Plant Res. 2(1) 22~25(1999)

Confirmation of F_1 Hybridity Using RAPD Markers in Soybean

Jong-Il Chung, Mi-Suk Ko, Jung-Hyun Shim, Seok-Hyeon Kim and Jin-Ho Kang
Department of Agronomy, Gyeongsang National University, Chinju 660-701, Korea

ABSTRACT

Molecular markers are useful to confirm the hybridity of F1 plant derived from cross of two homozygous parents with similar morphological traits. RAPD markers were used to test F1 hybrid plant obtained from cross of two homozygous soybean (*Glycine max*) parents. F1 plant for cross I was made from the mating of Hobbit87 (female) and L63-1889 (male) and F1 plant for cross II was obtained from the mating of H1053 (female) and L63-1889 (male). Selfing plant per each cross was also obtained. Among 20 Operon primers used, OPA04 and OPA09 show polymorphism between cross I and II parent. Band in size 1Kb of OPA04 and 2.1Kb of OPA09 primer was polymorphic band. This fragment identified F1 hybrid plant and selfing plant in cross I and II. Female parent Hobbit87 in cross I and H1053 in cross II has no this fragment (recessive allele). However, male parent L63-1889 and F1 hybrid plant in cross I and II has this size of polymorphic band (dominant allele). This indicated that F1 hybrid and selfing plants were detected by RAPD marker before phenotypic marker would be used to identify F1 hybridity. Amplification products of selfing plant for cross I and II were completely same to the those of female parent. When mature, flower color of F1 hybrid plant in cross I and II was purple and flower color of selfing plant in cross I and II was white. Purple flower is dominant trait. F1 hybridity was successfully detected at very early growth stage using RAPD marker. Therefore, RAPD marker can be used broadly to confirm F1 hybridity in many crops.

Key word : soybean, F1 hybrid, RAPD marker

INTRODUCTION

Obtaining F1 hybrid plants in self-pollinated crops is a basic procedure in the genetics and breeding program of these crops. Hybridity of F1 plant was normally confirmed based on morphological traits such as flower, pubscence, pod, hilum, seed coat color (Woodworth 1923). In early growth stage, most phenotypical traits can not be used to confirm F1 hybridity. Also, in case of the parental cross with similar traits, we cannot use these morphological traits to identify F1 hybridity. Alternative is to use molecular markers to test F1 hybridity.

Molecular markers have become fundamental tools for research involving plant genomes. Random amplified polymorphic DNA (RAPD) markers prepared by the polymerase chain reaction (PCR) are useful for fingerprinting varieties, tagging desirable genes and gene mapping because RAPD markers are more rapidly and more easily

Corresponding author: **Jong-Il Chung,** Department of Agronomy, Gyeongsang National University, Chinju 660-701, Korea

detectable than are RFLP markers (Welsh and McClelland 1990, Williams et al. 1990). Several researcher reported the inheritance and utilization of RAPD markers in F1 generation. Carlson et al. (1991) confirmed the inheritance of random amplified DNA markers in the F1 generation through diallel study of conifers. Heun and Helentjaris (1993) observed the inheritance of RAPDs in F1 hybrids of corn. In soybean, there was little study on the utilization of molecular markers to identify F1 hybridity derived from the mating of two homozygous parents. The objective of this research was to use RAPD marker to confirm F1 hybridity in cross of two homozygous parents.

MATERIALS AND METHODS

Plant materials

The study was conducted with three soybean genotypes. Three parents, Hobbit87, H1053, and L63-1889 were planted on 18 April 1998 in the Agronomy greenhouse. Flower color for Hobbit87 and H1053 was white and flower color for L63-1889 was purple. Purple flower is dominant trait. Two different crosses were made between three genotypes in flowering stage. Cross I was made from the mating of Hobbit87 (female) and L63-1889 (male) and cross II was obtained from the mating of H1053 (female) and L63-1889 (male). Selfing plant per each cross was also obtained. When mature, the hybrid and selfing seed borne on each female parent was collected and hand threshed. F1 hybrid and selfing seed and three parent seeds were planted in the Agronomy greenhouse. At early stage, young leaf tissue of each cross and parent was collected for RAPD analysis. The hybridity of F1 plant per each cross was verified by observing phenotypic marker, flower color.

Genomic DNA extraction and RAPD analysis

Total genomic DNA was isolated from young leaf tissue of the F1 hybrid and selfing plant and parent per each cross. DNA was isolated from finely ground leaf tissue by means of a modified CTAB procedure (Saghai Maroof et al., 1984). For the analysis of random amplified polymorphic DNA (RAPD) markers, 10-mer oligonucleotide primers were obtained from Operon Technologies, INC. (Alameda, Calif.). The RAPD-PCR protocol followed that described by Williams et al. (1990) except for minor modifications in the thermocycler temperatures and times that were needed to optimize the amplification of soybean DNA. The PCR reaction was performed in Perkin-Elmer Thermocycler. The thermal profile programmed for 38 cycles of 16 second at 92° C, 22 second at 42° C, and 70 second at 72° C, before ending with 1 cycle of 5 minutes at 72° C. The reaction mixture was then cooled to 4oC and maintained at this temperature until gel electrophoresis. Amplification products were electrophoresed in 1.2 % TBE agarose gels and stained with ethidium bromide to reveal DNA segments (amplicons) of varying sizes (i.e., number of base pairs) that had been amplified by the PCR. Gels were photographed under transmitted UV light. RAPD primers were first tested on the parental DNAs. Primer producing parental polymorphisms (female parent has no band, recessive allele and male parent has band, dominant allele) was then tested on the F1 hybrid and selfing plant per each cross.

RESULTS AND DISCUSSION

Three parents were first analyzed to find parental polymorphism with twenty Operon random primers. 20 primers used produced discrete PCR products with three parents and their size ranged from 0.3 to 3.2 Kb. The average number of band amplified per primer and their size in this study was similar to the results of another research (Chung and Specht 1997). OPA04(5' - AATCGGGCTG-3') and OPA09 (5' - GGGTA ACGCC-3') primers show polymorphism between cross I parent, Hobbit87 (recessive allele) and L63-1889 (dominant

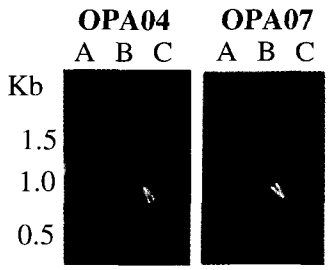

Fig. 1. DNA amplification patterns obtained with RAPD primers OPA04 and OPA07. A is Hobbit87, B is L63-1889, and C is H1053 parent. Polymorphic bands are indicated by arrows.

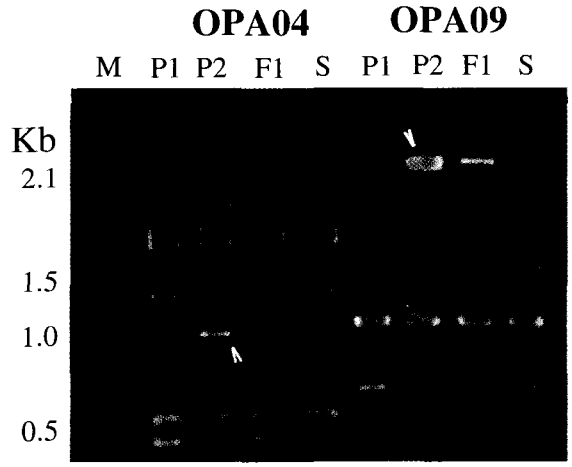

Fig. 2. RAPD markers obtained with OPA04 and OPA09 primers for cross I. M is molecular marker. P1 is Hobbit87 (female) and P2 is L63-1889 (male) parent. F1 is F1 hybrid plant and S is selfing plant obtained from cross of P1 and P2. Polymorphic bands indicated by arrows were used to confirm F1 hybridity.

allele) and cross II parent, H1053 (recessive allele) and L63-1889 (domianant allele). Although OPA07 and OPA15 primers show polymorphism between cross I and II parents, both primers were not used in F1 and selfing progeny screening because female parent has band (dominant allele). Figure 1 presents the results obtained with OPA04 and OPA07 primer. Arrow indicates polymorphic band. F1 and selfing plants of cross I and II were tested with primer OPA04 and OPA09. Figure 2 shows DNA amplification patterns of parent, F1 and selfing plant for cross I obtained with OPA04 and OPA09 primer. Among amplification products using OPA04 and OPA09 primer, band in size 1Kb of OPA04 primer and 2.1Kb of OPA09 primer was polymorphic band. F1 hybrid and selfing plants were identified by this fragment in cross I and II. Female parent Hobbit87 in cross I and H1053 in cross II has no this fragment. However, male parent L63-1889 and F1 hybrid plant in cross I and II has this size of polymorphic band. This indicated that F1 hybrid and selfing plant was detected by RAPD marker before phenotypic marker would be used to identify F1 hybridity. We never observed a fragment in F1 plant that was not observed in parent for cross I and II. This result is consistent with the result observed by Heun and Helentjaris (1993), Riedy et al (1992). Also, OPA04 and OPA09 primers were mapped in soybean mapping

Table 1. Flower color and RAPD marker for parents, Fl hybrid and selfing plant.

Cross	Parent	Flower color	RAPD marker	
			$OPA04_{1000}$	$OPA09_{2100}$
I	Female/Hobbit87	White	-	-
	Male/L63-1889	Purple	+	+
	F1	Purple	+	+
	Selfing	White	-	-
II	Female/H1053	White	-	-
	Male/L63-1889	Purple	+	+
	F1	Purple	+	+
	Selfing	White	-	-

-: no band, +: band.

population as linkage group 1 and 2, respectively (Chung 1996). Amplification products of selfing plant for cross I and II were completely same to the those of female parent. Table 1 shows flower colors, RAPD markers obtained with OPA04 and OPA09 primers for parent, Fl hybrid and selfing plant in both crosses. In both crosses, flower color of female parent was white and that of male parent was purple flower color. Purple flower color in F1 hybrid plant and white flower color in selfing plant was expected. When matured, flower color of F1 hybrid plants in cross I and II were purple, and flower color of selfing plant in cross I and II was white.

Three soybean parents used in this study have very similar morphological traits except for flower color. Hybridity of F1 plant derived from these parents was not confirmed until flowering stage. However, F1 hybridity was successfully detected at very early growth stage using RAPD marker. Therefore, RAPD marker can be used broadly to confirm F1 hybridity in many crops.

REFERENCES

Carlson J.E., Tulsieram L.K., Glaubitz J.C., Luk V.W.K., Kauffeldt C., and Rutledge R. 1991. Segregation of random amplified DNA markers in F1 progeny of conifers. Theor. Appl. Genet. 83: 194-200.

Chung JongIl and Specht J.E. 1997. Construction of linkage map using RAPD and SSR markers in soybean. Korean J. Plant Res. 10(3):241-246.

Chung JongIl. 1996. Genetic variance components and quantitative trait loci mapping for soybean seed size. Ph.D. thesis.

Heun M. and Helentjaris T. 1993. Inheritance of RAPDs in F1 hybrids of corn. Theor. Appl. Genet. 85:961-968.

Riedy M.F., Hamilton W.J., and Aquadro C.F. 1992. Excess of non-parental bands in offspring from known primate pedigrees assayed using RAPD PCR. Nucleic Acids Res. 20:918.

Saghai Maroof A.A., Soliman K.M., Jorgenson R., and Allard R.W. 1984. Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. USA 81:8014-8018.

Welsh J. and McClelland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 24:7213-7218.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J. A., and Tingey S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.

Woodworth C.M. 1921. Inheritance of cotyledon, seed-coat, hilum, and pubscence colors in soybeans. Genetics 6:486-553.

Received January 10, 1999
Accepted April 10, 1999