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Diversity analysis of commercial pearl millet [*Pennisetum glaucum* (L.) R Br.] hybrids based on RAPD markers

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SUMMARY

Random amplified polymorphic DNA (RAPD) markers were employed to assess genetic diversity in commercially cultivated pearl millet hybrids. A primer specific amplification profile was observed with minimum four amplicons with OPD-07 primer and a maximum of twelve amplified fragments with OPD-19 across seven varieties. The similarity index values ranged from 0.10 to 0.56 and the consensus tree exhibited two distinct groups. The late and mid-late maturing hybrids each formed a separate cluster whereas early maturing and drought tolerant hybrids formed another cluster. Hybrid GHB 577 was found to be most divergent. Use of such more polymorphic RAPD markers for screening pearl millet hybrids with characters of economic importance can help in identifying divergent cross combinations for achieving increased heterotic effects.

Key words: Pearl millet, RAPD, DNA polymorphism and Genetic diversity.

A high degree of genetic diversity among the commercial varieties, hybrids and breeding population is a prerequisite for achieving improved productivity. Use of molecular markers for estimating genetic distance among breeding stocks for estimating heterosis has been estimated in several crops (Witcombe, 2000). In pearl millet, a large number of crosses and evaluated each year; but the frequency of each hybrid released for commercial production is less. Pearl millet breeders need both variety and trait specific markers for accelerating the breeding efforts in view of the long span needed for varietal improvement. Complex hybrids with different levels of polyploidy are frequent among genetic use in pearl millet breeding and are difficult to identify using a few morphological and agronomic descriptors. Molecular diversity in pearl millet hybrids has been analyzed using RAPDs reflecting limited genetic base and necessity of diversifying genetic base. Commercial pearl millet hybrids have been analyzed using RAPD techniques to measure their genetic similarity in order to identify contrasting progenitors to be included in the breeding programme. The present work is aimed at analyzing the genetic diversity within the commercial hybrids of pearl millet developed over the years and which have been widely exploited in pearl millet breeding due to some desirable characters, by using RAPD markers.

MATERIALS AND METHODS

Seven pearl millet hybrids (released and promising) *viz.* GHB 577, GHB 538, GHB 558, GHB 526 MH 169, GHB 720 and GHB 664 were used for analysis of diversity using Operon random primers. Those primers showing polymorphic amplification profiles were used after initial screening (Table 1). The genomic DNA from the seedlings of each hybrid was extracted and after treatment with RNAase A (50 microgram/ ml) for 30 min. at 37^{0}c, the DNA was re-extracted with chloroform: isoamyl alcohol (24:1) and precipitated with 95 % ethanol (Zidani Sghaier *et al.* 2005). The pellets were washed with 70 % (v/v) ethanol and dried under vacuum and dissolved in TE (pH 8.0) buffer. The quantification of DNA was performed by running DNA samples on 0.8% agarose gel with known concentration of uncut lambda DNA and also by taking absorbance at 260 and 280 nm. Concentration was adjusted to 12.5 ng/ml. for use in a PCR reaction. PCR was performed in 25 ml. reaction volume containing, 0.5 ml of Taq DNA polymerase, d NTPs mix (0.2 Mm each of d CTP, d TTP, d ATP and d GTP), 20 mM Tris-HCL 2.5 ml, 1 ml primer and 25 ng of genomic DNA in 1 ml. The reaction mixture was vortexes and centrifuged briefly. Amplification was performed in a Thermal Cycler (Techne, UK) with the following temperature profiles, 94^{0}c for 5 minutes to denature genomic DNA, followed by 40 cycles of; 94^{0}c for 45 seconds, 38^{0}c for 30 seconds. 72^{0}c for 45 seconds. An additional cycle at 72^{0}c for 10 minutes was run at the end of these cycles. The PCR products were resolved by electrophoresis using 1.2% agarose gel in 0.5x Tris-Borate EDTA (TBE) buffer at 60 volt for 60 minutes. Gels were stained with ethidium bromide, visualized with UV light and photographed using gel-documentation system (Advanced American Biotechnology Lab, USA).

RESULTS AND DISCUSSION

The primer specific amplification profile was observed with minimum of 4 amplicons using OPD-07 primer across seven hybrids and a maximum of 12 amplification with OPD-19 primer. The polymorphism shown by five primers varied from 33.34 to 70.0 per cent (Table-1). While, the genotype specific amplification profile was observed with minimum of 15 amplicons by GHB 577 genotype across five primers and a maximum of 24 amplification with GHB 538 genotype.

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