*Journal of Plant Biology*, March 2001, 44(1) : 61-64 **SHORT COMMUNICATION**

# Assessment of Random Amplified Polymorphic DNA (RAPD) for Determining the Origin of *Youngia koidzumiana* Kitamura (Compositae)

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**We determined the parental species of *Youngia koidzumiana* (a natural interspecific hybrid) using PCR and arbitrary 10-mer primers to generate random amplified polymorphic DNA (RAPD) markers. These markers, generated by three primers, were sufficient to distinguish *Youngia sonchifolia*, *Youngia denticulata*, *Youngia chelidoniifolia*, and *Y. koidzumiana*. The electrophoresis profiles of the amplified products from each of the four species were then compared. Three primers produced a total of 42 scorable markers; nine were specific markers for *Y. denticulata* and *Y. chelidoniifolia*. The length of the amplified DNA fragments ranged from 370 to 2500 b p. The three primers revealed polymorphic bands, which were indicators of the parental species of *Y. koidzumiana*. These bands showed a combination of specific profiles for *Y. denticulata* and *Y. chelidoniifolia*. Our results also were comparable to the data obtained for flowering times, floret numbers, and chromosome numbers of the four species. Therefore, we suggest that *Y. koidzumiana* is a hybrid between *Y. denticulata* and *Y. chelidoniifolia*, and that RAPD markers are well suited for assessing the origins of plant species.**

*Keywords*: genetic markers, interspecific hybrid, random amplified polymorphic DNA (RAPD), *Youngia koidzumiana*

*Youngia koidzumiana* Kitamura belongs to the tribe Lactuceae under the family Compositae (Pak, 1991). Kitamura (1942) recorded *Y. koidzumiana* as a new species, using specimens of Koidzumi that were collected from Mt. Chiri, Sanchung-gun, Kyungnam, Korea. Because Kitamura (1942) had reported that *Y. koidzumiana* was endemic to Korea, Lee (1979) treated this species as a presumed natural hybrid between *Youngia sonchifolia* Maxim. and *Youngia chelidoniifolia* (Makino) Kitamura because of its morphological characters. However, Pak (1991) suggested that the hybrid origin of *Y. koidzumiana* was ambiguous, based on karyomorphological data of chromosome number, prochromosome type, genome size, centromere position, and secondary constriction from its somatic cells.

Recently, we found *Y. denticulata* (Houtt) Kitamura, *Y. chelidoniifolia*, and *Y. koidzumiana* on separate sites at Mt. Chiri, but these species also existed in the same location at Mt. Duckyu. Therefore, we investigated several characters of those three species. According to Chung (1957) and Lee (1996) flowering time for *Y. sonchifolia* is from May to September, but we failed to observe it from August to September. *Y. sonchifolia* blooms in spring and summer (May to July), whereas *Y. denticulata*, *Y. chelidoniifolia*, and *Y. koidzumiana* blooms in the autumn (September and October). Therefore, the probability that *Y. sonchifolia* was the parent species was very low, based on flowering-time data.

Putative hybrid plants are widely identified by one or more of the following methods: intermediate morphology (Fish et al., 1988), restriction fragment length polymorphisms (RFLP; Pental et al., 1988; Catalan et al., 1995), isoenzymes (Heun et al., 1994), or species-specific probes (Pehu et al., 1990). Detection of variability has been fine-tuned with the advent of new molecular techniques. Williams et al. (1990) and Welsh and McClelland (1990) have reported that DNA polymorphisms, arbitrarily amplified by 10-bp primers with specific sequences, are very useful as genetic markers. Random amplified polymorphic DNA (RAPD) analysis does not require any prior knowledge of the target genome, and only a small amount of DNA is used (Welsh and McClelland, 1990). RAPD analysis for useful genetic markers can help determine the relationship, variation, and differentiation within and between species and populations (Adams and Demeke, 1993; Russell et al., 1993; Lynch and Milligan, 1994; Rossetto et al., 1995; Cho et al., 1996; Tae and Ko, 1997; Han et al., 1998; Kim et al., 1998; Heibel et al., 1999; Tae et al., 1999). RAPDs

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also have been used for molecular characterization of inter- and intraspecific hybrids of potato (Baird et al., 1992), determining the origin of interspecific lilac hybrids (Marsolais et al., 1993), identifying the somatic hybrids between *Solanum tuberosum* and *Solanum brevidens* (Xu et al., 1993), assessing the origin of cultivars and hybrids of many plant species (Marsolais et al., 1993), providing evidence for the hybrid origin of interspecific relationships in *Asphodelus* (Lifante and Aguinagalde, 1996), and discovering the hybrid origin of *Nuphar* (Padgett et al., 1998). Furthermore, Takemori et al. (1994) has reported that RAPD analysis is better than RFLP analysis for confirming hybridity. Catalan et al. (1995) also have recently shown that RAPD data were suitable for resolving evolutionary pathways.

Here we demonstrate the benefits of using RAPDs in *Youngia* species identification via genome-specific markers pare. These markers can be applied as indicators of the ntal species for *Y. koidzumiana*. We also have compared these results with data from the morphological and cytological studies.

## MATERIALS AND METHODS

### Plant Material and DNA Extraction

The four species of *Youngia* were collected from two sites in Korea from June to September of 1999 (Table 1). Five individuals per species were sampled from each site for DNA extractions, and voucher specimens were deposited in the herbarium of the Hannam University (Table 1). Leaf tissues were stored at –70°C. Total DNA was extracted from this fresh tissue, using the CTAB procedure of Doyle and Doyle (1987) with the addition of phenol extraction. The quality and concentration of the DNA were assessed by agarose gel electrophoresis.

### DNA Amplification

Three arbitrary 10-mer primers (A07, B05, B19; Operon Technologies) were used for PCR amplification. Their sequences are 5'-GAAACGGGTG-3', 5'-TGCGCCCTTC-3', and 5'-ACCCCCGAAG-3', respectively. The reaction components were AccuPower PCR Premix (Bioneer, Cat. No. K-2014), 1 μL (10 pmol) oligonucleotide primer (Operon Tech. Inc. A, B series), and 2 μL (50 pg) of DNA in sterile distilled water. The final volume for each amplification reaction was 20 μL. A DNA thermalcycler (Perkin Elmer Cetus) was programmed for an initial denaturation step of 92°C for 1 min, then 40 cycles of 92°C for 1 min, 72°C for 1 min, and 35°C for 1 min. The amplifications finished with an incubation at 72°C for 10 min, followed by a 4-°C soak program until recovery. PCR products were separated by electrophoresis of 1.0% agarose gels for 1h at 50V. The gels were then photographed under UV light with Polaroid film 667. A 1-kb DNA ladder (MBI Co. Ltd.) was used as a molecular standard. Before this study, we had tested five individuals per species, and had obtained the same band patterns within each species.

## RESULTS AND DISCUSSION

We identified RAPD markers that could be used to confirm the genetic constitution of an inter-specific natural hybrid. The profiles of the amplified products from *Y. sonchifolia* , *Y. denticulata* , *Y. chelidoniifolia* , and *Y. koidzumiana* were compared here. Among the thirty primers, three (A07, B05, and B19) revealed polymorphic bands as indicators of the parental species for *Y. koidzumiana* (Fig. 1). These primers produced a total of 42 scorable bands; nine were specific markers for *Y. denticulata* and *Y. chelidoniifolia*. The length of the amplified DNA fragments ranged from 370 to

**Table 1.** Materials used in this study. Voucher specimens have been deposited in the herbarium of the Hannam University (HNU).

| Species | Symbol | Locality | Date | Voucher Specimens |
|---|---|---|---|---|
| *Y. sonchifolia* Maximowicz | S | Chungnam Mt. Kyeryoung | 20 June 1999 | HNU (26684) |
| *Y. denticulata* (Houttuyn) Kitamura | D | Chungnam Mt. Kyeryoung | 15 Sept. 1999 | HNU (26685) |
| *Y. chelidoniifolia* (Makino) Kitamura | C | Chonbuk Mt. Duckyu | 9 Sept. 1999 | HNU (26686) |
| *Y. koidzumiana* Kitamura | K | Chonbuk Mt. Duckyu | 9 Sept. 1999 | HNU (26687) |

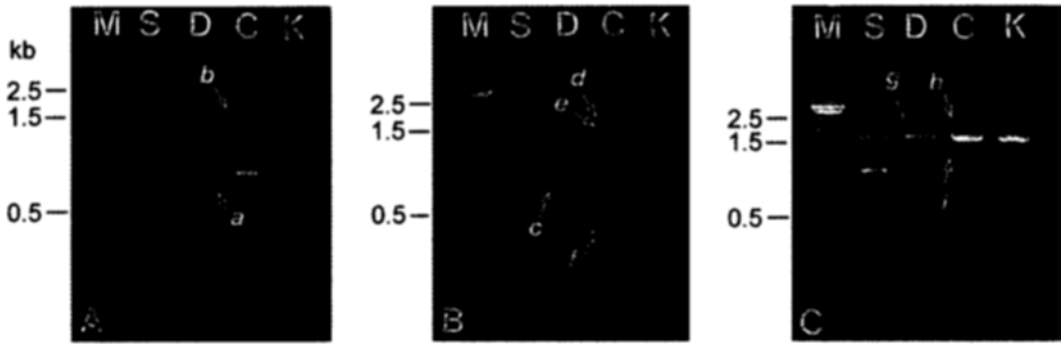


**Figure 1.** RAPD profiles generated by primer OPA-07 (A), OPB-05 (B), and OPB-19 (C). The symbols designating the lanes are explained in Table 1. Arrows a, c, and g, band specific for *Y. denticulata* (D); arrows b, d, e, f, h, and i, band specific for *Y. chelidoniifolia* (C), *Y. sonchifolia* (S), and *Y. koidzumiana* (K). On the left side, the size of the standards is indicated. M, DNA size markers (1 kb).

**Table 2.** Comparison of some characters among *Y. sonchifolia, Y. denticulata, Y. chelidoniifolia*, and *Y. koidzumiana*.

| Characters/Species | *Y. sonchifolia* | *Y. denticulata* | *Y. chelidoniifolia* | *Y. koidzumiana* |
|---|---|---|---|---|
| Floret number | 15-17 | 10-15 | 5-6 | 6-8 |
| Leaf | divided | entire | divided | divided |
| Flowering period | May-July | Sept.-Oct. | Sept.-Oct. | Sept.-Oct. |

2500 bp (Fig. 1). *Y. denticulata* could be separated by three specific markers to *Y. chelidoniifolia*, while *Y. chelidoniifolia* (Fig. 1C) produced six markers to *Y. denticulata*. The specific RAPD markers appeared on the *Y. koidzumiana* lanes of each primer (Fig. 1), but *Y. sonchifolia* had no specific markers. Here, RAPD markers appeared to provide a good basis for confirming the parental species of *Y. koidzumiana*. Therefore, we considered this species to be a natural hybrid between *Y. denticulata* and *Y. chelidoniifolia*.

Flowering times of the four *Youngia* species also were compared (Table 2). We observed that *Y. sonchifolia* bloomed from May to July, whereas *Y. denticulata*, *Y. chelidoniifolia*, and *Y. koidzumiana* flowered between September and November (Table 2). Because on this, we again determined that *Y. koidzumiana* was a natural hybrid between *Y. denticulata* and *Y. chelidoniifolia* because the probability of being hybridized from *Y. sonchifolia*, based on flowering time, was very low.

According to Makino (1989), *Crepidiastrum platyphyllum* x *Paraixeris denticulata* is a natural hybrid generated from *C. platyphyllum* and *P. denticulata* in Japan, with the three species having the same somatic chromosome numbers (all 2n = 10). Likewise, their floret numbers are 5, 10, and 8, respectively, for *C. platyphyllum*, *P. denticulata*, and *C. platyphyllum* x *P. denticulata*. In other words, the number of florets for a natural hybrid is the average between parental species. For our four *Youngia* species, Kitamura (1955) and Pak (1991) have used the somatic chromosome numbers of *Y. sonchifolia*, *Y. denticulata*, *Y. chelidoniifolia*, and *Y. koidzumiana* to classify them as diploids of x = 5, i.e., 2n = 10. Using dried specimens and living materials in the current study, we determined that the floret numbers were 15 to 17 for *Y. sonchifolia*, 10 to 12 for *Y. denticulata*, 5 for *Y. chelidoniifolia*, and 6 to 8 for *Y. koidzumiana*. Therefore, we again considered the parental species of *Y. koidzumiana* to be *Y. denticulata* and *Y. chelidoniifolia*, based on the average of the parents' floret numbers.

In summary, the results of our RAPD assessment agreed with our further investigation of the morphological and cytological characters of the four *Youngia* species (i.e., flowering times and numbers of florets). Therefore, RAPD analysis can provide an efficient tool for determining the parents of natural hybrids.

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