RESEARCH PAPER

Asian Journal of Bio Science (April, 2010) Vol. 5 Issue 1 : 138-139

Pearl millet characterization by using ISSR marker

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(Accepted : March, 2010)

The present study was carried out with emphasis on ISSR profiling and genetic relationship studies on pearl millet using ISSR marker. The experimental material consisted of 15 genotypes of pearl millet. The seeds of 15 genotypes of pearl millet were grown in petriplates and the young leaves from them were used for DNA extraction. Extraction of total genomic DNA was carried out following the methods suggested by Saghai-Maroof *et al.* (1984) with minor modification. The selected primers generated 123 ISSR bands and the size of the amplification products ranged from 150-35000 bp. The number of bands generated per primer ranged from 09 $(CA)_6$RG to 19 $(AC)_8$YA with a mean of 13.6 bands per primer. Out of the bands generated 80 were polymorphic and 43 were monomorphic. The genotype GHB 235 showed maximum similarity (0.92) whereas, minimum was for ICMV 155 (0.55) among the genotypes studied.

Key words : Pearl millet, Genetic diversity, ISSR

INTRODUCTION

After wheat, rice and sorghum, pearl millet is the fourth most important cereal crop in India which has the distinction of having the highest acreage (908m ha) under this crop in the world. The crop productivity has increased from 303 kg ha^{-1} in 1951-55 to 851 kg ha^{-1} in 2002 (Anonymous, 2005). This is a commendable increase given that Pearl millet is largely grown as a rainfed crop on soils starved of nutrients. This increase in productivity has been mainly achieved through development of high yielding and disease resistant cultivators coupled with suitable and improved agro-techniques. Large numbers of varieties have been recommended for cultivation and large numbers of genotypes are available.

However, morphological characterization is an important first step in description of germplasm or various genotypes because a breeding program mainly depends upon the magnitude of genetic variability. Their expression is simple, irreplaceable and helps to understand the magnitude of environmental influence on the characters, Biochemical markers are useful to study the variability, as they are based on the expressed loci of the genome but, may be biased by general consideration that only a small portion of genome is represented by these markers and the low polymorphism they exhibit relative to molecular markers. Therefore the present study was carried out with emphasis on ISSR profiling and genetic relationship studies on pearl millet using ISSR marker.

MATERIALS AND METHODS

The seeds of 15 pearl millet genotypes were grown in petriplates and the young leaves from them were used for DNA extraction. Extraction of total genomic DNA was carried out following the methods suggested by Saghai-Maroof *et al.* (1984) with minor modification.

All samples were treated with RNAse (100mg/ml) depending on the concentration of RNA detected through gel electrophoresis and were incubated for 60 minutes at 37^0C. Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added to it and mixed by swirling for 5 minutes. This was centrifuged at 12000 rpm for 10 minutes and the supernant was collected in a fresh tube. This was followed by one more extraction with chloroform: isoamyl alcohol (24:1). The purified DNA was precipitated by adding 1/10 volume of 3M sodium acetate (pH 5.6) and 2.5 times (v/v) chilled ethanol (100%). Extra salts were removed by washing with 70 per cent ethanol and DNA was pelleted and air-dried overnight. The pellet was dissolved in minimum volume (100µl) of TE (pH 8.0) buffer at room temperature and stored at -20^0C. DNA quantification was done using DyNA quant 200 fluorimeter (Hoefer Instrument, USA). It worked on the principle of fluorescence emitted by the double stranded DNA-Hoechest 33258 dye complex, which is directly proportional to the amount of DNA in the sample. Part of the DNA sample was diluted appropriated of TE (pH 8.0) buffer to yield a working concentration of 50 ng/µl

Table 1 : Details of ISSR bands generated by 9 primers in 16 pearl millet genotypes

Sr. No.	Primers	Total No. of bands	No. of monomorphic bands	No. of polymorphic bands	Polymorphism (%)	Molecular size amplicons (bp)
1.	$(GACA)_4$	18	05	13	72.33	400-3500
2.	$(CA)_6$ RG	09	03	06	66.6	560-1400
3.	BDB $(CA)_7$	12	06	06	50.0	330-1900
4.	VHV$(GT)_7$	10	02	08	80.0	400-770
5.	$(AC)_8$ T	16	06	10	62.5	350-1450
6.	$(AG)_8$ T	15	10	05	33.33	375-1400
7.	$(GA)_8$ C	10	01	09	90	500-1100
8.	$(AC)_8$ YA	19	05	14	73.68	150-1400
9.	$(GA)_9$ AC	14	05	09	64.28	430-1750

of DNA and stored at 4^0C. Sixteen primers were tested for amplification at different annealing temperatures of genomic DNA of two randomly chosen genotypes (81 A, ICTP 8203) included in the study. Out of these, nine consisting of both anchored and non- anchored ISSR that gave satisfactory amplification and band resolution were chosen for the study. The computations were performed using the program NTSYS-PC. Version.1.7 (Rohlf, 1993).

RESULTS AND DISCUSSION

Out of sixteen ISSR primers, nine of them gave satisfactory amplification and band resolutions. For further study nine primers were selected. The selected primers generated 123 ISSR bands and the size of the amplification products ranged from 150-3500 bp. The number of bands generated per primer ranged from 09 $(CA)_6$ RG to 19 $(AC)_8$YA with a mean of 13.6 bands per primer. Out of the bands generated 80 were polymorphic and 43 were monomorphic. The primers which showed maximum number of polymorphic bands was $(AC)_8$YA. It is revealed that primer $(GA)_8$C had the highest polymorphism percentage 90.0% whereas, the lowest polymorphism percentage 33.33% was observed in primer $(AG)_8$T. 0,1 profiles of ISSR primers the gel areas showing hazy bands and or positional non-clarity were not scored for final analysis (Table 1).

The genetic similarity in pearl millet genotypes was determined on the basis of Jaccard's similarity coefficients. Average Jaccard's similarity was calculated among all the genotypes and it was found to be 0.072.

The genotype GHB 235 showed maximum similarity (0.92) whereas, minimum was for ICMV 155 (0.55) among the genotypes studied. The genotypes were grouped by subjecting the Jaccard's similarity values to UPGMA clustering. Fifteen genotypes divided in to two clusters. The Ist cluster consisting of two genotypes *viz.*, L-111 A and 20 K 86. Cluster II was the largest which comprises thirteen genotypes. The II cluster subdivided into four sub groups II a consisting of 81 A, HHB-60, GHB 235 and GHB 183, II b (ICMP 451), II c comprised two genotypes (WCC 75 and ICTP 8203), II d (PCB 164). Whereas, the cluster II e found largest as compared to remaining clusters which had 843 A, PB 172, PT 1890, FBC 16 and ICMV 155 genotypes. For ISSR marker, two major clusters at 0.70 similarity level were obtained. All the three hybrids HHB 60, GHB 235 and GHB 183 having common parent 81 A were grouped together in to sub cluster II a. The genotypes WCC 75 and ICTP 8203 from ICRISAT were grouped together in cluster II c Probably they have common parent age DNA fingerprinting is an important tool for characterization of germplasm and establishment the identify of varieties/ hybrids/ parental sources etc. ISSR polymorphism is useful in areas of genetic diversity, phylogenic studies, gene tagging, genome mapping and evolutionary biology in a wide range of species (Joshi *et al*., 2005).

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