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Genetic Diversity of North American Soybean (*Glycine max* L.) Cultivars as Revealed by RAPD Markers

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ABSTRACT

The objectives of this study were to evaluate the genetic diversity of the North American soybean (*Glycine max* L.) accessions using Random Amplified Polymorphic DNA (RAPD) molecular marker; and to evaluate RAPD markers to be used in soybean as genetic markers and improve such techniques as suitable strategies for soybean germplasm characterization. Twenty soybean accessions were included in this study and were subjected to RAPD molecular markers analysis. Twenty-seven RAPD primers produced 210 amplification products of which 78 (27.3%) were polymorphic. In conclusion, this study reported a successful fingerprinting of *G. max* accessions using RAPD markers and demonstrated the usefulness of these markers in estimating the extent of genetic variation in *Soybean* germplasm.

Key words: RAPD, soybean, North America, *Glycine max*, genetic diversity, UPGMA

INTRODUCTION

Soybean is one of the important oil and protein crops in the world. Soybean breeders in the USA have successfully developed hundreds of improved cultivars (Fehr, 1984; Sneller, 1994; Ude *et al*., 2003). Knowledge of diversity patterns will allow breeders to better understand the evolutionary relationships among accessions, to sample germplasm in a more systematic fashion and to develop strategies to incorporate useful diversity in their breeding programs (Bretting and Widrlechner, 1995).

Different approaches were used to assay genetic diversity in crop plants including morphological traits and isozyme electrophoresis, however, these techniques are insufficient to serve as accurate markers due to environmental influences on morphological traits and insufficient polymoprhism produced among closely related genotypes (Li and Nelson, 2001; Matus and Hayes, 2002).

Certain properties are desirable for a molecular marker such as highly polymorphic behavior, co dominant inheritance and frequent occurrence in the genome, even distribution throughout the genome, selectively neutral behavior, easy access, easy and fast assay and high reproducibility (Weining and Langridge, 1991; Weining and Henry, 1995). Examples of such DNA molecular markers are: Random Amplified Polymorphic DNA (RAPDs) (Welsh and McCelland, 1990; Williams *et al.,* 1990). RAPD markers had proved to be good genetic markers to assay and evaluate the genetic diversity between and within the same species, populations and individuals (Weising *et al*., 1995; Warburton and Bliss, 1996). RAPD marker depends on the amplification of

DNA sequence by polymerase chain reaction using only a single primer of arbitrary nucleotide sequence. The technique has proved to be fast and simple needs small quantities of template DNA, beside its ability to detect relatively small amounts of genetic variation (Williams *et al.*, 1990; Warburton and Bliss, 1996).

A series of studies have been conducted on soybean using isozymes (Griffin and Palmer, 1995; Li and Nelson, 2001; Malik *et al.*, 2009), Microsatellites (Narvel *et al.*, 2000; Li and Nelson, 2001; Li *et al.*, 2010) and AFLPs (Ude *et al.*, 2003).

The objectives of this study were to characterize the molecular diversity of the soybean accessions by analyzing the DNA amplification products using RAPD molecular markers; evaluate RAPD method to be used in soybean as genetic markers and improve such techniques as suitable strategies for soybean germplasm characterization.

MATERIALS AND METHODS

Plant material and DNA extraction: This study was conducted between 04/2009 and 04/2010 at Ohio University Zanesville. Seeds represented 20 genotypes of soybean were obtained from US department of Agriculture (USDA-ARS germplasm) (Table 1).

DNA was extracted from the young leaves of green house planted seedlings (4 week old) using DNeasy Plant Mini Kit (QIAGEN Inc., Valenica, CA, USA).

RAPD amplification: Thirty random primers (10-mer) from two kits (A and B) (Eurfins MWG Operon, Huntsville, AL, USA) of arbitrary sequence were used in this study (Table 2).

RAPD reactions were done in a total volume of 20 μL containing 20-60 ng of template DNA, 60 ng of each primer, 10 mL of Taq PCR Master Mix (QIAGEN Inc., Valencia, CA, USA) and

Table 1: USDA *Glycine max* germplasm collection used in this study

# Designation	Accession	State	Developed
S-01	548297	New Jersey	
S-02	614732	N/A	N/A
S-03	548301	Virginia	USDA Arlington Farm
S-04	548510	Indiana	Purdue University
S-05	548512	Indiana	Purdue University
S-06	548531	Illinois	USDA: ARS
S-07	548533	Illinois	University of Illinois
S-08	548536	Iowa	Iowa State University
S-09	518669	N/A	N/A
S-10	553039	NA	NA
S-11	552538	N/A	N/A
S-12	548560	Minnesota	University of Minnesota
S-13	548325	Wisconsin	University of Wisconsin
S-14	595843	N/A	N/A
S-15	548565	Ohio	Ohio University
S-16	548654	N/A	N/A
S-17	508267	North Carolina	North Carolina University
S-18	548656	N/A	N/A
S-19	633609	N/A	N/A
S-20	559370	N/A	N/A

Table 2: Random primers used to screen soybean germplasm for RAPDs

Primer	Sequence 5` to 3`
OPA-01	CAGGCCCTTC
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPA-04	AATCGGGCTG
OPA-05	AGGGGTCTTG
OPA-06	GGTCCCTGAC
OPA-07	GAAACGGGTG
OPA-08	GTGACGTAGG
OPA-09	GGGTAACGCC
OPA-10	GTGATCGCAG
OPA-11	CAATCGCCGT
OPA-12	TCGGCGATAG
OPA-13	CAGCACCCAC
OPA-14	TCTGTGCTGG
OPA-15	TTCCGAACCC
OPA-16	AGCCAGCGAA
OPA-17	GACCGCTTGT
OPA-18	AGGTGACCGT
OPA-19	CAAACGTCGG
OPA-20	GTTGCGATCC
OPB-01	GTTTCGCTCC
OPB-02	TGATCCCTGG
OPB-03	CATCCCCCTG
OPB-04	GGACTGGAGT
OPB-05	TGCGCCCTTC
OPB-06	TGCTCTGCCC
OPB-07	GGTGACGCAG
OPB-08	GTCCACACGG
OPB-09	TGGGGGACTC
OPB-10	CTGCTGGGAC
OPB-11	GTAGACCCGT
OPB-12	CCTTGACGCA
OPB-13	TTCCCCCGCT
OPB-14	TCCGCTCTGG
OPB-15	GGAGGGTGTT
OPB-16	TTTGCCCGGA
OPB-17	AGGGAACGAG
OPB-18	CCACAGCAGT
OPB-19	ACCCCCGAAG
OPB-20	GGACCCTTAC

8 mL of PCR water (QIAGEN Inc., Valencia, CA, USA). Template DNA was initially denatured at 94°C for 2 min followed by 35 cycles for 1 min at 94°C, at annealing temperature of 37°C for 1 min and at 72°C for 2 min as an extension step. The final extension step was done for 8 min at 72°C and the reactions were kept at soak file at 4°C.

The RAPD-PCR amplified products were analyzed by gel electrophoresis in 1.5% ultrapure agarose in 1X TBE buffer stained with ethidium bromide (0.5 μg mL) at 100 volts using horizontal gel electrophoresis apparatus (Sigma Chemical Co. Louis, MO, USA). The amplified products were

visualized under UV light and photographed with digital Olympus C-7070 camera (Olympus imaging America Inc., Melville, NY, USA). One kilobite ladder was used as a DNA standard to estimate the molecular weights of the amplified products.

Statistical analysis: For each individual RAPD primer, PCR amplified products were designated. Data were scored on the basis of the presence or absence of the amplified products. If the product is present in a genotype, it was scored as 1, if absent, it was designated as 0. Using the SAS statistical computer program SAS 9.2 (SAS Institute Inc., 2000-2009), genetic similarities between the soybean genotypes were calculated using the simple matching coefficient and clustered by unweighted pairs group method with arithmetic average (UPGMA) based on the average linkage method of calculating distance between clusters in hierarchical cluster analysis to construct dendrograms (Rohlf, 1993).

RESULTS AND DISCUSSION

Out of a total of 210 amplification products (0.2 to 4 kilo base pairs) using twenty-seven primers, 78 (27.3%) were polymorphic and 132 products were shared among all genotypes (not polymorphic). The twenty-four primers (OPA-02, OPA-03, OPA-04, OPA-05, OPA-07, OPA-09, OPA-10, OPA-11, OPA-12, OPA-13, OPA-14, OPA-15, OPA-16, OPA-17, OPA-18, OPA-19, OPA-20, OPB-03, OPB-04, OPB-05, OPB-06, OPB-07, OPB-10, OPB-11, OPB-12) produced different banding patterns for all genotypes. While sixteen primers (OPA-01, OPA-06, OPA-08, OPB-01, OPB-02, OPB-08, OPB-09, OPB-13, OPB-14, OPB-15, OPB-16, OPB-17, OPB-18, OPB-19 and OPB-20) detected no polymorphism although they did successfully amplify a range of monomorphic bands.

Two major clusters resulted: One cluster included by itself (S1 to S5) and the second cluster included the rest of the accessions (S6 to S20). This second cluster was subdivided into two sub clusters (sub cluster included S6 to S10 and the other sub cluster included S11 to S20) (Fig. 1). The genetic distance between first and the second cluster was 4.1.

Cluster analysis of each RAPD profiles (Fig. 1) showed that a significant genetic variation was detected in soybean accessions in particular samples S1 to S10 which could reflect different genetic background. Moreover, cluster analysis showed that samples S11 to S20 were genetically close (they showed 96% similarity).

The dendrogram (Fig. 1) showed that the RAPD methodology was sensitive enough to detect low levels of variation in soybean accessions.

The current study aimed on using the PCR-based protocols to assess genetic variability and to fingerprint genotypes of the North American soybean. Random amplified polymorphic DNA (RAPD) had been used effectively to assess the amount of genetic diversity in germplasm collections. Using wheat, barley, rye and wheat-barley addition lines, Weining and Langridge (1991) detected polymorphism using conserved, semi random and random primers. With different combinations of primers, they were able to detect both inter and intra specific diversity. In this study it was possible to show that the amplification products from 27 random primers (RAPD assay) were sufficient to discriminate among individual genotypes of soybean.

Few genetic studies have been conducted on North American soybean cultivars. In recent studies, fingerprints based on different markers were compared using genotypes from different species. There were both agreements and disagreements in findings based on different markers and species: The study of *Brassica oleracea* by Lanner-Herrera *et al.* (1996), a moderate spearman's

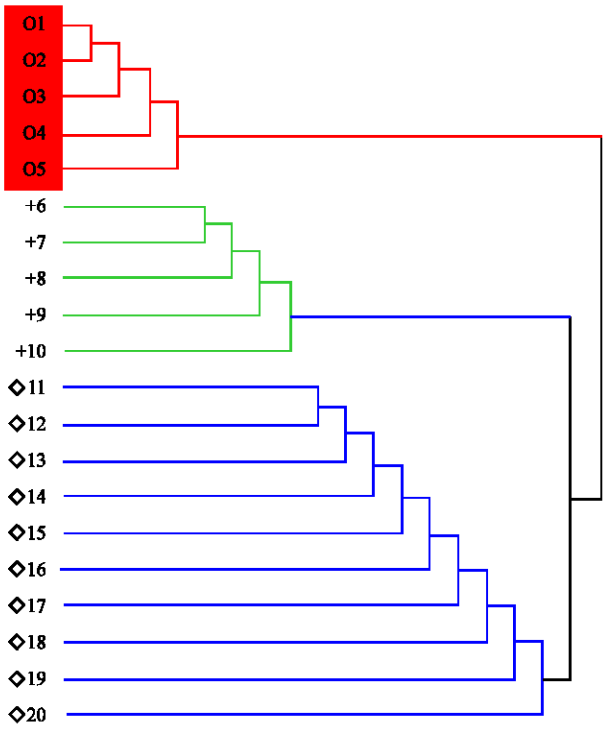

Fig. 1: Dendrogram derived from the UPGMA procedure using genetic distances generated from the AMOVA program depicting the relationships among soybean accessions. Genetic distances are estimated from RAPD markers. Red = First cluster; Green and Blue collectively = Second cluster

rank correlation (r = 0.38) between RAPD and isozyme distances was found. Russell *et al.* (1997) compared the levels of genetic variation among barley accessions revealed by RFLP, AFLP, SSRand RAPD and reported that when the spearman's rank correlation was used, the correlation between SSR and RAPD was 0.235, the highest correlation was found between RFLP and AFLP (0.708).

Because of the low number of the genetic studies and the poor resolution of some employed molecular markers in these studies, more studies like the current study are urgently needed in order to evaluate and estimate the genetic diversity in the North American soybean germplasm.

In conclusion, this study reported a successful fingerprinting of *G. max* accessions using RAPD and demonstrated the usefulness of these markers in estimating the extent of genetic variation in *soybean* germplasm. The current results are supported by many recent molecular studies (Thompson and Nelson, 1998a, b; Thompson *et al.*, 1998; Li and Nelson, 2001; Ude *et al.*, 2003).

The use of RAPD markers in *Glycine* must be further continued in order to drive specific linkage between RAPD markers and genes controlling agronomically important characters. These diagnostic molecular tools will greatly assist in the identification of new and different sources of diversity which may help breeders to decide what genotypes to cross for making new genetic combinations and to determine which genetic resources should be retained in a collection in order to conserve maximum genetic diversity in the gene bank.

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