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Screening and identification of molecular markers for genetic diversity among Cowpea genotypes

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ABSTRACT: *Cowpea is highlyproteinaceous and it is also tolerant to drought and acidic soil. Consequently, it is a best source to satisfy the food demand of escalating population of the world. For the study of genetic diversity mostly molecular markers are being used. ISSR markers are highly variable in the genome and also reproducible and cost effective. Therefore, it is the perfectmarker for the genetic diversity analysis. There were 52 ISSR primers in which 2 primers i.e. UBC807, UBC812 were scorableon agarose gel and showed polymorphism. UBC807 used for the identification of the line Babita (BB) and shows 810bp polymorphic band. To identify the line Lali (LL) Primer UBC812 was used and shows 600bp polymorphic band.These highly informative primers easily differentiated the parent genotypes.Genetic diversity helps the plant breeders to select lines which improve the food security.*
KEYWORDS:*Genetic diversity, ISSR, PCR, polymorphism, genotype, molecular markers.*
Abbreviation: CTAB-*Cetyltrimethyl ammonium bromide;* ***ISSR****-Inter-simple sequence repeat;*
PCR*- Polymerase chain reaction.*

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I. Introduction

Cowpea (*Vignaunguiculata* L. Walp.) is an annual herbaceous legume andpredominantly cultivated in the semi-arid regions across Africa and other countries such as Asia, Central and South America (Prasanthi et al. 2012). Mostly it is used as grain crop although it is used as animal silage or as a vegetable crop. Therefore, it can be used at all stages of growthfor both human and animals (Odireleng et al. 2016). The crop is mainly grown for its seeds but the leaves,young fresh or dry and immature seeds can also be consumed (Jinggui et al. 2003). Cowpea is so rich in protein that it is frequently referred to as the “poor man’s meat” (Ali et al. 2015). It has better tolerance for acid soil and drought. Its capability to fix nitrogen helps to restore fertility in soil (Aaron et al. 2010). Due to these properties Cowpea is an essential crop in many underdeveloped parts of the world. The major production areas of Cowpea are Asia, Central America and South America. According to the data released by the Food and Agriculture organization (FAO) at least 5.8 million tons of dry Cowpea cereal is produced annually with a minimum of 11 million hectares worldwide (Xiong et al. 2016).

The genetic diversity within the varieties of Cowpea is comparatively low because it is predominantly self-pollinating (Emily et al.,2016).Genetic diversity is crucial for good plant breeding practices. (Xiong et al. 2016). Excellent knowledge about genetic diversity in available germplasm is very beneficial for plant breeders (Prasanthi et al.2012). Moleculargenetics techniques have a remarkable effect on the conservation and use of genetic resources and have become popular for the study of phylogeny and evolution of species and to characterize and depict germplasm (Tosti and Negri 2002).For many genetic variation studies, high genetic variability and the ability to generate multilocus data from the genome under study defines a good genetic marker. Inter-simple sequence repeats (ISSRs) are those regions which are flanked by microsatellite sequences in the genome. Priorknowledge of the genome to be analyzed is not required for the development of ISSR markers; thus, it has universal application for plant genome analysis (Vijayan 2005).ISSR markers are highly variable, highly reproducible and ubiquitously distributed across the genome.All these characteristics make ISSR an ideal genetic marker for various studies, mostly on geneticvariation/diversity (e.g. Wang *et al*. 2012; Shafiei-Astani*et al*. 2015), DNA fingerprinting (e.g. Shen*et al*. 2006), andphylogenetics (e.g. Iruela*et al*. 2002)(Ng and Tan 2015). The objective of this study was to screen and identify theISSR molecular markers for the analysis of genetic diversity among genotypes of Cowpea.

II. Materials And Methods

2.1 Plant material

Seedlings of all four Cowpea lines used in the present study were grown in pots of coco-pit in a greenhouse at Adithya Seeds Pvt. Ltd, Raipur.Fresh young leaves were punchedand collected from three plants per line from 15-days-old seedlings.

2.2 DNA extraction

Thecollected leaves were frozen in liquid nitrogen and the genomicDNA was isolated according to the CTAB (cetyltrimethylammonium bromide) protocol (Zhu et al. 2010) with some modifications.Thequality of the isolated genomic DNA samples were checked by electrophoresis in 0.8% agarose gel stained withethidium bromide (1 mg/mL) in 1× TAE buffer.

2.3 ISSR assay

The ISSR primers were obtained from Eurofins Genomics India Pvt. Ltd, Bangalore. Total of 52 primers were screened by polymerase chain reaction. PCR wascarried out in 0.2mL tubes with a reaction volume of 20μL containing 2μL 10x PCR buffer, 6 picomoles of primer, 2mM of each dNTPs, 1U Taq DNA polymerase, and 30ng DNA.Thetubes were placed in a Thermal cycler (BIORAD), programmed for initial denaturation at 94^{0}Cfor 1 minute, followed by 35 cycles for 20 seconds at 94^{0}C,30 seconds at 50^{0}C, 1:30 minutes at 75^{0}C, and final extension for 5 minutes at 72^{0}C. The PCR amplicons were analyzed on an (2%) agarose gel using containing 1mg/mL ethidium bromide in 1X TBE buffer. The gel was photographed using gel documentation system (BIORAD).

III. Results And Discussion

3.ISSR PCR amplification

For the identification of different Cowpea lines (Table 1) ISSR markers were used as shown in (Table 2). There were 52 ISSR primers screened and out of which 2 primers i.e. UBC807, UBC812. They were reproducible and showed polymorphism [Fig. 1(A),1(B) respectively].UBC807 (5'AGAGAGAGAGAGAGAGT3') was used for the identification of the line Babita (BB) and shows 810bp polymorphic band. However, it was absent in the other three lines. Therefore,it is the precised marker for Babita [Fig. 1(A)]. To identify the line Lali (LL) primer UBC812 (5'GAGAGAGAGAGAGAGAA3') was usedand shows 600bp polymorphic band. However, it was absent in other three lines. Therefore, it is definite marker for Lali [Fig. 1(B)]. In the present study, ISSR markers have been proved efficient for the analysis of genetic diversity. ISSR primers UBC807, UBC812 robustlysupports the genetic diversity.

S.No.	Genotypes/ Lines	Abbreviations
1.	Lali	LL
2.	Babita	BB
3.	44	
4.	77	

Table 1. List of Cowpea genotypes.

S.No	Primer	Sequence
1.	UBC807	^{5}AGAGAGAGAGAGAGAGT^3
2.	UBC812	$^{5'}$GAGAGAGAGAGAGAGAA$^{3'}$

Table 2. List of primers and their sequences which show polymorphism in Cowpea lines.

(A) (B)

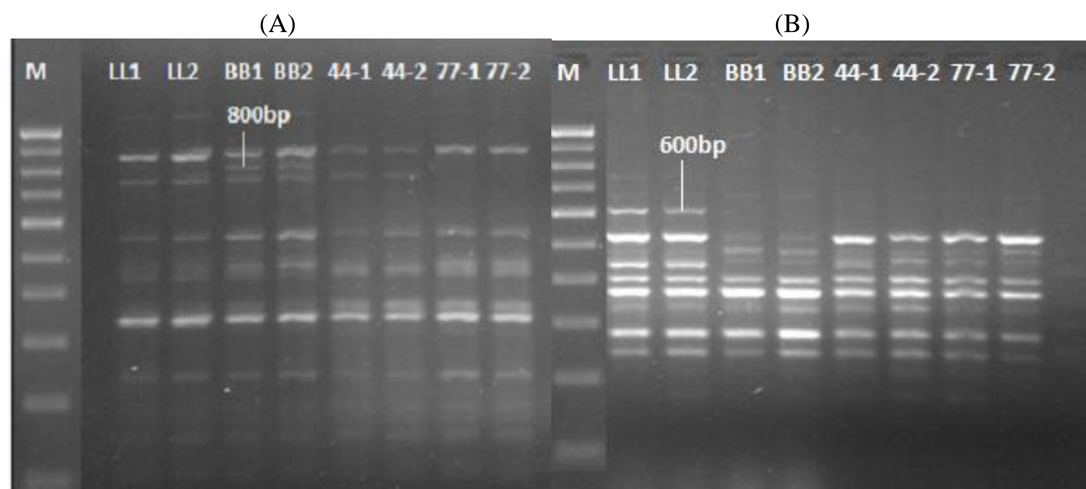

Figure1. PCR amplification results of primers UBC807 (A) and UBC812 (B) from Cowpea lines. Lane M represents marker (100-1000bp), Lanes 1-2 represent line Lali, Lanes 3-4 represent line Babita, lanes 5-6 represent line 44, and lanes 7-8 represent line 77.

IV. Conclusion

The results of the present study suggest that molecular markers can be used as the more accurate and efficienttoolfor the differentiation among Cowpea genotypes.Mainly in plants genetics, on the application of ISSR markers, many reviews have been published over the years. (Ng and Tan, 2015).Association of conventional breeding programs with modern agricultural practices has been responsible for the shrinkage of genetic diversity for many cultivated species (Cheng et al.,2001).The molecular markersobtained in the present study are identified specific for the particular line. Therefore it could be beneficial for plant breedersfor the utilization and improvement of the genotypes.

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