To Study the role of molecular marker for genetic purity analysis of chilli germplasm and their hybrids

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ABSTRACT *Capsicum annum is the brand name of elite variety of chilli used in the case of marketing. For theuse of morphological differences between true hybrids and off types in grow out test (GOT) for genetic purity analysis, are not always apparent and cannot be recognized easily. There are the several molecular maker used for the genetic purity testing and Grow out test (GOT) for analysis of genetic purity which show the polymorphism of different parental lines and in their hybrids. In these paper we have been study and identify the PCR based DNA marker such as ISSR(UBC815) and RAPD(B20) for the identify the polymorphic band in the DNA of different parental lines and hybrids of chilli. These maker differentiate the different size of amplicon for two hybrid of chilli used in these study. The present report is, therefore, a step to protect the Plant Breeder's Rights by making use of reliable and modern DNA technologies.*

Keywords:*Chilli ; Capsicum annum ; ISSR ; RAPD ; PCR ; Polymorphism.*
Abbreviation*:CTAB-N-Cetyltrimethyl ammonium Bromide; GOT-Grow out test; PCR- Polymerase chain reaction ; ISSR-Intersimple sequence repeat; RAPDRandom amplified polymorphic DNA.*

Introduction:

Chilli, a genus of the family Solanaceae,is highly cultivated species that has been usedin food flavoring and human health(Gomez H B, Moreno et.al, 2013).The cultivated species of chilli are *C. annuum, C.frutescens, C.baccatum, C. pubescens, and C. chinense*(Kumar L D et.al, 2001). In Worldwide 2014, 32.3 million tonnes of green chili peppers and 3.8 million tonnes of dried chili peppers were produced. China is the world's largest producer of green chillies, providing half of the globaltotal. The level of polymorphism may greatly decrease in crosses between lines of C. annuum. There are several molecular marker such as Simple sequence repeats (SSRs)/microsatellite (TautzD,1989-Broun P, Tanksley S D, 1996), inter simple sequence repeats-PCR (ISSR-PCR) (Zietkienicz E, Rafalksi A, et.al,1994) Amplified fragment length polymorphisms (AFLP) analysis(Vos P. et.al,1995)and random amplified polymorphic DNA (RAPD)marker is used for various purpose ranging for forensic and agricultural science to identifying the gene responsible for genetic disease and inferring evolutionary relationship among species(PatersonA H,1996).The first DNA marker applied for the analysis of plant is RFLP (Beckman J S &Soller M, 1983).There will be the PCR based molecular marker which are used as the major molecular tools for the various genetic analysis such as its application include DNA fingerprinting for identification and protection of plant variety rights, phylogenetic and different hybrid diversity analysis and also for the genomic mapping and gene tagging(Williams J G K,et.al ,1990) .Several report describe the number of publicly available molecular markers based on polymerase chain reaction (PCR) for pepper(Beckman J S &Soller M, 1983).The potential of molecular markers as a tool to detect polymorphism among closely related commercial sweet pepper cultivars has not been determined yet(Weber J L, May P E, 1989) . In the present study we determine the different polymorphic band in the different germplasm and their hybrid using ISSR and RAPD marker. In the diverse species of animals ISSR-PCR marker also be shown for the finger printing (Zietkienicz E, et.al, 1994) .The Inter-SSR-PCR (ISSR-PCR) strategy is especially attractive because it avoids the need to carry out costly cloning and sequencing inherent in the original microsatellite-based approach (Reddy K D et.al,1999) . ISSR-PCR has been portably used for genetic linkage analysis of various plant species (Kantety et al., 1995; Charters et al., 1996; Provan et al., 1996; Tsumura et al., 1996).Furthermore, the technique of ISSR amplification is sensitive enough even to differentiate between closely related individuals (Zietkiewicz et al., 1994)RAPD analysis has been used to differentiate different forensic plant samples, which could not be differentiated by HPLC analysis (T. Halward, et.al,1992).

Materials and methods

Plant material

Leaf sample of commercial hybrid (ACH900 & 1500) and their parental lines (Table 1) used in this study were acquired from Adithya Seeds Pvt Ltd, Raipur. Fresh young leaves from all plants were collected for DNA extraction.

Chemicals :
All Chemicals were used in this study was molecular biology grade. And the PCR buffer and enzyme used is of bio labs chemical company. All glassware and plastic wares and the buffers prepared in Mille Q water were sterilized by autoclaving at 15 lbs for 15 min before using.

DNA extraction
Genomic DNA was isolated according to a modified CTAB method (Zhu et al., 2010). Quantification of DNA was accomplished by analyzing the DNA on 1% agarose gel using diluted uncut lambda DNA as a standard. Finally, all the genomic DNA samples were diluted to a final concentration of 40ng/µl with 1X TE buffer (10mM Tris-HCL; pH 8.0; 1mM EDTA). DNA samples were stored at -20ºC for further use.

RAPD & ISSR-PCR amplification and agarose gel electrophoresis analysis
Total of 15ISSR-PCR markers and 8 RAPD marker were used to identify polymorphic marker among the parental line and their hybrid. In these some show monomorphic band and UBC 815(Tm47C) shows polymorphic band for the ACH1500 hybrid and Primer B20 (Tm32C) shows polymorphic band for the ACH 900 hybrid of chilli. PCR amplification was conducted in a 20µl volume containing 40 ng of genomic DNA templet, 1X buffer, 1U *Taq*DNA polymerase, 1.5mM MgCl2, 2.5mM dNTPs, 10mg/ml BSA and 6pmol forward and reverse primer. The PCR protocol consisted of an initial denaturation at 94ºC for 5 min, followed by 38 cycles of 94ºC for 30 sec, primer annealing was conducted for 30 sec, 72ºC for 1 min30sec and final extension step of 72ºC for 5 min. The amplification reaction was carried out in thermo cycler (Applied Biosystems). The PCR products were analysed on 2% agarose gel along with 100bp molecular weight marker and photographed under UV light using Bio-Rad gel documentation system.

Result & Discussion
High molecular weight DNA extracted from the leaf material was found to be pure and free of RNA. DNA was diluted as a concentration of 40ng/ul and was amplified using the ISSR& RAPD marker for polymorphism. All the primer had good amplification.Out of 15 ISSRprimers&8 RAPD marker UBC 815 &B20 show the polymorphism in parental line of CH1500 & CH900 respectively. These were analyzed on 2%agarose gel and showed polymorphism in parents.The two markers collectively amplified a unique fingerprint for all hybrids and therefore, were effective in distinguishing them from one another. The polymorphisms observed between the parents are used as markers for hybrid identification.UBC 815 primer demonstrated an amplified allele of size 900bp in the male parent (CH15) and allele of size 1,200bp in the female parent (CH101). However, hybrid (CH1500) exhibited the alleles of both parents, confirming the heterozygosis of the hybrid with the presence of two bands at 900 and 1,200bp (Fig. 1).
Similarly, hybrid CH900 was identified and distinguished by the RAPD marker B20, as shown in (Fig. 2). The hybrid demonstrated the complementary banding pattern of both parents. The marker had an amplicon of 700 bp in its male parent (CH109); and amplicon of size 420bp in female parent (CH102); the hybrid showed both the amplicons at 700bp and 420bp. Thus, it confirmed the genuine nature of the hybrid.
In the present study the PCR based DNA marker were used to the analysis and identification of the parental line and the hybrid of chilli sample to show the polymorphism. For the genetic purity of seed hybrid, GOT has been employed to assess the purity of hybrid seeds using morphological traits. GOT is used to determine the genetic purity of hybrid. It is the tedious, space demanding and time consuming, hence the development of molecular marker has been suggested for the genetic purity testing, since they are used to assess the genotype, and not the phenotype.

Figure and Tables

Table 1: Name of chilli hybrid and their parental line used in this study

Hybrid	Female line	Male line
ACH 1500	ACH 115	ACH 101
ACH 900	ACH 109	ACH 102

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

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

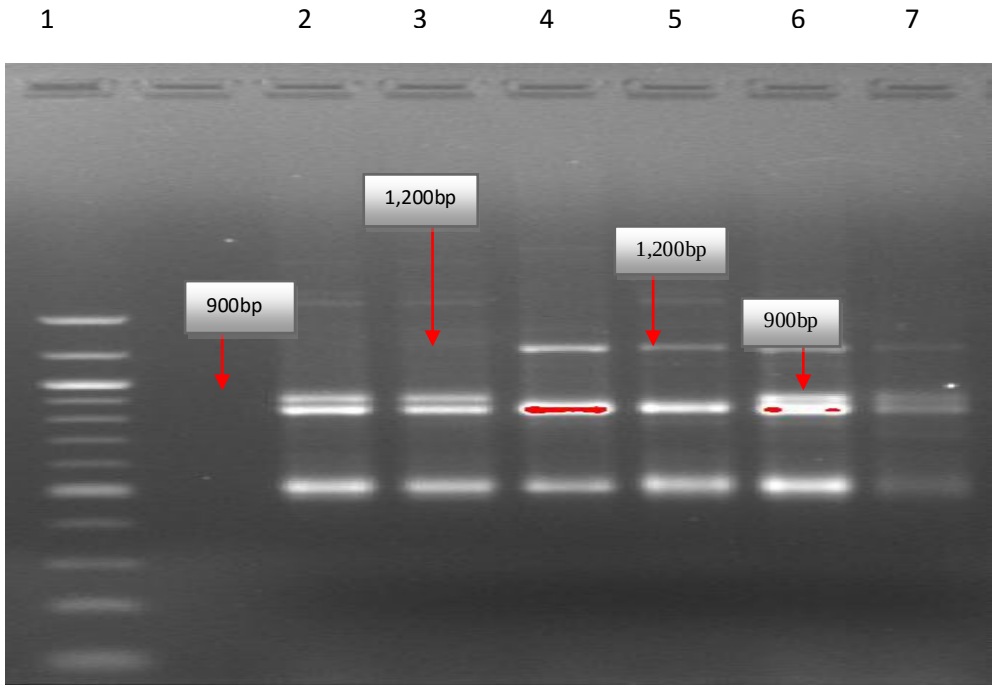

Fig 1. Amplification results of primer UBC 815 from Chilli hybrid CH1500 and their parental lines. Lane 1 represent the DNA ladder-100bp (100bp-1.5kb),Lane 2& 3 represent Male parent CH-115,amplification size 900bp and Lane 4 & 5 represent Female parent CH 101, amplification size 1,2000bp and Lane 6 & 7represent hybridCH-1500,amplification sizes 900:1200bp.

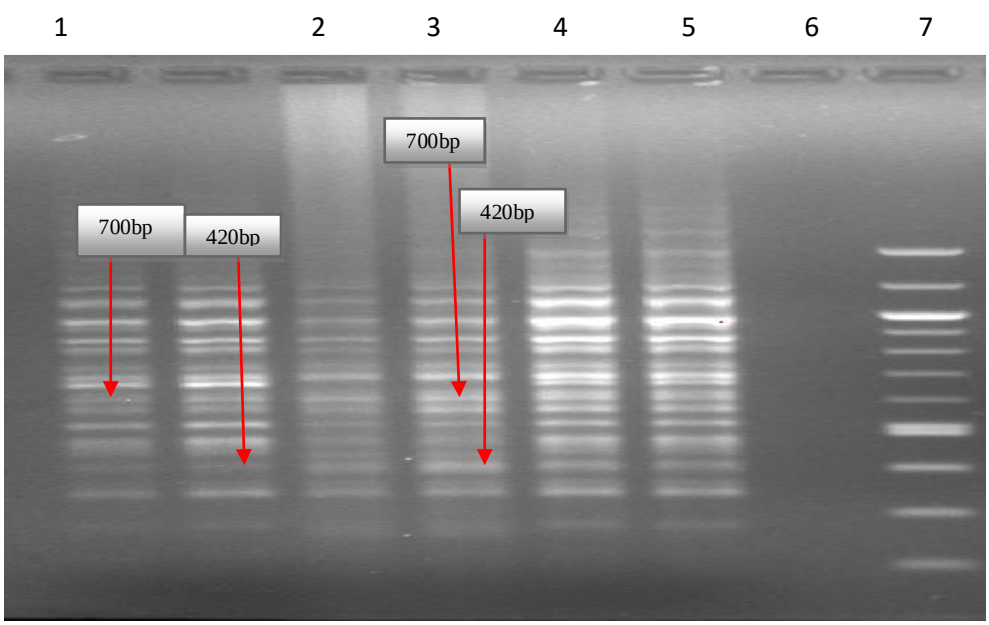

Fig 2. Amplification results of primer B20 from Chilli hybrid CH900 and their parental lines. Lane 1& 2 represent Male parent CH-109,amplification size 700bp and Lane 3& 4 represent Female parent CH 102, amplification size 420bp and Lane 5& 6represent hybrid CH-900, amplification sizes 700:420bp. Lane 7 represent the DNA ladder-100bp (100bp-1.5kb).

Future Aspect :

At present, the development of the molecular marker is the used for the genetic purity analysis, genetic diversity analysis and so on. Further these study will used in the linkage mapping, genotyping of mapping

population with DNA marker, construction of linkage map for the identification of closely linked DNA marker.

These technics also used in the study of development of F2 hybrid and subsequence generation. Phenotype characterization on the basis of morphological character of parent and mapping population.

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