

Crossability of the *Aconitum jaluense* Species Complex (Ranunculaceae) in Korea

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In the present study, we have examined the crossability of four species in the *A. jaluense* complex to provide additional information on their delimitation and levels of differentiation. Crosses between diploid *A. chiisanense* and the tetraploid species including *A. jaluense* s. str., *A. triphyllum*, and *A. pseudoproliferum* yielded no fruit- or seed-set, indicating that the former species is reproductively completely isolated from the latter three species. In interspecific crosses between the tetraploid species, combinations involving *A. jaluense* s. str. resulted in much reduced fruit- and seed-set, indicating that *A. jaluense* s. str. is more or less reproductively isolated from the other tetraploid species. However, crossing data revealed the absence of reproductive isolation between *A. triphyllum* and *A. pseudoproliferum*. These results strongly support the relationships that have been previously suggested for the *A. jaluense* complex on the basis of morphology, chromosome number, and flavonoid chemistry.

The genus *Aconitum* L. is morphologically distinct and characterized within the Ranunculaceae by its zygomorphic flowers and spurred, long-stipitate, nectariferous petals (Tamura, 1966b). The genus includes ca. 300 species and comprises three subgenera; *Aconitum* with biennial tubers, *Lycotconum* Tourn. with perennial rhizomes, and *Gymnaconitum* (Stapf) Rapaics with annual roots (Wang, 1979; Kadota, 1987). The species of *Aconitum* are found in Asia, North America, and Europe, with the center of diversity in eastern Asia (Hardin, 1964; Tamura, 1966a).

The *Aconitum jaluense* Komarov complex belongs to the subgn. *Aconitum*, and in Korea it includes seven morphologically closely related species; *A. jaluense* s. str. (Komarov, 1901), *A. uchiyamae* Nakai (1909), *A. seoulense* Nakai (1911), *A. triphyllum* Nakai (1914), *A. chiisanense* Nakai (1935a), *A. proliferum* Nakai (1935b), and *A. pseudoproliferum* Nakai (1937), all of which were described from the Korean peninsula. These species are, however, highly variable in morphology, resulting in taxonomic confusion in delimiting species boundaries and determining relationships (Nakai, 1950, 1953; Chung, 1957; Park, 1974; Lee, 1980; Kadota, 1987). In particular, Kadota (1987), in his revision of subgn. *Aconitum* of east Asia, recognized two subspecies within *A. jaluense*, and placed *A. uchiyamae*, *A. seoulense*, *A. triphyllum*, *A. chiisanense*, *A. proliferum*, and *A. pseudoproliferum* in synonymy under *A. jaluense* subsp. *jaluense* on the basis of the notion that all

these taxa are well within the range of variability of *A. jaluense* subsp. *jaluense*. However, Park and Oh (1997) pointed out that *A. chiisanense* is a distinct species that can be distinguished from *A. jaluense* subsp. *jaluense* sensu Kadota by a diploid chromosome number of $2n=16$ and a number of morphological characters related to the size and shape of leaves and pedicel hairs; all other species in the complex are tetraploid with the chromosome number of $2n=32$ (Park and Oh, unpubl. data). On the basis of flavonoid chemistry, Lim et al. (1998) also strongly suggested the presence of two major chemical entities in the tetraploid species of the complex; one includes *A. jaluense* s. str. and the other includes the remaining species.

In the present study, we have examined the crossability of the species in the *A. jaluense* complex in Korea to provide additional information on their delimitation and levels of differentiation. The main objective of this paper is to report the degree of interfertility among the four species of the complex and to discuss its taxonomic significance.

Materials and Methods

The plants used for the crossing program were collected from 16 populations in Korea, and grown in the experimental garden at Seoul National University (Table 1). The plants represented four sexually reproducing species in the *A. jaluense* complex, including *A. chiisanense*, *A. jaluense* s. str., *A. triphyllum*, and *A. pseudoproliferum*. *Aconitum proliferum* was not included in the program, since it was described by Nakai (1935b) on the basis that the plants of this taxon reproduce only vegetatively

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Table 1. Collection data for plants used for the crossing program of the *Aconitum jaluense* complex*

<i>A. chiisanense</i> . KOREA. Kyungnam Prov. Mt. Chiri: Changteomok, Kang & Oh D1006, D1007, D4004, D4008, D4010, E3001, E3003; Chotdaebong, Kang & Oh GCH1, GCO1.
<i>A. jaluense</i> s. str. KOREA. Kangwon Prov.: Mt. Kwangduck, Kang & Oh B1003, C1003. Kyunggi Prov.: Mt. Kukmang, Kang & Oh A2002, A2003, A2005, C2001, C2003, C2004, C5001-C5003, C6002, C6003, C6008, C6013, C6014, C6015, C6013, GKM1. Kyunggi Prov.: Mt. Myungji, Kang & Oh A1105.
<i>A. triphyllum</i> . KOREA. Chungnam Prov.: Cheollipo, Kang & Oh B7003, B7004, B7007, B7009, B7013. Kyungbuk Prov.: Mt. Chuheul, Kang & Oh A1003, A1101, A1102, A1302, A1303; Mt. Hwanghak, Kang & Oh B4001, B4003, B4005-B4010. Kyunggi Prov.: Mt. Chunma, Kang & Oh A5001-A5004, A6002-A6005, B3010-B3012; Mt. Unak, Kang & Oh B5001-B5003, B5005; Kwangreung, Kang & Oh B6001, B6005-B6008, GKW1, KG3; Mt. Yongmun, Kang & Oh B8001-B8004, B8006, B8007, GYM2.
<i>A. pseudoproliferum</i> . KOREA. Chubuk Prov.: Byunsan peninsula, Kang & Oh C3001, C3008, C3010, C4002, C4004, C4006. Kyungbuk Prov.: Mt. Chuheul, Kang & Oh A1102, A1103, A1203, A1205. Kyunggi Prov.: Mt. Chunma, Kang & Oh GCM1-GCM3; Kwangreung, Kang & Oh A7002-A7004, GKW2, KWA58, KWA59, KWA68, KWA69, KWA73, KWA75, KWA77, KWA79, KWA84, KWA85.

* All vouchers are at SNU.

by bulbils formed from the stem apices and axils of upper leaves. *Aconitum uchiyamae* was also excluded, because no individuals matching with Nakai's description (1909) were found; Kadota (1987) suggested that the type specimens of this taxon are probably interspecific hybrids. In the case of *A. seoulense*, no healthy living individuals were available when artificial crossings were made.

The crossing program of the *A. jaluense* complex employed 103 parental plants (Table 1) in 337 crosses, and reciprocal cross-pollinations were performed for all possible pair-wise combinations among four species. For each pair of species, 4-43 crosses were performed. In addition, 71 reciprocal crosses involving 231 pollinations were made between individuals of different populations within each species to estimate intraspecific compatibility. To test for self-compatibility, 7-26 self-pollinations were made on plants of each species. The total number of pollinations made for the crossing program was 1268.

Flowers were emasculated prior to anthesis using a pair of fine forceps sterilized in 70% ethanol and the inflorescences were bagged until flowering. Then the inflorescence was uncovered, fresh pollen was applied directly to the receptive stigma by brushing dehiscing anthers, and the bag replaced.

Follicles were harvested as they matured, usually after five to six weeks. For each follicle, mature seeds and unfertilized ovules (i.e., ovules failed to develop into seeds) were counted, and percent seed-set was determined by the proportion of treated ovules that actually developed into seeds (McDade and Lundberg, 1982). Since the reciprocal crosses in each species pair show no significant differences in fruit- and seed-set, the data from reciprocal crosses in each pair were pooled. Crossability index (CI) values were calculated for interspecific crosses using a method similar to that

of McDade and Lundberg (1982), in which percent seed-set is divided by mean percent seed-set from intraspecific crosses for each ovulate parent.

Results and Discussion

Self-pollinations and intraspecific crosses

Self-pollinations were successful for all species in the *A. jaluense* complex, yielding 100% fruit-set. The average percent seed-set for self-pollinations ranged from 73.2% (*A. jaluense* s. str.) to 88.4% (*A. chiisanense*) (Table 2), indicating that all these species are self-compatible. *Aconitum* species are, however, protandrous, and pollinated primarily by bumblebees; Brink (1980) strongly suggested that protandry and foraging behavior of bumblebees promote outcrossing of *Aconitum* plants in nature.

Intraspecific crosses also resulted in complete fruit-set (Table 2). The average percent seed-set for intraspecific crosses was from 74.4% (*A. jaluense* s. str.) to 82.6% (*A. pseudoproliferum*), and did not significantly differ from those for self-pollinations. Although Pellmyr (1995) reported that bagged flowers of *Aconitum* species yielded complete fruit-set, all bagged flowers without artificial pollinations failed to produce any fruits in this study.

Interspecific crosses

A total of 247 interspecific crosses comprising 977 pollinations was performed, and the results are given in Table 3 and Fig. 1. The crossing data presented here appear to be very useful for recognizing the levels of differentiation among the species within the *A. jaluense* complex in Korea. Crosses between diploid *A. chiisanense* and the tetraploid species in the *A. jaluense* complex yielded no fruit- or seed-set

Table 2. Self-pollinations and intraspecific crosses within each of four species of the *Aconitum jaluense* complex

Species	Self-pollination			Intraspecific cross		
	N*	Percent fruit-set	Percent seed-set	N*	Percent fruit-set	Percent seed-set
<i>A. chiisanense</i>	14	100	88.4	22	100	80.7
<i>A. jaluense</i> s. str.	13	100	73.2	57	100	74.4
<i>A. triphyllum</i>	26	100	85.5	84	100	80.1
<i>A. pseudoproliferum</i>	7	100	88.1	68	100	82.6

* Number of pollinations made.

Table 3. Interspecific crosses of the *Aconitum jaluense* complex*

Cross	N	Percent fruit-set	Percent seed-set	CI
JAL-TRI	411	42.9	18.7	0.24
JAL-PSE	147	34.0	18.5	0.24
JAL-CHI	106	0	0	0
TRI-PSE	179	100.0	76.3	0.94
TRI-CHI	101	0	0	0
CHI-PSE	33	0	0	0

*N, number of pollinations made. CI, crossability index. JAL, *A. jaluense* s. str. TRI, *A. triphyllum*. PSE, *A. pseudoproliferum*. CHI, *A. chiisanense*

(Table 3, Fig. 1), indicating that *A. chiisanense* is reproductively completely isolated from the other species in the complex. The crossing data, therefore, strongly support the recent contention (Park and Oh, 1997) that the merger of *A. chiisanense* with *A. jaluense* subsp. *jaluense* by Kadota (1987) is not warranted and the former taxon should be recognized as a distinct species. Morphologically, *A. chiisanense* is distinct from the other species in the complex by its smaller, deeply incised leaves with narrowly lanceolate laciniae and long caudate apices and by its short, swollen-based pedicel hairs (Park and Oh, 1997). *Aconitum chiisanense* is a rare endemic species of Korea, and only a few isolated populations are found in shady, moist habitats with rich soils along the ridges of Mt. Chiri and Mt. Sorak.

Crosses between the tetraploid species, however, yielded varying degrees of fruit- and seed-set (Table 3). The percent fruit-set ranged from 34.0% (JAL-PSE) to 100% (TRI-PSE), and the average percent seed-set was from 18.5% (JAL-PSE) to 76.3% (TRI-PSE). In these crosses, combinations involving *A. jaluense* s. str. with the other tetraploid species resulted in much lower fruit- and seed-set. The CI for these combinations appeared to be less than 0.24 (Table 3, Fig. 1), strongly indicating that *A. jaluense* s. str. is more or less reproductively isolated from the other two tetraploid species. This result parallels the morphological and flavonoid evidence (Lim et al., 1998) that the divergence between *A. jaluense* s. str. and the other tetraploid species in the complex is substantial. The flavonoid profile of *A. jaluense* s. str. is characterized by the presence of both flavonol- and flavone-glycosides, but those of the other tetraploid species in the complex are based entirely on the flavonols kaempferol and quercetin glycosides (Lim et al., 1998). In addition, individuals of *A. jaluense* s. str. are morphologically separable from those of the other tetraploid species in having densely pubescent pistils and more deeply incised leaves with relatively long caudate apices.

Aconitum pseudoproliferum is very similar to *A. triphyllum* in morphology; the former is indistinguishable from the latter in other morphological characters than aerial bulbils at the stem apices (Lim et al., 1998), formation of which shows a wide range of intraspecific variation in subgn. *Aconitum* (Brink, 1982; Kadota, 1987). On the basis of flavonoid chemistry, Lim et al.

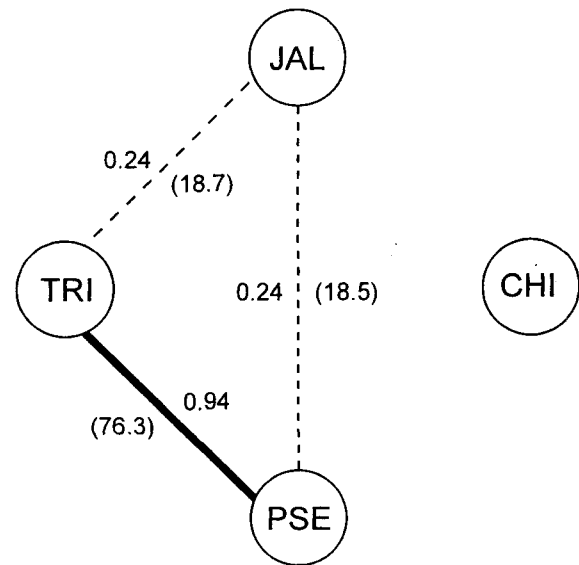


Fig. 1. Crossing polygon showing crossability indices between the species of the *Aconitum jaluense* complex. Numbers in parentheses represent the average percent seed set. CHI, *A. chiisanense*. JAL, *A. jaluense* s. str. TRI, *A. triphyllum*. PSE, *A. pseudoproliferum*.

(1998) strongly suggested that *A. triphyllum* and *A. pseudoproliferum* are conspecific. Crossing data provide further support; crosses between these two species were highly successful, and the average percent fruit-set and seed-set for these crosses were 100% and 76.3%, respectively. The CI calculated for these crosses appeared to be 0.94, indicating the absence of reproductive isolation between *A. triphyllum* and *A. pseudoproliferum* (Table 3, Fig. 1).

In conclusion, the crossing data from the present study strongly support the relationships that have been previously suggested for the *A. jaluense* complex on the basis of morphology, chromosome number, and flavonoid chemistry (Park and Oh, 1997; Lim et al., 1998). Crossing data reassert the distinctness of diploid *A. chiisanense* from the other tetraploid species in the complex. In addition, crossing data divide the tetraploid species of the complex into two groups; one includes *A. jaluense* s. str. and the other includes *A. triphyllum* and *A. pseudoproliferum* (Fig. 1). These groupings parallel major entities recognized previously on the basis of flavonoid chemistry (Lim et al., 1998).

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