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Prospects of endosperm DNA in maize seed characterization

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Abstract

DNA based characterisation of maize germplasm has become the easiest and fastest approach to identify genetic diversity as compared to phenotyping. The conventional DNA source for genotyping is the leaf which required at least 2 weeks waiting period from seed planting to leaves sampling. This work exploits the use of endosperm DNA (EDNA) for the genotyping of maize germplasm. Maize endosperm was excised from maize seeds using pliers, ground and used for Genomic DNA extraction (gDNA). Leaves DNA (LDNA) was also extracted concurrently. The extracted LDNA and EDNA were quantified and subjected to SSR-PCR. The mean concentrations of DNA extracted were 1575 ng/ul for the leaves and 526 ng/ul for endosperm. Though the difference in quantity of EDNA and LDNA were highly significant, the quality (A260/A280) for both EDNA and LDNA fall within 1.6-1.8 range of pure DNA index. SSR-PCR products using phi032 were similar for both EDNA and LDNA, indicating the usability of EDNA in genotyping. This seed based method of gDNA extraction takes less than 24 hours from sampling to quantification and genotyping. It also allows germination of sampled seeds, selection before planting, avoids the delay of planting and waiting in leaf sampling and saves field space.

Keywords: genomic DNA, maize endosperm, genotyping, SSR-PCR

Introduction

DNA markers have the advantage of detecting unlimited number of polymorphisms randomly distributed in the genome without environmental effects and without influence of plant physiological development. Thus DNA based methodologies for seed purity assessment, genetic diversity study and selections are becoming more popular (Salgado et al, 2006; Menkir et al, 2005; Senior et al, 1998).

Simple sequence repeat (SSR) markers have been extensively used for most of these studies in maize (Smith et al, 1997; Reif et al, 2003; Pinto et al, 2003; Menkir et al, 2004). SSR motifs are 2, 3, or 4 nucleotides that are found in abundance in the genomes of eukaryotic plant species and these units are tandemly repeated many times in the DNA sequence (Morgante and Olivieri, 1993; Enoki et al, 2002; Menkir et al, 2004).

However, extracting DNA samples from leaves, the conventional method, which include collecting, processing leaf tissue and tracking back to source plants is the most significant rate limiting factor in genotyping and marker assisted selection. It is also time consuming and costly. According to Crouch (2007) 'use of leaf tissue means that lab analysis of markers has been "after the fact" in essence, scientists need to wait for plants to develop to obtain samples'. Meanwhile, only a few plants may contain the desired genes from the large number that must be grown. Seed DNA-based genotyping is now considered an important alternative (Salgado et al, 2006; Gao et al, 2008). It involves a non-destructive sampling method in extracting DNA that allows germination of sampled seeds and permits selection to be carried out in advance of planting. It also saves field space and creates the possibility of working with larger effective populations for complex agronomic traits.

This study was carried out to extract DNA from maize endosperm using simpler, non-destructive and cheaper sampling technique. In Salgado et al (2006), whole seeds were ground in liquid nitrogen while Gao et al (2008) soaked the seeds for few hours to soften without stimulating germination for sampling.

Materials and Methods

Genetic materials

Five open pollinating maize varieties derived from the maize breeding unit of Institute for Agricultural Research, Samaru Nigeria, were used for this study.

Endosperm Sampling

Seed endosperm sampling was done using plier to excise small pieces of the endosperm from the other side of the embryo. The seeds were not soaked for this sampling. 50 mg of dry endosperm pieces excised from 5 seeds per varieties were ground into fine powder using genogrinder at 1,000 strokes min^{-1} for 2 minutes.

Leaves Sampling

20 seeds of each variety were planted separately in a screen house at room temperature using normal garden soil. Leaves from 2 weeks old seedlings were freeze dried and ground using genogrinder at 1,200 strokes minute^{-1} for 2 minutes.

Table 1 - Mean squares for DNA concentrations and quality for DNA extracted from Endosperm and Leaves of Maize (2 repeated extractions).

Source of Variation	Df	DNA conc (ng μl⁻¹)	A260/A280	A260/A230
Genotype	4	191881.63	0.06	0.06
DNA source	1	4517891.73**	3.64*	12.85
Extraction buffer	1	1139702.49**	1.25	2.35*
Error	53	91252.83	0.11	0.20

**, * significant at P< 0.01and P<0.05, respectively

DNA extraction

For each variety, DNA was extracted in duplicate from the ground endosperm and leaves using both SDS protocol (0.1 M Tris-HCl pH 8.0; 0.02 M EDTA pH 8.0; 0.1 M NaCl; 1% SDS) (Salgado et al, 2006) and CTAB protocol (0.2 M Tris-HCl pH 8.0; 0.05 M EDTA pH 8.0; 2.0 M NaCl; 2% CTAB; 1% 2-mer-captoethanol) (Mace et al, 2004). Concentration and quality of the DNA was assessed using a Nanodrop (ND) 1000® spectrophotometer and by electrophoresis of 1 μl on a 0.7% agarose gel. The DNA concentration were standardised at 25 ng μl⁻¹ for SSR PCR.

PCR and electrophoresis for SSR analysis

Maize SSR oligonucleotide primer phi032 was diluted to a working concentration of 5 μM with sterile water and stored at -20°C. PCR conditions and gel visualisation were performed as described by Senior et al (1998). A "touch down" PCR profile, with annealing temperatures from 65°C to 55°C, was used for the SSR PCR analysis. The SSR loci amplified were separated on 2% (w/v) superfine agarose gels (Amresco). The gel was stained with ethidium bromide solution (500 μg ml⁻¹) and photographed under UV light attached to a gel documentation system (Bio-Rad, Hercules, CA).

Results and Discussion

Mean squares from ANOVA for complete random design (CRD) (Table 1) showed that there were significant differences in the quantity of DNA extracted for sources of DNA (leaves and endosperm) and for extraction buffers (SDS and CTAB). However, no significant difference in the quality of DNA extracted except between sources of DNA. Higher quantity of DNA is extracted using leaves samples than using the endosperm (Table 2). Although there was significant difference in the A260/A280 values between the LDNA and the EDNA the values fall within the range of acceptable DNA purity of 1.6-1.8. On the other hand the A260/A230 value indicating levels of impurity in the DNA sample showed that the EDNA

Table 2 - Comparison of concentration and quality of DNA extracted from leaves and endosperm of maize using CTAB based extraction buffer.

| DNA source	DNA conc (ng μl⁻¹)	A260/A280	A260/A230
Leaves	1575.24	1.81067	1.626
Endosperm	526.43	1.61833	0.7003
LSD (0.05)	356.44	0.1713	0.234
CV	5.7	2.865	12.302
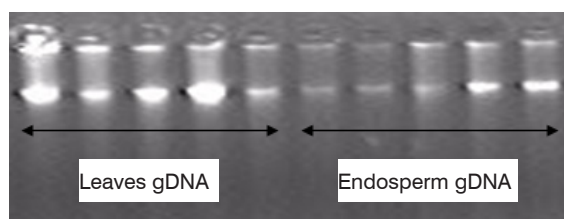
Figure 1 - GDNA extracted from maize endosperm and leaves using SDS extraction buffer.

had higher levels of carbohydrate. Yet this did not affect the quality of the gDNA (Figure 1) and its usability in further DNA analysis for characterisation of genotypes (Figure 2). Figure 1 showed the intactness of the DNA from leaves and endosperm samples of maize. The sizes were the same except the quality as indicated by intensity of the bands. These DNA samples worked well for further characterisation using SSR markers. The results from LDNA and EDNA were similar (Figure 2). Thus, EDNA is as useful as LDNA in SSR based characterisation of maize genotypes. This offered the opportunity for time saving and non-destructive genotyping of maize as compared to use of LDNA. These results were similar to those obtained in Gao et al (2008) and Salgado et al (2006). Moreover, the method of endosperm sampling used in this report would prevent the possible loss of seed and/or viability as compared to when seeds were soaked to soften for sampling (Gao et al, 2008) and the used of whole seeds (Salgado et al, 2006). It was also relative cheaper as grinding did not require liquid nitrogen.

Acknowledgements
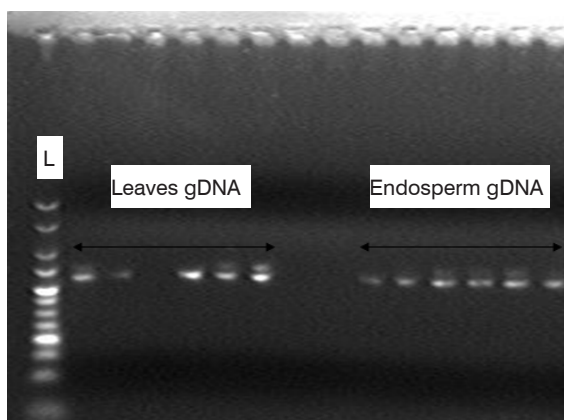
Figure 2 - SSR PCR amplification of maize endosperm and leaves DNA using Phi032 SSR primer.

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