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Genetic Relationships among Korean Adlay, *Coix lachryma-jobi* L., Landraces Based on AFLPs

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ABSTRACT: Thirty-two germplasms of Korean adlay landraces were examined to analyse the genetic relationship through the amplified fragment length polymorphism (AFLP) approach. Total number of AFLP products generated by 12 selective primer combinations was 882. The number of polymorphic fragments by each primer combination greatly varied from 4 to 51with a mean of 20.3, bands visible on the polyacrylamide gel. A genetic similarity coefficient was used for cluster analysis following UPGMA (unweighted pair grouping method of averages) method. The resulting clusters were represented in the form of a dendrogram. The clustering was not tight in the dendrogram. There was generally no clear grouping of the adlay according to the geographic regions in which germplasms were collected. The present AFLP analysis imply that although Korean adlay displayed a larger amount of AFLP variation within germplasms, the variation was shown independently without reflecting a clinal variation. This study demonstrated that AFLP method can be used to examine the genetic relationships among different germplasms of adlay.

Keywords: adlay, germplasm, AFLP, genetic relationship

Adlay (*Coix lachrymal-jobi* L.) is a type of wild millet grass, related to maize (McKevith, 2004). Adlay has long been used as a traditional medicinal and nourishing food, due to its high nutritional value and special biological and functional effects on the human body (Tseng *et al*, 2004). It is widely planted in Taiwan, China, and Japan, where it is considered as a healthy food supplement.

Over the years, adlay has been used as a food source for human and livestock. It has also been utilized as a diuretic, stomach medicine, analgesic, antispasmodic and hypoglycemic agent in oriental folk medicine. However, there is no scientific verification of it having any medicinal properties (Cho & Lee, 1997). Studies for adlay have been focused on nutritional, protein trait and agronomic characteristics (Cho & Lee, 1997; Woo, 1991; Kim *et al*, 1996; Kim *et al*., 1997). Recently, Yi *et al* (2004) reported to evaluate on agronomic traits of Korean local adlay germplasms.

Amplified fragment length polymorphisms (AFLP) analysis is based on the selective PCR, using selective primer combinations, with a high number of restriction fragments, which is obtained through a total digestion of genomic DNA (Vos *et al*, 1995). The AFLP approach is especially powerful because it can be used without a prior sequence characterization of the target genome (Park & Kim, 1999). Additionally, it detects a ten-fold greater number of loci than those detected by RAPD analysis (Sandhu *et al*, 1992; van Heusden *et al*, 2000). Since the AFLP analysis can rapidly screen thousands of independent genetic loci, it is useful to distinguish the closely related species, varieties, and cultivars having similar phenotypes (Russell *et al*., 1997). Roa *et al* (1997) evaluated a representative sample of the crop's diversity and six wild taxa with AFLPs to estimate genetic relationships within a genus.

The objectives of this study were to test the effectiveness of AFLP analysis in identifying genetic diversity of adlay, and to determine the molecular genetic variations and relationships among 32 germplasms of adlay collected from different regions.

MATERIALS AND METHODS

Plant materials

Seeds of 32 collected adlay germplasms were provided by the Gyeonggido Agricultural Research & Extension Service in Hwaseong, Korea (Table 1). Adlays were grown in a growth room under conditions of 16 h / 8 h (day / night) at 25 °C. Leaves of four-week-old seedling were harvested.

AFLP analysis

Genomic DNA was extracted from 100 mg of fresh young leaf tissues using plant genomic DNA extraction miniprep system (VIOGENE). AFLP analysis was conducted using AFLP Analysis system (Invitrogen™ Life Technology).

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Table 1. Distribution of collected region and 32 adlay cultivars used in the experiments.

No.	Local	Province
C1	Anseong	Gyeonggi
C2	Boseong	Jeonnam
C3	Buan	Jeonbuk
C4	Bukjeju	Jeju
C5	Cheongsong	Gyeongbuk
C6	Dangjin	Chungnam
C7	Geochang-1	Gyeongnam
C8	Geochang-2	Gyeongnam
C9	Gimje	Jeonbuk
C10	Gochang	Jeonbuk
C11	Goesan	Chungbuk
C12	Goheung	Jeonnam
C13	Gokseong	Jeonnam
C14	Gongju	Chungnam
C15	Goseong	Gyeongnam
C16	Gunwi	Gyeongbuk
C17	Gwangju	Jeonnam
C18	Gyeongju	Gyeongbuk
C19	Gyeongsan	Gyeongbuk
C20	Hadong	Gyeongnam
C21	Hwaseong	Gyeonggi
C22	Icheon	Gyeonggi
C23	Jeongeup	Jeonbuk
C24	Jinan	Jeonbuk
C25	Kumrung	Gyeongbuk
C26	Kumsan	Chungnam
C27	Miryang	Gyeongnam
C28	Muju	Jeonbuk
C29	Yangyang	Gangwon
C30	Yecheon	Gyeongbuk
C31	Yeoncheon-1	Gyeonggi
C32	Yeoncheon-2	Gyeonggi

Some 250 ng genomic DNA of each cultivar was restricted with 2.5 U *Eco*R, 2.5 U *Mse*, and 5 μl 5× reaction buffer [50 mM Tris-HCl (pH 7.5), 50 mM magnesium acetate, and 250 mM potassium acetate] in 25 μl total volume for 2.5 h at 37 °C, then tube incubated for 15 min at 70 °C. For the adapter ligation reaction, the following mixture was added to the restriction reaction mixture: *Eco*R/*Mse*I adapters, 0.4 mM ATP, 10 mM Tris-HCl (pH 7.5), 10 mM magnesium acetate, and 50 mM postassium acetate. This mixture was incubated for 3 h at 30 °C. After the ligated DNA was

Table 2. Sequence of primers and adapters used for AFLP analysis.

Primers / adapters	Sequences
*Eco*RI adapters	5'-CTCGTAGACTGCGTACC-3' 3'-CTGACGCATGGTTAA-5'
E (universal primer)	GACTGCGTACCAATTC
*Eco*RI + 1 mer	*E* + A
*Eco*RI + 2 mers†	*E* + AA
	E + AC
	E + AT
	E + TA
	E + TC
	E + TG
	E + TT
*Mse*I adapters	5'-GACGATGAGTCCTGAG-3' 3'-TACTCAGGACTCAT-5'
M (universal primer)	GATGAGTCCTGAGTAA
*Mse*I + 1 mer	*M* + C
*Mse*I + 3 mers	*M* + CAA
	M + CAC
	M + CAT
	M + CTA
	M + CTC
	M + CTT
	M + CTG

†Total of 12 primer combinations were applied for selective amplification

diluted to 1/10 in TE buffer, the DNA was used as template for pre-amplification. The pre-amplification was performed in a 51 μl volume containing 5 μl diluted adapter-ligated DNA, 40 μl of pre-amplification primer mixture (AFLP Small Genomic primer kit, Invitrogene™ Life Techonolgy), 10× buffer [100 mM Tris-HCl, 400 mM KCl, and 15 mM $MgCl_2$, pH 9.0], and 1 U *Taq* polymerase (Bioneer) by using a PTC-100TM Programmable Thermal Controller (MJ research, Inc.) following the 30 cycles profile: a 30 sec DNA denaturation step at 94 °C, a 30 sec annealing step at 56 °C, and a 60 sec extension step at 72 °C. Pre-amplified DNA was diluted to 1/50 and prepared DNA was used as a template for selective amplification. Twelve *Eco*RI / *Mse*I primer combinations were used for selective amplification (AFLP small genome primer kit, Invitrogene™ Life Technology). The oligonucleotide sequences used for AFLP analysis and primers are listed in Table 2. PCR was carried out in a 25 μl volume containing 2 μl diluted pre-amplified DNA, 0.5 μl *Eco*RI primer, 4.5 μl *Mse*I primer, 2.5 mM dNTP, 10× buffer, and 2 U of *Taq* polymerase. The PCR program for selective amplification was followed 10 cycles

of 60 sec at 94 °C, 60 sec at 65 °C, and 90 sec at 72 °C, annealing temperature was reduced each cycles by 1 °C step by step, and then followed 33 cycles of 30 sec at 94 °C, 30 sec at 56 °C, and 60 sec at 72 °C. PCR products were analyzed on 1.5% agarose gels.

Selective amplified products were fractionated on 6% denaturing polyacrylamide gels. The gel was pre-runned in 1× TBE buffer at 1500 V. Products were electrophoresed at 1600 V for 3.5 h. Silver staining of PCR products was performed as described by Bassam *et al* (1991).

Data analysis

Polymorphism was scored on a presence or absence of bands in each line and data were analyzed for clustering using the NTSYSpc version 2.0 (Exeter Software). The results were converted into a similarity matrix utilizing the SIMQUAL (similarity for qualitative data) method. A similarity coefficient was used for cluster analysis following UPGMA (unweighted pair grouping method of averages) method, which in one of the several SHAN (sequential, hierarchical, agglomerative, and nested) clustering methods that are available. The resulting clusters were represented in the form of a dendrogram.

RESULTS AND DISCUSSION

AFLP markers can be generated for any organism using digested genomic DNA and no prior knowledge about the genomic make up of the organism is needed.

AFLP analysis of 32 germplasms of adlay with 12 AFLP primer combinations detected a total of 882 amplified fragments, of which 223 (25.3%) were polymorphic at species level (Table 3). Eleven primer combinations amplified polymorphic fragments. The size of the AFLP fragments was determined by comparing AFLP patterns with sequential ladders of control template DNA (1 kb DNA ladder). The sizes of polymorphic fragments ranged from about 100 to 1,000 bp. Polymorphic fragments were distributed across the entire size range with majority being 400 – 800 bp (Fig. 1). The remaining polymorphic fragments were shared equally among the remaining size ranges of 800 – 1,000 and < 400 bp. However the bands larger than 1,000 bp were ignored because of difficulty for identifying each band.

The number of fragments detected by individual primer combinations ranged from 6 (E + AT / M + CAT) to 116 (E + AA / M + CAC). The number of polymorphic fragments for each primer combination varied from 4 (E + AT / M + CAT) to 51 (E + AC / M + CAC) with an average of 20.3 polymorphic fragments per primer combination. The percentage of polymorphic fragments ranged from 9.5% (E + TA / M + CTC) to 66.7% (E + AT / M + CAT) (Table 3).

AFLP analysis has been used to detect genetic variations of cultivated crops (Mackill *et al*, 1996) and closely related species that and had been very difficult to resolve with morphological features (Russell *et al*, 1997). Utilization of AFLP markers for detecting genetic diversity and relationships among cultivars and their wild relatives is now well established and many examples are now available (Maughan *et al*, 1996; Sharma *et al*, 1996; Mace *et al*, 1999).

The resultant dendrogram constructed from cluster analysis through UPGMA was shown in Fig. 2. Levels of genetic similarity indices ranged from 0.72 to 0.94 in 32 germplasms of adaly. In all cases, the clustering was not tight in the dendrogram. There was generally no clear grouping of the adlay into different geographic regions. Genetic exchanges between locally collected adlays caused by long distance seed dispersal, associated with cross-pollinate and human activity, as well as short distance pollen and seed dispersal may be responsible for the higher level of genetic variation between landraces. The present AFLP analyses imply that although Korean adlay displayed a higher degree of AFLP variation within germplasms and was shown inde-

Table 3. Number of polymorphic AFLP bands observed using 12 AFLP primer combinations.

Primer combinations	Total bands	Polymorphic bands (%)†	Primer combinations	Total bands	Polymorphic bands (%)
E + AA / *M* + CAA	50	5 (10.0)	*E* + TA / *M* + CTA	71	17(24.0)
E + AA / *M* + CAC	116	23 (19.8)	*E* + TA / *M* + CTC	63	6 (9.6)
E + AC / *M* + CAC	98	51 (52.0)	*E* + TC / *M* + CTT	78	26 (33.3)
E + AC / *M* + CAT	97	21 (21.7)	*E* + TG / *M* + CAC	92	23 (25.0)
E + AC / *M* + CTA	89	19 (21.3)	*E* + TG / *M* + CTT	83	29 (35.0)
E + AT / *M* + CAT	6	4 (66.7)	*E* + TT / *M* + CTG	38	0 (0.0)
Total	881	223 (25 3)			

†Polymorphic rates (%)

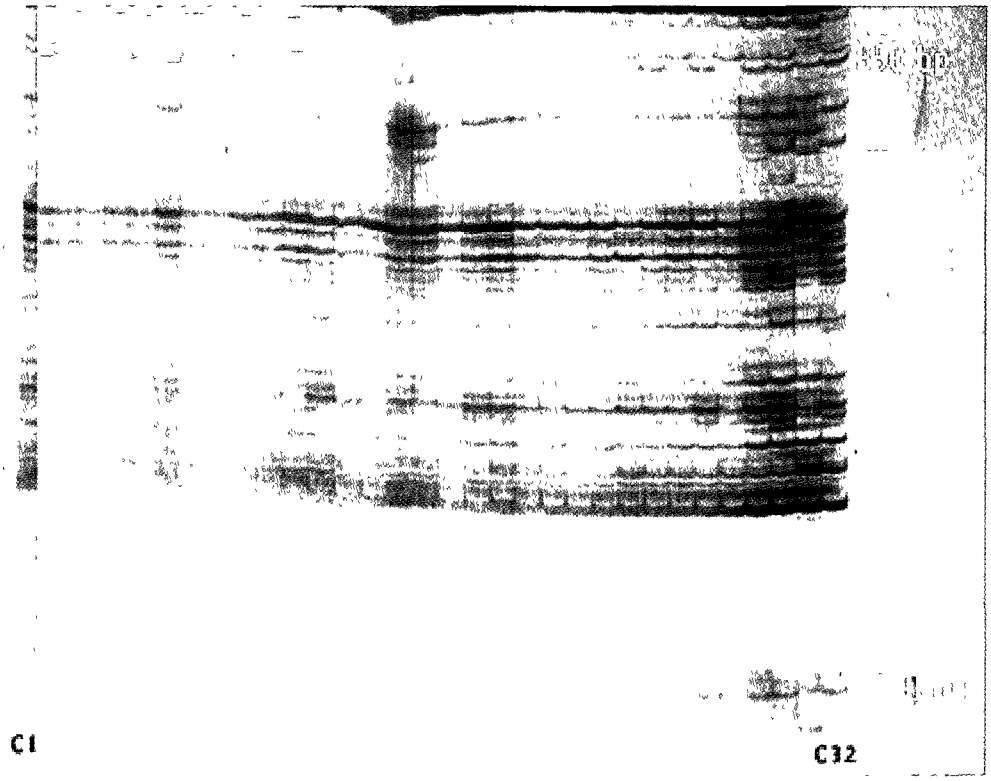

Fig. 1. An example of AFLP profile with primer pair E + AC / M + CAC in 32 germplasms of adaly.

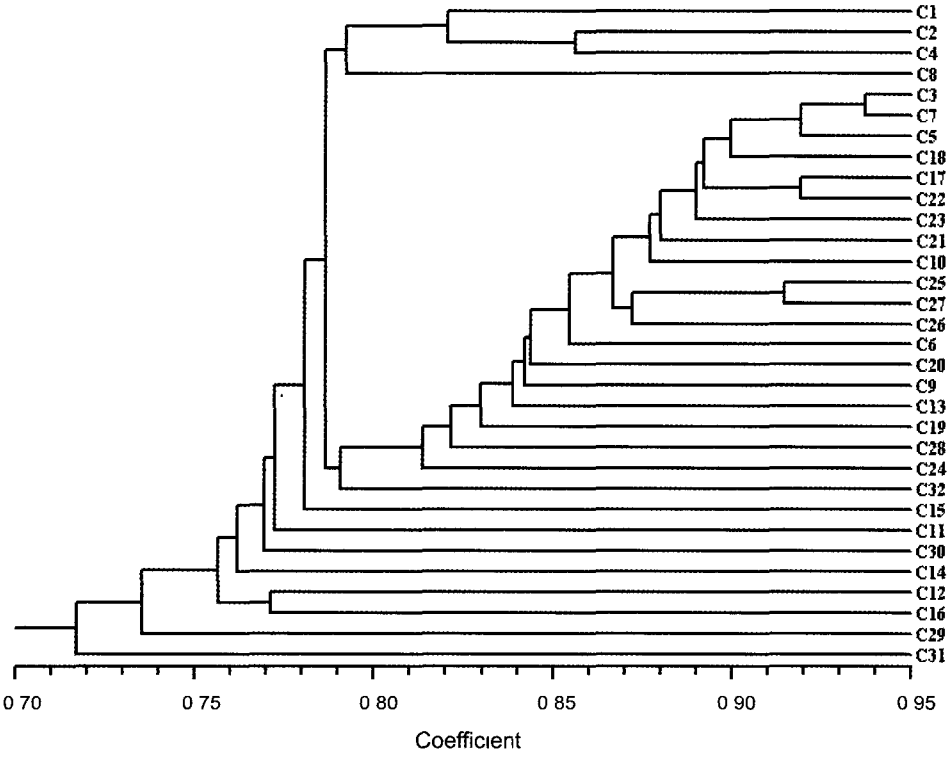

Fig. 2. Dendrogram of the 32 adlay germplasms by UPGMA cluster analysis of AFLP-based genetic similarity estimates using 223 AFLP polymorphic bands obtained by 12 primer pairs.

pendently without reflecting a clinal variation. Huh & Ohnishi (2002) reported that the allozyme diversity of natural populations of radish species from Japan and Korea, and revealed that natural populations maintained a higher allozyme variation than the other insect-pollinated outcrossing annual species. The differences between rye genotypes could be originated from the natural variability of allogamous rye, rather than by genetic drift during reproduction. It is also possible that the identified DNA fragments are linked to some important genome regions of unknown nature (Chwedorzewsk *et al*, 2002). This study demonstrated that AFLP method can be used to examine genetic polymorphism and relationships in different local germplasms of adlay.

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