Soybean Cultivar Identification Within A Selected Group Using Only An Agarose Gel System With Simple Sequence Repeat DNA Markers

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Abstract

This research focuses on the feasibility of using only high-resolution agarose gel electrophoresis with simple sequence repeat (SSR) DNA markers to distinguish elite soybean [*Glycine max* (L.) Merr.] cultivars. Most of the soybean research with SSR markers has utilized sequencing polyacrylamide gel electrophoresis. We tested our ability to distinguish between four elite cultivars using only high-resolution agarose electrophoresis with eleven SSR makers. Fragment sizes differing by seven base pairs or more could be easily separated. Of the eleven SSR markers, five markers exhibited visible polymorphisms between the four cultivars. Two SSR markers were found that could distinguish between all four cultivars. These results indicate that this method could be used to identify cultivars within a much larger selected group. Although agarose gel electrophoresis does not have the precision of polyacrylamide gel electrophoresis, it might find great utility with SSR markers for soybean cultivar identification.

Introduction

Molecular genetic markers have brought phenomenal changes in the area of plant biotechnology by their ability to produce unique DNA profiles in various crops. Simple sequence repeat markers are being extensively used in genome studies, marker-assisted selection, and cultivar identification and are well-known for their versatility in providing a quick assay and for their highly informative data (Song et al., 1999; Cregan et al., 1999). Simple sequence repeat (SSR) markers have provided an excellent and powerful method for distinguishing between different cultivars (Akkaya et al., 1992; Morgante and Olivieri, 1993; Song et al., 1999). There are many reasons for cultivar identification. We had a need to verify the originating cultivar for specific segregated lots of soybean [*Glycine max* (L.) Merr.] for a biodiesel research project.

Most of the published SSR marker methods for soybean cultivar identification used primarily sequencing polyacrylamide gel electrophoresis for separating the amplified SSR fragments (Akkaya et al., 1992; Rongwen et al., 1995). Unfortunately, our lab did not have equipment for sequencing polyacrylamide gels; however, we did have equipment for agarose gel electrophoresis. We found only a few reports of where researchers relied solely on agarose gels with SSR markers in soybeans (Morgante and Olivieri, 1993; Burnham et al., 2002); whereas, there are numerous reports of researchers utilizing polyacrylamide gels with soybeans (Akkaya et al., 1992; Maughan et al., 1995; Rongwen et al., 1995; Song et al., 1999; Narvel et al., 2000). When considering other crops or plants, there are reports where other researchers successfully utilized high-resolution agarose gels with SSR markers: in *Arabidopsis thaliana* by Bell and Ecker (1994), in maize (*Zea mays* L.) by Senior et al., (1998), and in wheat (*Triticum aestivum* L.) by Poland (2003). The objective of this study was to test the feasibility of using SSR markers to distinguish between four elite cultivars using only high-resolution agarose gel electrophoresis with a limited number of markers. It is likely that many other labs might utilize SSR markers if it was demonstrated that agarose electrophoresis equipment could be effectively utilized for cultivar identification.

Materials and Methods

Four elite cultivars were initially selected for possible use in the proposed biodiesel project because of their high combined protein and oil content (Lacefield and Pfeiffer, 2002). The four cultivars tested were Asgrow AG4201, Asgrow AG3703, Croplan Genetics RC4432, and FFR RT446. We chose to test the feasibility of distinguishing between these cultivars using only high-resolution agarose gel electrophoresis with a limited number of SSR markers. One hundred mg of epicotyl tissue from germinated seeds was used for DNA extraction. The tissue was ground in a mortar and pestle with liquid nitrogen prior to being extracted using the DNeasy plant mini extraction kit and protocol (Qiagen, Valencia, CA). The DNA concentration was quantified using absorbance at A_{260} and then stored in a freezer at -20^{o}C until used for polymerase chain reaction (PCR). Two replications were conducted for this experiment.

Each replication began with the extraction of DNA from each cultivar and ended with the analysis of each cultivar with all eleven SSR markers.

Eleven SSR marker primers were selected based upon the previous research of Morgante and Olivieri (1993), Rongwen et al. (1995), Akkaya et al. (1992 and 1995), and Narvel et al. (2000). The markers selected for testing were those that had potentially large differences in the amplified fragments to aid in separation using only agarose gels. Pre-synthesized primers were obtained from ResGen MapPairs (Invitrogen Life Technologies, Chicago, IL). The primer pairs used were: Satt001, Satt002, Satt005, Satt009, Satt173, Satt175, Satt181, Satt185, Satt586, SOYHSP176, and SOYRPRP1 (Soybase, 2002). Reaction mixes containing 90 ng of soybean genomic DNA, 1.5 m*M* Mg^{2+}, 0.1 µ*M* each of forward and reverse primers, 200 µ*M* of each dNTP, 1X PCR buffer containing 200 m*M* Tris-HCl, pH 8.4, 500 m*M* KCl, 15 m*M* $MgCl_2$, and 1 unit of *Taq* DNA polymerase (Invitrogen Life Technologies, Chicago, IL) in a total volume of 30 µL. Thermocycling consisted of a initial denaturing for 2 min at 95^{o}C, followed by 33 cycles of denaturing (25-s at 92^{o}C); annealing (25-s at 47^{o}C); and extension (25-s at 68^{o}C), followed by a final extension step (3 min at 72^{o}C) (Chung et al., 2003) on a MJ Research model PTC-200 thermocycler (MJ Research, Inc., Watertown, MA). For primer SOYHSP176, annealing and extension times were doubled to 50-s and also the annealing temperature was raised to 58^{o}C in order to provide optimum amplification conditions during PCR.

PCR products were separated on high-resolution 5% (w/v) agarose gels (SFR Agarose, Amresco, Solon, OH) using standard methods of DNA agarose gel electrophoresis (Asubel et al., 1994). The entire 30 µL PCR reactions were loaded on the gels after being mixed with a loading dye. In the first replication the electrophoresis was conducted for four hours using horizontal gel containing 1X TBE buffer, at 74 constant volts (Chung et al., 2003; Matthews et al., 2001). However, for the second replication electrophoresis was run for eight hours for better separation, visualization, and sizing of amplified fragments. Gels were stained with 0.15 µg/mL ethidium bromide (Asubel et al., 1994), visualized under UV source and photographed using Bio-Rad Gel Doc 2000 (Bio-Rad Inc., Hercules, CA). When shadowing or multiple bands were present, the most dominant band was used for analysis. DNA standards derived from a *Hae* III digest of

pUC 18 plasmid (Amresco, Solon, OH) were used as molecular weight standards and DNA fragments were sized according to relative mobility using a computer software program Quantity One (Bio-Rad Inc., Hercules, CA).

Results and Discussion

Out of the eleven markers tested, five markers (Satt005, Satt173, Satt181, Satt185, and SOYHSP176) were able to produce polymorphisms with significant differences in their fragment sizes. The results from both replications were nearly identical; however, the 8h electrophoresis runs in the second replication greatly improved the separation of the DNA fragments. The results of the amplification of microsatellite markers showing polymorphisms are presented in Table 1. No visible polymorphisms were detected between these four cultivars using Satt001, Satt002, Satt009, Satt175, Satt586, and SOYRPRP1 markers.

Table 1. Differences in SSR fragment sizes (bp) between four selected cultivars for five SSR markers.

	SSR Markers				
Cultivar:	**Satt005**	**Satt173**	**Satt181**	**Satt185**	**SOYHSP176**
Asgrow AG4201	**155†**	**233**	**211†**	**227**	**166**
Asgrow AG3703	**167**	**257†**	**213†**	**247†**	**117†**
CPL RC4432	**174**	**206**	**172**	**252†**	**131**
FFR RT446	**157†**	**261†**	**211†**	**252†**	**117†**

† When the difference between two fragment sizes was < 7 base pairs, no distinction was made between the compared cultivars using that respective marker

Figure 1 shows the results of analysis of Satt005 and Satt173 SSR markers. These two markers were able to distinguish between the four cultivars by themselves. Using Satt005 marker, the fragment lengths could be separated into three groups. In the first group, fragments from cultivars Asgrow AG4201 (lane 1) and FFR RT446 (lane 4) were similar. Asgrow AG3703 (lane 2) was by itself in the second group, and Croplan Genetics RC4432 (lane 3) was in the third group. To further distinguish these cultivars, Satt173 marker was then able to distinguish between cultivar Asgrow AG4201 (lane6)

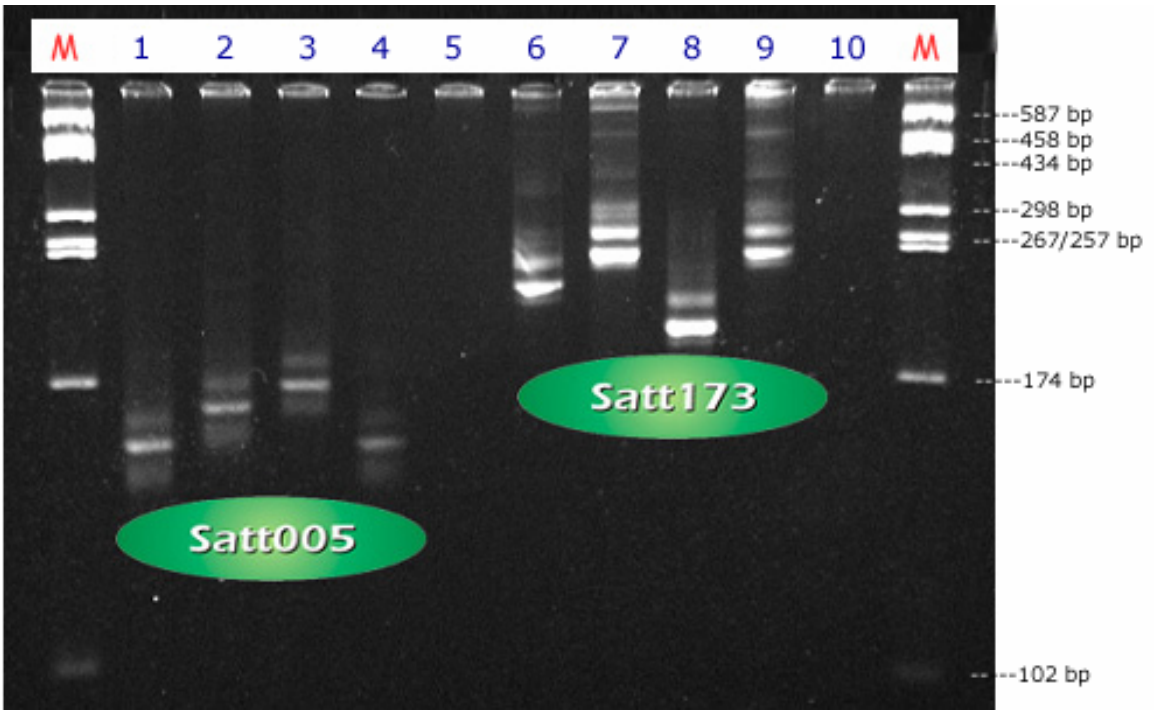

Figure 1. SSR marker polymorphisms detected at locus Satt005 (lanes 1 – 4) and Satt173 (lanes 6 – 9). M – denotes molecular marker. Lanes 1 and 6 represent the cultivar Asgrow AG4201; lanes 2 and 7 represent the cultivar Asgrow AG3703; lanes 3 and 8 represent the cultivar Croplan Genetics RC4432; and lanes 4 and 9 represent the cultivar FFR RT446. Lanes 5 and 10 were control PCR reactions where *Taq* DNA polymerase was omitted from the PCR reaction.

and FFR RT446 (lane 9). The other SSR markers that showed polymorphisms between these cultivars could then easily be used for further verification.

Using only high-resolution agarose gels, we tested and demonstrated that SSR markers can be used to distinguish cultivars of a selected group. High-resolution agarose gels were easily able to distinguish length of fragments differing by 7 bp or more (Fig. 1, Table 1). Another advantage of using agarose gel system is the ease of incorporating this method in undergraduate courses teaching plant breeding or biotechnology, without having to teach students how to pour polyacrylamide gels. This advantage may allow more instructors to utilize SSR markers in their classes. It is acknowledged that we selected SSR markers with the potential to have large differences in the fragment sizes.

Some disadvantages are acknowledged. This agarose method required more PCR supplies, since a greater volume of PCR reactions were loaded on each lane. Other limitations include the obvious inability to separate fragments that are very close in their size. These limitations, however, did not prevent us from utilizing this method.

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