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Assessment of molecular diversity in selected maize inbreds*

D. S. MUKHARIB, V. C. PATIL[1], D. P. BIRADAR, P. M. SALIMATH AND V. P. CHIMMAD

Department of Agronomy
University of Agricultural Sciences, Dharwad-580005, India.
Email: mukarib@gmail.com
[1]Precision Agriculture Research Chair (PARC), King Saud University, Riyadh, Saudi Arabia
Email: vcpatiluasd@gmail.com

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Abstract: Analysis of the extent and distribution of genetic diversity in crop plants is essential for optimizing sampling and breeding strategies. Randomly amplified polymorphic DNA marker was used to assess genetic diversity in a selected group of maize inbred lines. A high level of polymorphism of 73.02 per cent was detected among the genotypes. The maximum genetic distance of 29.7 per cent was detected between CM-202 and KDMI-16. While, the minimum genetic distance of 12.8 per cent was observed between KDMI-04 and CI-05. The results indicated that variation can be attributed to use of random primers as well as variation that naturally occur in the genotypes. Therefore, the study indicated that RAPD marker are successful in identifying genetic diversity in maize inbred lines.

Key words: RAPD markers, DNA polymorphism, maize genetic diversity

Introduction

Assessment of the extent and distribution of genetic variation in a crop species and its relatives is essential in understanding pattern of diversity and evolutionary relationship between accessions that helps to sample genetic resources in a more systematic fashion for conservation and plant improvement. Ideally, the study of polymorphism is best done at the level of arrangement of nucleotide bases in DNA, the primary source of all biological information. At this level, even seemingly identical accessions could display enormous differences, if only we could employ appropriate DNA profiling techniques. Besides, availability of virtually unlimited number of markers in any study group and the fact that they can be determined unequally at any stage in the life cycle of an organism under any growth environment is of great practical value.

Randomly amplified polymorphic DNA (RAPD) is one of the polymerase chain reaction (PCR) based DNA markers, which is an assay based on the amplification of genomic DNA with single primer of arbitrary nucleotide sequence. RAPD can be used in studying genetic diversity, phylogeny, quantitative tract loci, varietal identification etc. (Weising *et al.*, 1995). In the present study eight inbreds and Arabhavi local were assessed for diversity using RAPD analysis to use them in evolving heterotic hybrids.

Material and methods

A set of eight maize inbred lines and Arabhavi local were used in the study. DNA was extracted by CTAB method. Seed samples were germinated in plastic tea cups until seedlings were 2-3 leaf stage. At least 5-6 such seedlings were harvested from each genotype with a razor blade and were placed in an autoclaved pestle and mortar containing liquid nitrogen. The lypholized tissue sample was ground into fine powder. The ground tissue was transferred to tubes containing about 10 ml of 2 per cent CTAB solution. The tubes containing ground tissue samples were placed in water bath (with gentle shaking) for 10-15 minutes at 65°C with periodical shaking at an interval of 5 minutes. Later, the tubes with tissue extract were incubated at room temperature for 15 min. About 10 ml of chloroform and isoamyl alcohol mixture (24:1) was added to the tissue extract and the contents were mixed by shaking gently. The contents were then transferred to fresh centrifuge tubes and spinned for 10 minutes at 5000 rpm at room temperature.

The supernatant was transferred to fresh centrifuge tube, 10 ml of chillied isopropanol was added to each tube, mixed by inverting and incubated at -20°C for over night. Thereafter the content was centrifuged for 20 min with 5000 rpm at 4°C. The supernatant was discarded and the DNA pellet obtained was washed with 70 per cent ethanol and the tubes were inverted on blotter paper to dry the pellet. The DNA was dissolved in TE buffer (50 µl) and incubated at 4°C.

RAPD analysis was done individually with 28 random decamer primers (OPP 01-10; OPK 01-10; OPO 01-06 and OPJ 01-06) obtained from Operon Technologies, Almeda, USA.

The amplification products were separated on 1.2 per cent agarose gel. Electrophoresis was done until the tracking dye (Bromophenol blue) reached the edge of gel. The bands were visualized under UV-transilluminator and photographed. All primers produced recognizable bands. Scorable bands for a primer in each genotype were compared and allotted '0' (absent) or '1' (present) values. Band patterns ('0', '1' matrix) were tabulated for individual primers separately and the data pooled to obtain a combined matrix for genotypes.

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