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Intraspecific Genetic Diversity in Different Capsicum Frutescense Germplasm Revealed by Molecular Markers

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ABSTRACT

The Capsicum frutescence is one of the major spice crop that belongs to family Solanaceae. Capsicum is a highly value crop, as a fruit it is rich in vitamin B, C & E. The objective of this study was to show genetic diversity within different Capsicum frutescence germplasm by using DNA markers. ISSR and RAPD molecular marker used for the analysis of the genetic diversity of Capsicum frutescence. These markers involve Polymerase Chain Reaction (PCR) amplification of DNA using a single primer composed of a microsatellite sequence. ISSR and RAPD technology rapidly reveals high polymorphism and determines genetic diversity. Among 15-20 primers used only ISSR primer UBC 815 and RAPD primer B-20 amplified alleles showed the specific polymorphism within the germplasm of chilli.

Keywords: Chilli, capsicum frutescens, agarose gel electrophoresis, polymorphism, Rapd, Pcr, Issr , Got, genetic diversity

INTRODUCTION

Chilli pepper is an important commercial crop cultivated exclusively in tropical and temperate zones of the world. It is good source of vitamin C, A and E .India being the largest producer of chilli has tremendous potentiality to increase production in order to promote export besides meeting is domestic requirement.Chilli(pepper) is a predominant variety of spices originate from the Western Hemisphere and other American cities, which is scientifically known as Capsicum annuum. Capsicum is widely spread all over the world both wild and commercial one, among which commercial variety belonging to the five major species of cultivated peppers (genus Capsicum): C.annum, C.chinense, C.baccatum, C.frutescens and C.pubescens has been used for various purposes (G.S.C.Buso et.al., 2016). Capsicum frutescence has a smaller variety of shapes compared to other capsicum species. Peppers, Capsicum spp., are grown worldwide for vegetable, spice, ornamental, medicinal and lachrymator uses and are a significant source of vitamins A and C Peppers have been found along with other food fossils from as early as 6,000 years ago and are considered the first spice to have been used by humans (Sahel Miladi Lari et.al.2014) . Chilli is one of the most cultivated spices in World and in India it is the most grown berries used in green, ripped and powdered form. 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 nutraceutical properties. The demand of this crop is increasing because of its beneficial properties and good market demands for the high yielding crop, which has led its production to the 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

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plants, so diversification and purification among the different breeding lines are important to protect the breeder's right. Today technology has built that far that it is so easy to distinguish diversity and purity in plant with the help molecular marker. In early times to check the yield, production, variety, purity of true line etc. several morphological characteristic were compared by growing the plant to maturity and assessing them, which results in a tedious and time taking process and the method so-called was GOT(Grow Out Test).

Pepper belongs to the genus Capsicum, which includes about 25 wild species and five (Capsicum annuum L., Capsicum frutescens L., Capsicum chinense Jacq., Capsicum baccatum L. and Capsicum pubescens Ruiz and Pavon) cultivated species (Kumar et al. 2006a). C. annuum species bearing pungent (chilli, chili or hot pepper) and non-pungent (sweet pepper) fruits are the most widely cultivated worldwide (Ved Prakash Rai et.al.2013).. Besides being one of the most commonly used spices, condiments and vegetables, peppers have several versatile and innovative food and non-food uses (Ved Prakash Rai et.al.2013). Chilli is an important cash crop for small and marginal farmers in many developing countries across Asia (China, India, Pakistan, Bangladesh, Thailand and Indonesia) and Africa (Egypt, Ethiopia, Nigeria and Ghana). India is the largest producer of dry chilli fruits, accounting for more than 43 % of the world's total dry chilli production (FAOSTAT 2011).

MATERIAL AND METHODS

Plant material

For this experiment, leaf sample of chilli genotypes (AS-45,AS-705,AS-709) are collected from Aditya Seeds pvt.Ltd, Raipur Chhattisgarh .These all fresh leaves are collect from plants for the 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

Extraction of genomic DNA was done by CTAB (Cetyl Trimethyl Ammonium Bromide) method. The DNA was quantified by using 1% agarose gel in 1X TAE buffer (10mM Tris-HCL; pH 8.0; 1mM EDTA). Photograph was observed under UV light of Bio-Rad gel documentation system.

PCR amplification and electrophoresis

25 ISSR-PCR markers and 10 RAPD markers were used to identify polymorphic marker among the chilli parental line. UBC 815(Tm47 °C) shows polymorphic band for the AS-709 & AS-45. Primer B20 (Tm32C) shows polymorphic band for the AS-705.

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Polymerase chain reactions (PCR) were carried out in Applied Bio system thermo cycler. PCR reactions were performed on each DNA sample in a 20 µl reaction mixture containing 1µl of DNA sample, 2µl of 10X PCR buffer, 0.5µl MgCl2 (25mM), 1 µl dNTP's (5mM), 1µl BSA(10mg), 0.3µl Tween 20, Primer (ISSR/RAPD) 2.0µl/ 1.5µl (3-5 pM), 0.2µl(1Unit) of Taq polymerase. PCR thermal profile consist of initial denaturation at 94ºC for 4min, followed by 38 cycles at 94ºC for 20sec, annealing at different tm of primer for 30sec, initial extension at 72ºC for 1min 50sec and final extension step at 72ºC for 5min. The PCR product was analyzed in 1.8% agarose gel in 1X TBE buffer and photographed under UV light of Bio-Rad gel documentation system.

RESULT

The RAPD and ISSR primer were used for diversity test. All the 10 RAPD primers successfully amplified the Capsicum DNA of all Varieties but only B-20 shows polymorphic band. And Of the 25 UBC primers (ISSR), 20 successfully amplified all the Capsicum DNA, of which two detected polymorphism for the different lines of chilli.These were analyzed on 1.8%agarose gel and showed polymorphism in parents.

UBC-815 was able to detect the polymorphic band in the chilli parent line AS-709 and AS-45. The specific band size is 1200bp for AS-709 and 500bp for AS-45. (fig:1)
B-20 was able to detect the polymorphic band in the chilli parent line (705), The specific band size is 1300bp for AS-705 (fig:2)

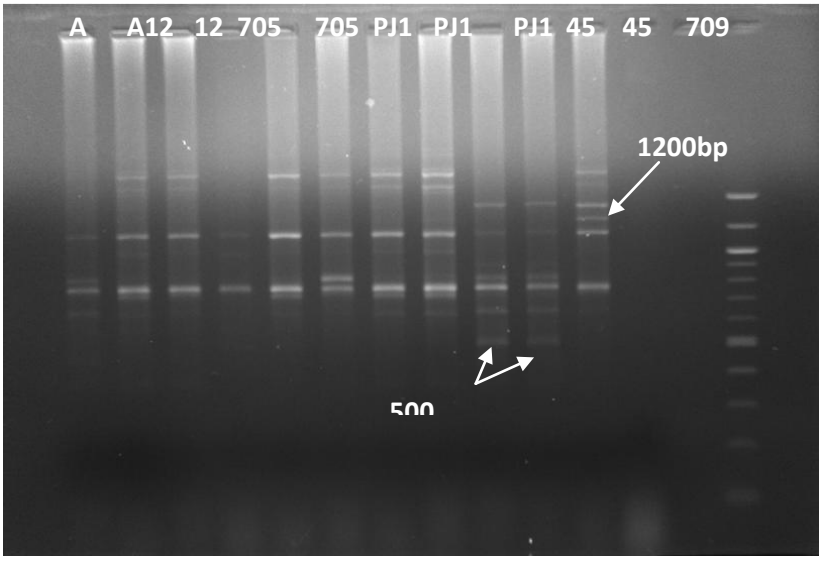

Figure 1:- PCR amplification result of primer UBC 815. Lane 1 and 2 stand for AS- A, lane 3-8 stand for AS-12, 705,PJ1 respectively and lane 9-10 stand for AS-45 (Polymorfic band of 500 bp), lane 11 stand for AS-709 (Polymorfic band of 1200 bp). Lane 14 stands for ladder (100-1500 bp).

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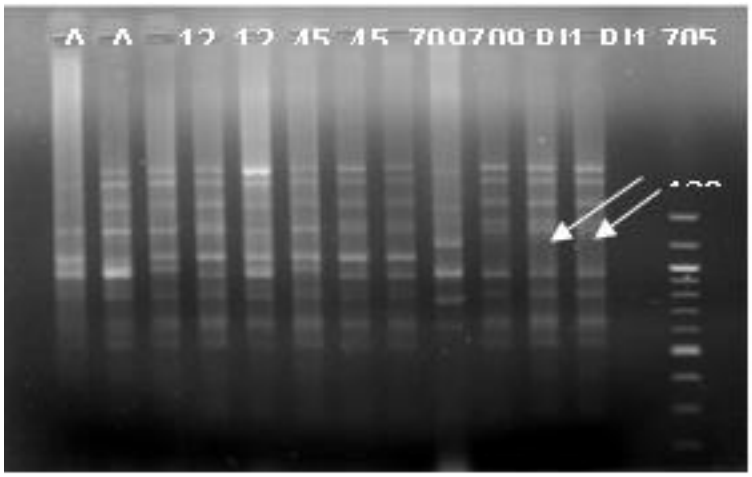

Figure 2:- PCR amplification result of primer RAPD B20. Lane 1-2 stand for AS-A , lane 3-4 stand for 12, lane 5-6 stand for 45, lane 7-10 stand for 709, PJ1 and lane 11-12 stand for AS-705 which is (Polymorphic band of 1300 bp), and L stand for ladder (100-1500 bp). All the leaves were collected from the fields of Aditya Seeds Pvt Ltd, Raipur (C.G.).

Figures and Tables:-

Tab-1 Name of chilli and their parental line used in this study (Aditya seed chilli sample)

PARENTAL LINE	PRIMER
AS709	UBC-815
AS 45	UBC-815
705	B-20

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

Primer	Sequences Text(5'-3')
ISSR UBC-815	CTCTCTCTCTCTCTCTG
ISSR UBC-807	AGAGAGAGAGAGAGAGT
RAPD B-20	GGACCCTTAC

DISCUSSION

In the present study the molecular marker ISSR and RAPD is used to detect the genetic diversity of the chilli plant. The conventional method involves GOT for the study of morphological characters and the biochemical test for the identification of true line breeds. In the PCR based DNA markers were used to the analysis and identification of the parental line of chilli sample to show the polymorphism. For the genetic diversity of parental lines, to assess the diversity of seeds using morphological traits. It is the tedious, space demanding and time consuming, hence the development of molecular marker has been suggested for the genetic diversity testing, since they are used to assess the genotype, and not the phenotype. As the result obtain the ISSR and RAPD both amplify the DNA of chilli, the ISSR markers UBC-815 and RAPD B-20 for the parents 709,45 and 705 respectively are found scorable for the diversity assessment of chilli.

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 diversity exists among pepper accessions analyzed by RAPD and ISSR markers, and this information is very important to choose the specific from the parent is correct for breeding programs. It can also be stated that these methods of seed diversity test can be more useful as it suppresses the conventional method which is time-consuming and laborious in the genetic diversity test. The chilli 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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