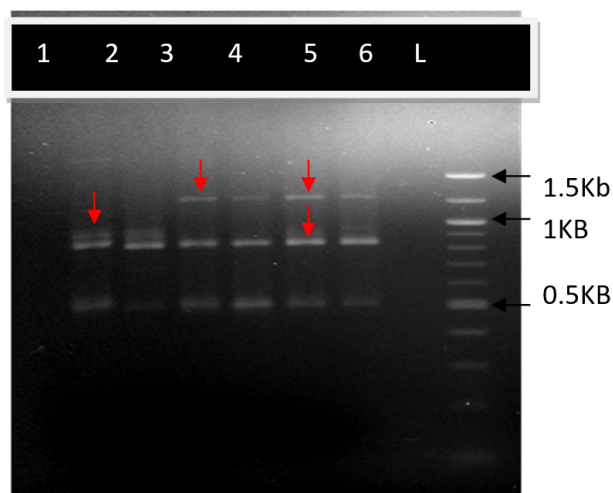
UBC-815 was able to detect the polymorphic band in the male parent line(CH12), in female parent line(CH10) the size of these band were 900bp and 1200bp respectively and in their hybrid line(ACH112) both polymorphic bands were present. The presence of similar size band in parent and hybrid represent that this is pure lines and shows genetic relationships among them(genetic purity) (Figure 1).

**Table 2: Sequence of ISSR & RAPD primer used in this study which shows polymorphism in parental lines**

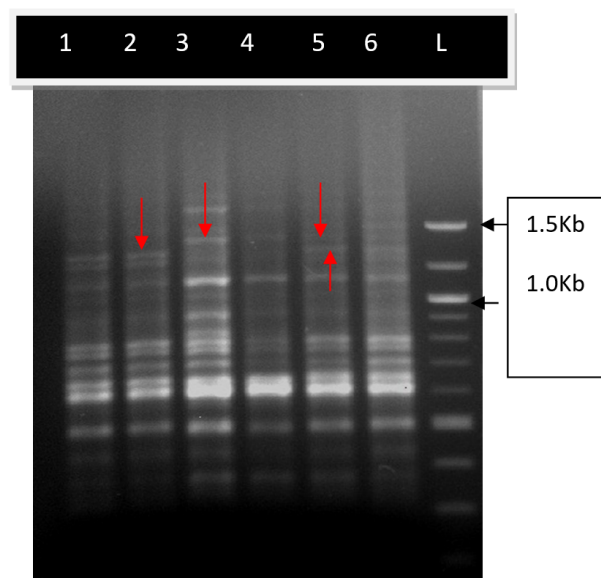
Primer Name	Seq (5'- 3')
UBC 815	CTCTCTCTCTCTCTCTG
UBC-827	ACACACACACACACAG
B 20	GGACCCTTAC

UBC-827 was able to detect the polymorphic band in the male parent line(CH709), in female parent line(CH12) the size of these band were 1250bp and 1350bp respectively and in their hybrid line(ACH179) both polymorphic bands were present (Figure 2). B-20 was able to detect the polymorphic band in the male parent line(CH530), in female parent line(CH734) the size of these band were 700bp and 400bp respectively and in their hybrid line(ACH753) both polymorphic bands were present (Figure 3).

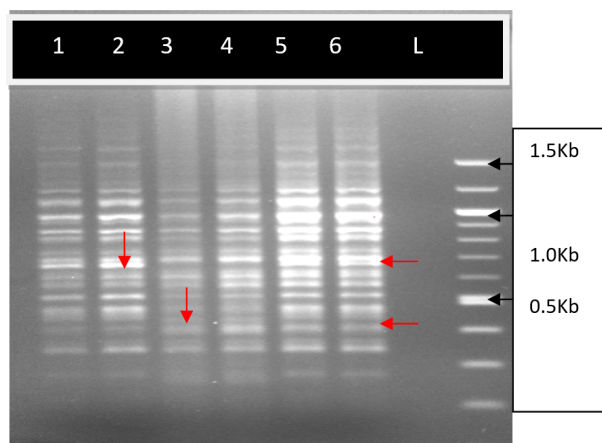
In the present study, the PCR based DNA markers were used to the analysis and identification of the parental line and the hybrid of chilli sample to show the polymorphism. To assess the genetic purity of hybrid seeds, GOT has been employed using morphological traits, but this technique is cannot be recognized easily and also costly, tedious, space



**Figure 1: Amplification results of primer UBC 815 from Chilli hybrid ACH112 and their parental lines. Lane L represent the DNA ladder - 100 Bp (100bp-1.5kb) Lane 1 & Lane 2 represent Female parent (CH-10) 900bp and Lane 3 & Lane 4 represent Male parent (CH12)1,200bp and Lane 5 & Lane 6 represent (Hybrid-ACH 112)**



**Figure 2: Amplification results of primer UBC 827 from Chilli hybrid ACH179 and their parental lines. Lane L represents the DNA ladder - 100 Bp (100bp-1.5kb) Lane1 & Lane 2 represent Female parent (CH-12) 1250bp and Lane 3 & Lane 4 represent Male parent (CH 709)1350bp and Lane 5 & Lane 6 represent (Hybrid-ACH179)**



**Figure 3: Amplification results of primer B20 from chilli hybrid ACH753 and their parental lines. Lane L represents the DNA ladder - 100 Bp (100bp-1.5kb) Lane 1 & Lane 2 represent Male parent (CH-530) 700bp and Lane 3 & Lane 4 represent Female parent (CH 734) 400bp and Lane 5 & Lane 6 represent (Hybrid-ACH 753)**

demanding, and time-consuming, hence the molecular marker analysis for the genetic purity testing used to assess the genotype.

As a result, obtain the ISSR and RAPD both amplify the DNA of chilli, the ISSR markers UBC-815, UBC-827 for the hybrid ACH 112, ACH 179, respectively, and RAPD markers B20 for the hybrid ACH 753 are found scorable for the purity assessment of chilli. The impurity in the parental or cultivar seeds with the hybrid can lower the genetic purity of the produced hybrids. The molecular markers can be a reliable source and can be used to check the hybrid and parental genetic purity for the particular chilli line, which will be a boon for the breeder's right. This technique reduces the cost and the time associated with the selection of suitable plants for hybrid production and can be effectively adapted by the breeders.

## CONCLUSION

We concluded from the present study that the investigation carried on checking the genetic purity of the chilli sample was found to be pure. The genetic purity exists among pepper accessions analyzed by RAPD and ISSR markers, and this information is very important to choose the hybrid from the parent is correct for breeding programs. It can also be stated that these methods of seed purity test can be more useful as it suppresses the conventional method, which is time-consuming and laborious in the seed purity test. The hybrid seed can be used by the industrialist to be launched in the market for further

use by the farmers and breeders.

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