RESEARCH PAPER

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Pearl millet characterization by using ISSR marker

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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