Saudi Journal of Biological Sciences (2009) **16**, 57–62

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Application of simple sequence repeat (SSR) markers for molecular diversity and heterozygosity analysis in maize inbred lines

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Available online 27 October 2009

KEYWORDS

Maize;
Inbred lines;
Molecular diversity;
SSR;
Homozygous;
Heterozygous alleles

Abstract There is an important role of understanding the genetic diversity among and within inbred lines at the molecular level for maize improvement in different breeding programs. The present study was devoted to estimate the level of genetic diversity among the inbred lines of maize using the simple sequence repeat analysis (SSR). The application of six different SSR markers successfully provided the information on similarity or diversity as well as the heterozygosity of the allelic loci for all the eight inbred line of maize.

1. Introduction

Genetic fingerprinting of maize is an efficient method for large scale application to aid breeders in the placement of breeding lines and populations into the correct heterotic group, to aid in the curation of gene bank collections by refining the core subsets formed from field evaluation and to have a better understanding of the evolution of major tropical maize races (Dubreuil and Charcosset, 1998; Franco et al., 2001; Warburton et al., 2002). Previous studies have used restriction fragment length polymorphism (RFLP) markers to place temperate line into known heterotic groups with considerable success (Anthony et al., 2001; Ajmone-Marsan et al., 1998; Dillman et al., 1997; Dubreuil et al., 1996; Jones et al., 1997). In a study involving 148 US maize inbred lines, Mumm and Dudlley (1994) used 46 RFLP markers to cluster all the inbred lines into the two major heterotic groups. They were also able to identify subgroups within the major heterotic groups. Dillman et al. (1997) used RFLPs and morphological distances to study 145 maize inbreds released in France. They concluded that RFLP markers could serve as tools to discriminate between closely related individuals from different breeding sources. Other investigators have used random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) for maize diversity analysis (Ajmone-Marsan et al., 1998; Castigloione et al., 1993; Jones et al., 1997; Hernandez et al., 2001; Gomez et al., 2001). In a study of 33 inbred lines, SSR produced twice as more information as AFLPs and RAPDs, and 40% more than RFLP's in terms of numbers of alleles per locus (Powell et al., 1996).

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doi:10.1016/j.sjbs.2009.10.001

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Many studies have reported genetic diversity or relatedness of maize inbred lines at the molecular level (Bornet and Branchard, 2001; Dangle et al., 2001; Kenis and Keulemans, 2000; Selvi et al., 2003; Senior et al., 1998). However, studies on measuring genetic variation at this level within and among identically named inbred lines maintained by different programs are lacking. The approach of molecular fingerprinting is complementary to phenotypic measures in quantifying genetic changes because it shows variations in DNA that may not be phenotypically expressed. Historically important public inbred lines continue to play an important role in maize improvement in many different breeding programs. Owing to their continued use, they have undergone numerous seed generations in diverse programs since their original release (Warburton et al., 2002). The objective of this study was to estimate the level of genetic diversity both among and within inbred lines of maize by means of SSR markers.

2. Materials and methods

2.1. Plant materials

Plant tissue samples were obtained from each plant per inbred of eight maize lines. The plants were grown in the greenhouse at 25 °C under four weeks of light and darkness. After that time, approximately 100 mg of plant tissue was harvested and stored at −80 °C until DNA was extracted.

2.2. Molecular analysis

Plant genomic DNA was extracted by a modified cetyltrimethylammonium bromide (CTAB) method (Mitchess et al., 1997). Leaf tissues (100 mg) were ground in 100 μm of CTAB extraction buffer (100 mM Tris, 1.4 M NaCl, 20 mM EDTA, 0.2% β-mercaptoethanol and 2% CTAB, pH 8.0) and heated at 60 °C for 30 min. DNA was extracted with one volume of chloroform: isoamyl/alcohol mix (24:1) and precipitated in presence of isopropanol (40% [v/v] final concentration). The DNA pellet was washed with 5 mM ammonium acetate and 70% ethanol, dried, and dissolved in 100 μL of TE (10 mM Tris–HCl, 1 mM EDTA pH 8.0). After addition of 1 μl of RNase (10 mg/mL), DNA concentrations were determined with fluorometer (Hoeffer TKO 100) using bisbenzimide as a fluorescent dye. DNA was quantified with the picogreen ds DNA quantification kit (Molecular probes, Eugene, OR) Table 1.

Six SSR markers were used for genotyping as reported earlier (Warburton et al., 2002). The sequences and length of primers as well as the fluorescent dye labeling strategy are given in Table 2. Primer pairs were chosen on the basis of their properties of detecting single loci, their broad coverage of the genome and their high levels of polymorphism when applied to a broad range of maize germplasm. The sequence of the six primer pairs were chosen from the maize database project, Mias DB at the University of Missouri (http://www.agron.Missouri.edu).

Table 1 Types of inbred maize lines.

Inbred maize lines	Variety
A	Coral
A1	Merit
A2	Iochief
A3	Ne + 7007
A4	Ne + 7007
A5	Panama
A6	Giubileo
A7	Bonanza F1

2.3. Polymerase chain reaction (PCR)

Each 20 μl PCR reaction consisted of 1 × PCR buffer, 0.4 mM dNTPs, 1.2 mM $MgCl_2$, 0.2 unit of Taq polymerase, 1 μl (4 pmul/μl) of each primer and 5 μl (25–50 ng) of DNA. The amplification conditions were 95 °C for 2 min, 55 °C for 1 min, 72 °C for 5 min and a terminal extension step at 72 °C for 10 min. To prepare the PCR products for detection 0.5 μl of the amplified DNA was mixed with 0.1 μl Genescan 500 XL RoX standard (Applied Biosystems, Foster City, CA) and 1 μl of 50% formamide loading buffer and the DNA was denatured by heating at 95 °C for 2 min and then promptly cooled on ice.

The samples were loaded and electrophoresed on 2% (w/v) denaturing long ranger (FMC) 36-cm well-to-read gels. DNA samples were electrophoresed in 1 × TBE buffer (pH 8.3) at constant voltage (3.00 kV) for 3 h. Microsalellite loci repeats were assayed on the basis of their observed hetorozygosity and number of alleles detected with the PCR amplification profile. All samples were replicated to verify the results.

2.4. Statistical analysis

Hetrerozygosity (H) was calculated using Neis (1973) formula $H = n\,(n-1) * (1-\sum P_i^2)$, where P_i is the frequency of alleles i in the analyzed trees and n is the number of alleles. The power of discrimination (PD) for each locus was calculated using the formula $PD = 1 - \sum P_i^2$, where P_i is the frequency of genotype i (Kloosterman et al., 1993).

3. Results

The determined concentration of DNA ranged between 125 ng/μl for line A7 and 560.5 ng/μl for line A1.The genetic relationship of eight inbred lines were analyzed by simple sequence repeats (SSRs) markers and the six SSR primers gave stable amplified band pattern detected over 50 alleles among the tested lines (Table 3). The average number of alleles per SSR locus was 4.35 with a range from 2 to 10. The value of polymorphism information content (PIC) for each SSR locus varied between 0.42 1 and 0.88 with an average of 0.58. Clustering analysis with UPGMA showed that Merit and Ne + 7007 could not be compartmentalized as other genes, but the genetic distance between BonanzaF1and one of the other was quite big. The difference in mean genetic distance between Merit and Ne + 7007 compared to the mean genetic distance between Merit and Ne + 7007 was significant at 0.01 levels. Among the eight germ plasmas, Ne + 7007 is more closed to Merit.

Tables 4 and 5 showed that the alleles length for sir locus UMC 1061 were equal in lines A, A2, A3 and A6 (105 bp) and for line A1, A5 and A7 was 102 BP.

For SSR locus UMC 1122, the lines A, A1 and A4 have similar allele length (164 BP), but the A2 and A5 have 156