Use of RAPD Markers to Screen Hybrids of Oilseed Sunflower

Liu Gongshe, Mo Jiesheng, Liu Jie
Institute of Botany, Chinese Academy of Sciences
20, Nanxincun, Xiangshan, 100093 Beijing, P.R.China
Fax : +861062590833; e-mail: liugs@public.east.cn.net

Sun Baoqi
China Agricultural University
Beijing 100094, China
Fax +861062891866; e-mail: sbq8@263.net

Summary:

The identification of sunflower hybrids can be carried out in using polymerase chain reaction (PCR) and arbitrary 10-mer primers to generate random amplified polymorphic DNA (RAPD) markers. The hybrids A15 (one commercial oilseed sunflower hybrid in China) and its parents (male sterile line A and restorer line R) were assessed for genetic diversity using RAPD analysis. DNA was extracted from young true leaves and purified. The PCR test system suited for RAPD analysis of A15 seeds purity was constructed. 171 RAPD primers (Operon series) were tested between A line and R line. Strong PCR products were produced by 154 (about 90 percent) of these primers. It was displayed that detecting genome of plant by RAPD primers is doable because these random primers can cover most part of the genome. 118 primers (76 percent of 154 primers) yielded polymorphic PCR products. 38 primers yielded same type of bands. All of the 154 primers produced 906 bands. 483 bands among them are common between A line and R line. The similarity of A line and R line is 0.5331 (simple matching coefficient). The genetic distance of A line and R line was calculated to be 0.6833 (average Euclidean distance). Besides, one primer H9 produced complementary bands in hybrid A15, which were not detected in other large cultivated varieties. The differential bands produced by this primer can be used to screen hybrid A15 and test seeds purity.

Key word: RAPD, oilseed sunflower

Introduction

The identification of the genetic purity of F1 hybrid seeds is essential for seed production. Before putting on the market F1 hybrid seeds, the genetic purity of the products usually must be determined in order to keep high yield of the crop. Morphological methods are traditionally used for the determination of the purity, these methods, however, have some constraints that involve in a lengthy survey of plant growth and are costly labor-intensive and sensitive to changes in the environmental conditions. Although some techniques such as isozyme analysis and electrophoresis of storage protein in the seed have been successfully developed (Bassiri, 1976; Ladizinsky & Hymowits, 1979), they can not be applied to the discrimination of closely genotypes because of relatively little polymorphism. To overcome the constraints associated with the use of morphological or biochemical markers (Tanksley, 1983), DNA polymorphism are used as molecular marker for detecting variation among plants and many corresponding new techniques such as RFLP, RAPD, AFLP, SSR, DAF etc. appeared and put to use in analyzing of germplasm, location of gene, construction of genetic map and many other field (Rogstad et al., 1989;Williams et al., 1990; Ajmone, et al., 1998; Russell,et al., 1997;). In the present paper, we report a application of the RAPD assay for sunflower cultivar identification and genetic purity determination of hybrid seeds in oilseed sunflower.

Materials and methods

Plant materials used in this study (table 1): The A line and R line are maintained as pure stocks and used to produce commercial A15 hybrid seeds. Other 10 hybrid verities of oilseed sunflower were obtained from France and USA. All the plants

Table 1: Plant materials used in this study

Symbol of the cultiva	Breeding Character	Source
A	Sterile line	France
R	Restoring line	France
A15	Hybrids	France
LG12028Q	Hybrids	France
CRN1435	Hybrids	France
CRN1445	Hybrids	France
SF9903	Hybrids	France
SF9902	Hybrids	France
S-3322	Hybrids	France
SH332	Hybrids	France
SH41	Hybrids	France
SF9901	Hybrids	France
G101	Hybrids	U. S. A.

were planted in greenhouse.50 plants were individually sampled to analyze the genetic variation in A and R lin. As essentially no variation was observed within the lines, DNA was extracted from 10 plants in bulk, and used for PCR reaction.

DNA extraction: DNA was extracted from young true leaves of 10-day-old seedling according to the method of Rogers and Bendich (1980). The DNA concentration was determined by band intensity on agarose minigel.

PCR conditions: the reaction mixture (20PCl) for PCR was composed of 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 20 mmol/L TrisHCl (pH: 8.0), 0.1% Triton-x-100, 200 , mol/L dNTPs (each), 0.25 momol/L 10 mer primer [purchased from Operon Technologies (Alameda,Calif.)], 50ng template DNA, 1 unit Tag Plus I DNA polymerase (Sangon, Canada). Amplification was carried

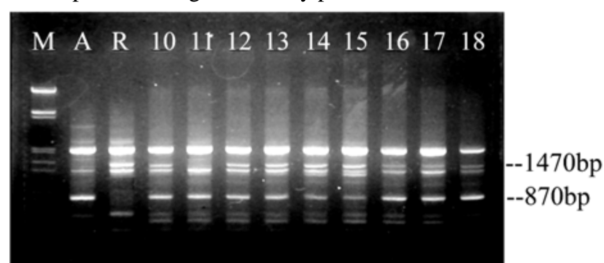
out in a AmpGene 4800 (N.J.Co.Ltd.) with preheating for 94po 4 min, 40 cycles of 40 sec at 94 4,1 min 30 sec at 38,1, 1 min at 72, , postheating for 7 min at 72, . After all the cycles, 6. l PCR products were loaded on 1.2% agarose gel with 0.5e g/ml ethidium bromide in 1 X TBE buffer and run electrophoresis at 80 V, 1.5 h and then take photograph under UV light.

Results and discussion

171 RAPD primers were used for detecting polymorphism between A line and R line. Strong PCR bands were produced by 154 (about 90 percent) of these primers. 118 primers (76 percent of 154 primers) yielded polymorphic PCR products, 38 primers yielded same type of bands. Approximately 906 products, ranging from 0.31 to 5.0 kilo base pairs (kbp), were amplified by the 154 primers tested. 483 bands among them are common between A line and R line. 423 out of a total of 906 PCR products observed were polymorphic between the parents, suggesting that 46.69% of the genome differs. The similarity of A line and R line is 0.5331 (simple matching coefficient). The genetic distance of A line and R line was calculated to be 0.6833 (average Euclidean distance). It showed that detecting genome of plant by random oligo-nucleotide short primers (RAPD primers) is doable because these random primers can cover most part of the genome.

The primer H9 produced a unique fragment of 0.87 kbp in the female parent line (A line) and a 1.47 kbp fragment in the male parent line (R line) (Fig. 1). Both of the complementary bands were produced in the F1 (A × R). The products from H9 primer were reproducible and, the female parent (A line), male parent (R line) and false individual hybrid (No.11) could be discriminated from the F1 (A × R) using this marker.

Fig .1: RAPD profile of parents and generated by primer H9



M: DNA markerM:DNA +Hind III +Ecor I; A: A line; R: R line; 10-18: A15 hybrids

Fig.2 showed that there was rich polymorphism between the 11 hybrids hybrids using of primer H9. The 0.87kb band was generated by the primer in CRN1435, SF9903, S-3322, SH3322 and meanwhile the 1.47 kb band in SF9902, SF9901, CRN1445, whereas all the other hybrids of oilseed sunflower in this test were without any of the two bands. The A15 can be discriminated from other 11 hybrids of oilseed sunflower with primer H9.

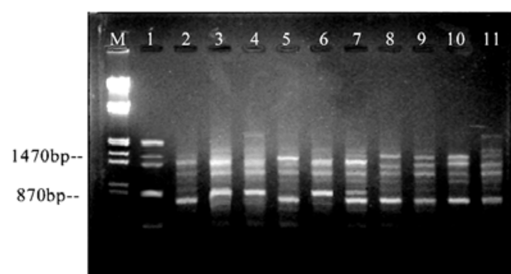


Fig.2. RAPD profiles of 11 oilseed sunflower hybrids generated by primer H9
M: DNA marker MDNA +Hind III +Ecor I; 1: A15 hybrid; 2: LG12028Q; 3: CRN1435; 4: SF9903; 5: SF9902; 6: S-3322; 7: SH332; 8: SH41; 9: SF9901; 10: CRN1445; 11: G101

The female parent A line is a male sterile line and the male parent R line is a restorer line. The combination of these two groups is extensively used for commercial F1 seed production in high-yield, high-oil-rate oilseed sunflower cultivars in China. Primer H9 is considered to be useful for the determination of the purity of seeds developed from this combination. As shown in the present study, RAPD as a technique could be used for the detection of genetic differences in the parental lines of oilseed sunflower, and is suitable for the determination of the genetic purity of hybrid seeds.

References

Ajmone marsan,P.,Castiglioni,P.,Fusari,F.,Kuiper,M.,Motto,M.,1998.Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. Theor Appl Genet. 96: 219-227

Bassiri,A., 1976. Barley cultivar identification by use of isozyme electrophoretic patterns. Can.J.Plant Sci. 56:1-6

Ladizinsky,G., Hymowitz,T., 1979. Seedprotein electrophoresis in taxonomic and evolutionary studies. Theor.Appl.Genet. 54:145-151

Rogers, S.O.,Bendich,A.J., 1985. Extraction of DNA from miligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol Bio.5: 69-76

Rogstad,Steven,H., 1989.The biosystematics and evolution of the polyalthia hypolenca complex (Annonaceae) of Malesia: I systematic treatment. J Arnold Arbor. Harv.Univ. 70(2):153-246

Russell,J.R., Fuller,J.D., Macaulay,M., Hatz,B.G., Jahoor,A.,Powell,W., Waugh,R., 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs,SSRs and RAPDs. Theor Appl Genet 95:714-722

Tanksley SD.,1983. Molecular markers in plant breeding. Plant. Mol.Biol.Rep. 1:3-8

Williams, F.G.K,Kubelik, A.R., Livak, K.J.,1990. DNA polymorphisms amplified by arbitary primers are useful as genetic markers. Nucl. Acids Res., 18:6531-6535