



Taxonomic position and genetic differentiation of Korean *Astragalus mongholicus* Bunge

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한국산 황기의 분류학적 위치 및 유전적 분화

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ABSTRACT: To clarify the taxonomic position for *Astragalus nakaianus* and provide correct scientific name for *A. mongholicus* cultivar in South Korea, we examined external morphological characters and sequence variations from ITS and five cp non-coding DNA regions. Genetic structure was also analyzed for 61 individuals from three populations using nine microsatellite loci. We found no significant difference between the South Korean cultivar and *A. mongholicus* var. *dahuricus* when morphology and ITS sequences were considered. Morphologically, *A. nakaianus* specimens varied somewhat from *A. mongholicus* var. *mongholicus* and var. *dahuricus* in habit, plant height, and lengths of leaf axis and leaflet. Although sequence data from ITS and cp non-coding DNA regions could not distinguished *A. nakaianus* from *A. mongholicus*, microsatellite analysis revealed strong structuring between the cultivar and *A. nakaianus*. Therefore, we conclude that the South Korean *A. mongholicus* cultivar should be treated as *A. mongholicus* var. *dahuricus* and that *A. nakaianus* should be merged into *A. mongholicus* as a variety, i.e., *A. mongholicus* var. *nakaianus*.

Keywords: Taxonomic position, *Astragalus mongholicus* cultivar, *Astragalus nakaianus*, external morphological characters, genetic differentiation

적 요: 본 연구에서는 제주황기(*Astragalus nakaianus*)의 분류학적 위치를 명확히 하고 한국산 재배황기(*A. mongholicus* cultivar)의 올바른 학명을 부여하기 위하여 외부형태형질과 ITS 그리고 5구간의 cp non-coding DNA의 염기서열을 조사하였다. 또한 9개의 마이크로새털라이트 마커를 이용하여 3집단 61개체에 대한 유전적 구조가 분석되었다. 그 결과, 남한산 재배황기와 *A. mongholicus* var. *dahuricus* 사이에서는 형태와 ITS 염기서열상에서 유의한 차이점이 없었다. 제주황기는 *A. mongholicus* var. *mongholicus* 그리고 var. *dahuricus*와 줄기의 습성, 식물체의 길이, 엽축의 길이, 소엽의 길이 등에서 형태적으로 차이를 보였다. ITS와 cp non-coding 구간 염기서열에서 제주황기는 황기(*A. mongholicus*)와 차이점을 보이지 않았지만, 마이크로새털라이트 분석에서 제주황기와 남한산 재배황기간에 뚜렷하게 구분된 구조를 보였다. 이러한 결과들을 보았을 때, 한국산 재배황기는 *A. mongholicus* var. *dahuricus*로 처리해야 하며 제주황기는 *A. mongholicus*의 변종으로서 취급되어야 한다.

주요어: 분류학적 위치, 재배황기, 제주황기, 외부형태형질, 유전적분화

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Astragalus L. (Fabaceae), the largest angiosperm genus in the world, comprises about 3,000 species (Lock and Simpson, 1991) and it is considered a remarkable example of adaptive radiation (Kazempour Osaloo et al., 2003). Members are found primarily in cold to warm arid and semiarid mountainous regions in the Northern Hemisphere and in South America (Chaudhary et al., 2008). *A. mongholicus* Bunge is a perennial herb growing on mountainous grasslands in East Asia (Xu and Podlech, 2010). In addition, it is often cultivated as an important pharmacological resource in East Asia (Guo et al., 2010). Because plants show great morphological variation, this species has been separated into geographical varieties (Chen and Zhu, 1990; Xu and Podlech, 2010). Although it has been known as *A. membranaceus* Bunge in regional flora (Fu et al., 1993; Ohashi, 2001; Lee, 2003), this name is illegitimate because it is a later homonym of *A. membranaceus* Moench (1794). Instead, Podlech (1999) has treated this species as a variety, i.e., *A. mongholicus* Bunge var. *dahuricus* (DC.) Podlech. This species is also sometimes considered a member of the *A. penduliflorus* complex (Chen and Zhu, 1990; Zhu and Chen, 1991; Zhu, 1996). In revision work of *A. penduliflorus*, Zhu (2005) has reduced this species to an intra-specific taxon of *A. penduliflorus*–*A. penduliflorus* subsp. *mongholicus* var. *dahuricus* X.Y. Zhu. However, Xu and Podlech (2010) treat it as a synonym of *A. mongholicus* Bunge. However, none of those studies have examined South Korean specimens.

Plants of *A. mongholicus* var. *dahuricus* are naturally distributed in North Korea as *A. penduliflorus* subsp. *mongholicus* var. *dahuricus* (Zhu, 2005) and also cultivated (Im, 1998). *A. mongholicus* cultivar also exists in South Korea (Kim et al., 2000). *Astragalus* plants have been found growing on the subalpine grassland of Mt. Halla (Jeju Island), where they are known as wild endemics (Nakai, 1914; Lee and Tho, 1959; Tho, 1971; Lee, 1981; Kim et al., 2006; Lee, 2006). This plant was first recorded, as *A. membranaceus* var. *alpinus* Nakai nom. nud. in a floristic list for Jeju Island (Nakai, 1914). Lee and Tho (1959) reported these plants from Mt. Halla as a distinct species, *A. hallasanensis* Y.N. Lee, but without description. This declaration was based on its histological differences from *A. membranaceus*, including features for the stomata, pollen, and the structure of leaves, stems, and roots. Moreover, Tho (1971) suggested that these Mt. Halla plants should be separated from *A. membranaceus* based on karyotype analysis, even though they have the same number of chromosomes ($2n = 16$). Lee (1981) described this plant as *A. nakaianus*, separate from *A. membranaceus*, because of its tufted stems and very small leaflets. Recent cytogenetic study by Kim et al. (2006) has also re-confirmed *A. nakaianus* as distinct from *A. mongholicus*

and *A. membranaceus* by karyotype. However, it has now been merged into *A. membranaceus*, i.e., *A. membranaceus* var. *nakaianus* Y.N. Lee (Lee, 2006). Thus, its taxonomic status is controversial. This species is sometimes regarded as a geographical form of *A. mongholicus* because its diagnostic characteristics are considered to be just vegetative traits. Moreover, it is listed as “critically endangered” in the Rare Plants Data Book in Korea (Korea National Arboretum, 2008).

Nuclear ribosomal ITS and Chloroplast non-coding DNA regions have been used in the analysis of angiosperms and in examinations of lower-level (genus and species) phylogeny (Baldwin, 1992; Baldwin et al., 1995; Mort et al. 2007). In particular, phylogenetic analysis based on ITS has been very successful in distinguishing *A. mongholicus* var. *mongholicus* and var. *dahuricus* from other related taxa at the species level (Dong et al., 2003; Yip and Kwan, 2006). A recent DNA barcoding investigation (Guo et al., 2010) of this species has also verified findings previously reported that ITS is effective as a species identification marker. However, the infra-specific ITS sequence variations seen in this species could not identify the intra-specific taxa (Dong et al., 2003; Yip and Kwan, 2006; Guo et al., 2010). Simple Sequence Repeats (SSRs), or microsatellites (Litt and Luty, 1989), are sequences of DNA made up of randomly repeated motifs one to six bases long (Hancock, 1999). They are useful for determining relationships among closely related species (Weising et al., 2005; Bowles et al., 2010) especially when sequence data have revealed little variation (Yao et al., 2008).

Because the taxonomic position of *A. nakaianus* is unclear and the scientific name (*A. membranaceus*) that is primarily applied to a Korean *A. mongholicus* cultivar is illegitimate, the objectives of the present study were to determine the taxonomic position of *A. nakaianus* and propose the correct nomenclature for that cultivar based on morphological and genetic variations.

Materials and Methods

1. Morphological observations

Materials for the *Astragalus mongholicus* cultivar and *A. nakaianus* were collected in South Korea from 2002 to 2011 (Appendix). Voucher specimens are kept in the Herbarium of Inha University (IUI). To observe their morphological characters, we loaned specimens including *A. mongholicus* cultivar, *A. mongholicus* var. *dahuricus*, and *A. nakaianus* from the following herbaria: Ewha Womans University (EWH), Kangwon National University (KWNNU), Sungkyunkwan University (SKK), Seoul National University, College of

National Sciences (SNU), Seoul National University, College of Agriculture Life Sciences (SNUA), Warm-Temperate Forest Research Center (WTFRC), Tohoku University (TUS), The New York Botanical Garden (NY), and Chinese Academy of Sciences, Beijing (PE).

2. Nuclear ribosomal ITS and cp non-coding DNA sequences

Genomic DNA was extracted from fresh or silica gel-dried leaves, using a G-spin™ Iip Kit for plants (iNtRON, Seongnam, Korea). The nuclear ribosomal region, including ITS1, 5.8S, and ITS2, was amplified with primer pair ITS-5/ITS-4 (White et al., 1990). One sample each was examined from the South Korean *A. mongholicus* cultivar and *A. nakaianus* (Table 1). We also analyzed ITS sequences reported previously (Guo et al., 2010). Five cp non-coding DNA regions were amplified via PCR from four individuals each of the cultivar and *A. nakaianus* (Table 2). Universal primers were used to amplify *trnL-F*, which included the *trnL* intron and *trnL-trnF* intergenic spacer (Taberlet et al., 1991), as well as intergenic spacers for *psbA-trnH* (Sang et al., 1997), *psbC-trnS* (Nishizawa and Watano, 2000), *rpoB-trnC* (Shaw et al., 2005), and *petD-rpoA* (Nishizawa and Watano, 2000). PCR was conducted with a GeneAmp® PCR System 2700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Each reaction mixture contained 200 μM dNTPs (GeneCraft, Lüdinghausen, Germany), 1x PCR buffer with 1.5 mM MgCl₂, 1 U of Taq DNA polymerase (TaKaRa, Seoul, Korea) 10 ng of DNA, and an appropriate concentration of primers in a total volume of 50 μL. PCR conditions included an initial denaturation at 94°C for 2 min; followed by 35 cycles at 94°C for 30 s, 52°C for 45 s, and 72°C for 1 min; with a final extension at 72°C for 10 min. PCR products were visualized on 2% agarose gels, purified by PCRquick-spin™ (iNtRON), and sequenced with an ABI 3100 Genetic Analyzer and an ABI BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). All sequences were aligned manually using the program Clustal

X ver. 1.83 (Thompson et al., 1997).

3. Microsatellite analysis

Genomic DNA was extracted according to the method described above for 18 individuals of the South Korean *A. mongholicus* cultivar collected at Cheoram-dong of Gangwon-do. Nine microsatellite markers were used which had previously been developed for Korean *A. mongholicus*: Astna1, Astna2, Astna4, Astna5, Astna6, Astna7, Astna8, Astna9, and Astna10 (Choi and Choi, in press). PCR reaction and genotyping were conducted according to the method of Choi and Choi (in press). To infer structuring between that cultivar and *A. nakaianus*, we used a Bayesian medel-based clustering method (STRUCTURE 2.0; Pritchard et al., 2000) based on data for nine microsatellite loci from 61 individuals of three *Astragalus* populations. Those populations included the data from Cheoram-dong as well as previously genotyped data (Choi and Choi, in press) from Bukdong-ri (*A. mongholicus* cultivar, n = 13) and Mt. Halla (*A. nakaianus*, n = 30). We performed 10 runs for each value of *K* (number of clusters) from 1 to 8. Each run employed the Markov chain method with 20,000 iterations (burn-in) and 10,000 Monte Chain Monte Carlo (MCMC) repetitions. The most appropriate number of clusters (*K*) was estimated based on the Evanno method (Evanno et al., 2005) as implemented in STRUCTURE HARVESTER (Earl and von Holdt, 2012). According to the program STRUCTURE, the most likely *K* as that wherein $\ln \Pr(X | K)$ is maximized. Analysis of molecular variance (AMOVA) was conducted to partition the genetic variation between *A. mongholicus* cultivar and *A. nakaianus* via *F*_{st}. Data were tested for significance using the AMOVA option within GenAlix 6.4 (Peakall and Smouse, 2006).

Results and Discussion

1. External morphology

Plant height: Samples were 50 to 100 cm tall for the *Astragalus mongholicus* cultivar and 15 to 40 (70) cm tall for

Table 1. Sites of variability in the aligned (ITS1, 5.8S, and ITS2) sequences of *A. mongholicus*.

Variety	Locality and Voucher No.	Position		GenBank Accession No.
		4	7	
<i>A. mongholicus</i> var. <i>nakaianus</i>	Mt. Halla, Jeju-do, Korea, <i>S.Y. Kim & J.S. Lee 307281</i>	G	C	KC262198
<i>A. mongholicus</i> var. <i>dahuricus</i> *	Xunyi, Shaanxi, China	G	C	HM142275.1
<i>A. mongholicus</i> var. <i>dahuricus</i>	Mt. Mullae, Gangwon-do, Korea, <i>S.Y. Kim et al. 308021</i>	-	T	KC262199
<i>A. mongholicus</i> var. <i>mongholicus</i> *	Daqingshan, Inner Mongolia, China	G	T	HM142288.1

*data obtained from Guo et al., 2010.

A. nakaianus. Plant specimens described as *A. mongholicus* from China (25-60 cm; Xu and Podlech, 2010) and Russia (25-40 cm; Shishkin, 1946) shared a resemblance with *A. nakaianus*. Those described as *A. membranaceus* from China (50-100 cm; Fu et al., 1993) were similar to the South Korean cultivar examined here. Plants from Japan, identified as *A. membranaceus* (40-70 cm; Ohashi, 2001) were intermediate in their overall variation. Although there was overlap among specimens, plants of *A. nakaianus* were somewhat smaller than those of *A. mongholicus* from East Asia.

Habit: The growth form of the South Korean cultivar was singular or several, with an erect stem form that was also characteristic of *A. membranaceus* from China (Fu et al., 2001). By comparison, plants of *A. membranaceus* from Japan (Ohashi, 2001) showed tufted and erect forms. Samples of *A. nakaianus* were tufted but had an ascending or decumbent form. Although one might regard this as an ecotype, it was a trait that distinguished *A. nakaianus* from other *A. mongholicus* in East Asia.

Leaf: Axis of leaf: The leaf axes were generally shorter for *A. nakaianus* [2-5 (9) cm] than for the *A. mongholicus* cultivar (6-10 cm), such that the latter resembled *A. penduliflorus* subsp. *mongholicus* var. *dahuricus* (5-9 cm; Zhu, 2005). Hairs on leaflet: Zhu (2005) has suggested that leaflets from both *A. penduliflorus* subsp. *mongholicus* var. *mongholicus* and var. *dahuricus* are glabrous on their upper surfaces, a trait coinciding with that of *A. nakaianus*. However, leaflets from the South Korean cultivar had pubescence on both surfaces, a trait also noted with *A. membranaceus* from Japan (Ohashi, 2001). Number of leaflets: At the intra-specific level, the number of leaflets has been used as a diagnostic characteristic among members of the *A. mongholicus* complex (Zhu, 2005;

Zhao, 2006). Leaflet counts did not differ significantly between the South Korean *A. mongholicus* cultivar (6-10 pairs) and *A. nakaianus* (7-10 pairs). Such variations have also been reported for plants of *A. penduliflorus* subsp. *mongholicus* var. *dahuricus* [(5) 7-11 (13) pairs; Zhu, 2005]. Length of leaflet: Leaflets were 15 to 20 mm long for the cultivar and 4 to 8 (9) mm long for *A. nakaianus*. Whereas the cultivar was similar in this trait to plants described as *A. membranaceus*, i.e., 15 mm long (Zhao, 2006), the leaflets from *A. nakaianus* resembled those from *A. mongholicus* in length, i.e., 10 mm (Zhao, 2006) and *A. penduliflorus* subsp. *mongholicus* var. *mongholicus*, 5 to 10 mm (Zhu, 2005).

Hairs of ovary and legume: Bunge (1868) has distinguished among species by the presence of hairs on the ovary, i.e., either pubescent (*A. membranaceus*) or glabrous (*A. mongholicus*). This separation in characteristics has been accepted by Fu et al. (1993) for *A. membranaceus* var. *membranaceus* and var. *mongholicus*, by Zhu (2005) for *A. penduliflorus* subsp. *mongholicus* var. *mongholicus* and var. *dahuricus*, and by Zhao (2006) for *A. membranaceus* and *A. mongholicus*. In the South Korean samples, those of the *A. mongholicus* cultivar and *A. nakaianus* had pubescent ovaries with very short, appressed black and white hairs.

2. Sequence variation

ITS: ITS sequences determined in this study were aligned and analyzed together with GenBank sequences from the *A. mongholicus* complex. The complete dataset included two taxa and 603 characters. Lengths were 227 to 228 bp for the ITS1 region, 164 bp for 5.8S, and 221 bp for the ITS2 region. Of the 603 initial alignment positions, 601 sites were identical while the remaining two were variable. The ITS sequence from *A. nakaianus* was identical to that of *A. mongholicus* var.

Table 2. Alignment of variable sites for chloroplast DNA from Korean *Astragalus mongholicus*.

Variety	Locality and Voucher No.	cpDNA regions	
		<i>trnL-F</i>	<i>trnC-rpoB</i>
		361	800
<i>A. mongholicus</i> var. <i>nakaianus</i>	Mt. Halla, Jeju-do, <i>I.S. Cho 1106208</i>	•	•
	Mt. Halla, Jeju-do, <i>I.S. Choi 1108030</i>	•	•
	Mt. Halla, Jeju-do, <i>I.S. Choi 1108040</i>	•	•
	Mt. Halla, Jeju-do, <i>I.S. Choi 1108049</i>	•	•
<i>A. mongholicus</i> var. <i>dahuricus</i>	Mt. Mullae, Gangwon-do, <i>S.Y. Kim et al. 308021</i>	1*	•
	Hwaamyaksu, Gangwon-do, <i>S.Y. Kim s.n.</i>	•	2*
	Bukdong-ri, Gangwon-do, <i>I.S. Choi 1110013</i>	•	•
	Cheoram-dong, Gangwon-do, <i>I.S. Choi 1110022</i>	•	•

*1, Duplication = ATGAA; 2, Duplication = AAACCCCGAA

dahuricus from China. Although the sample from Mt. Mullae had an indel at nucleotide position 94, the relevant ITS sequences showed few differences among *A. mongholicus* and did not reflect morphological or geographical variations. Our findings concur with those of Guo et al. (2010) in terms of the effectiveness of ITS as a species identification marker, but not in terms of its efficiency at the intra-specific level. These results suggested that both the Korean *A. mongholicus* cultivar and *A. nakaianus* should be regarded as members of the *A. mongholicus* complex.

CpDNA: Five regions were successfully sequenced - *psbA-trnH*, *trnL-F*, *psbC-trnS*, *rpoB-trnC*, and *petD-rpoA*. Intra-specific cpDNA variations were detected in *trnL-F* and *rpoB-trnC*. Both are non-coding regions. Yang et al. (2011) have suggested that the *psbA-trnH* region varies between Chinese *A. mongholicus* and the South Korean *A. mongholicus* cultivar. However, we found no such variation between that cultivar and the *A. nakaianus* samples. The *trnL-F* region has duplication in ATGAA at nucleotide position 361 in the inland population (Mt. Mullae). Similarly, the *rpoB-trnC* region has duplication in AAACCCCGAA at nucleotide position 800 in the inland population (Hwaamyaksu). However, in our data, that variation did not occur between the South Korean *A. mongholicus* cultivar and *A. nakaianus* but, instead, among samples of that cultivar (Table 2).

Microsatellite analysis: Cluster assignments were made for 30 individuals from *A. nakaianus* and 31 individuals from the South Korean *A. mongholicus* cultivar. According to the program STRUCTURE, the most likely K is the one where it is maximized in $\ln \Pr(X|K)$. Here, the likelihood for partitioning of the data increased sharply from $K=1$ to $K=2$ before increasing or decreasing from $K=2$ to $K=8$ (Fig. 1). The uppermost level of K indicated $K=2$ as the most probable

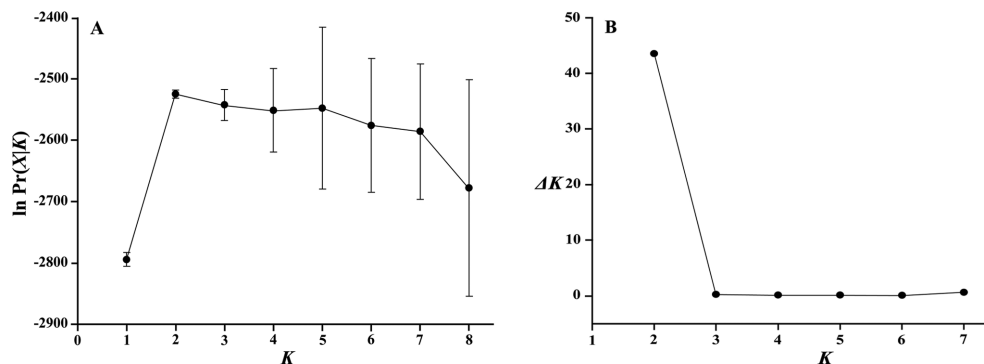


Fig. 1. Summary of runs for K values ranging from 1 to 8 by STRUCTURE. (A) Mean log likelihood for each K plotted against K , (B) ΔK plotted against K .

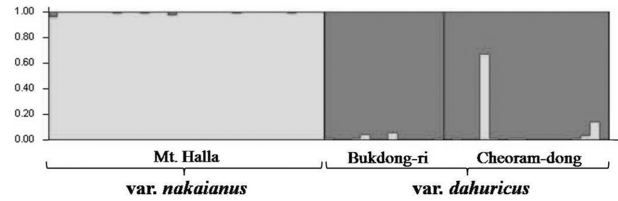


Fig. 2. Individual assignment of 61 *Astragalus mongholicus* individuals to population clusters, inferred by using STRUCTURE 2.2.3 based on nine SSR markers.

number of clusters to explain the observed genotypes (Fig. 1). Therefore, we used the result of $K=2$ in our analysis. In STRUCTURE analysis, all individuals within the *A. nakaianus* and *A. mongholicus* cultivar strongly assigned to different cluster each other (Fig. 2). The percentage of variance attributed to differentiation among populations was 5% while that between taxa was 9%. The value for F_{st} between taxa was 0.125 ($P < 0.001$).

4. Taxonomic treatment

Astragalus mongholicus Bunge, Mém. Acad. Imp. Sci. Saint-Petersbourg 11: 25. 1868 [and 15: 30. 1869]

var. dahuricus (DC.) Podlech, Sendtnera 6: 172. 1999-Basionym: *Phaca alpina* L. var. *dahurica* DC., Prodr. 2: 273. 1825. **Type:** Dahuria, ?, Fischer *s.n.* (Isotype, P, seen as a photo!).

Astragalus membranaceus Bunge, Mém. Acad. Imp. Sci. Saint-Petersbourg 11:25. 1868 [and 2: 30. 1869], nom. illeg., non Moench, Methodus (Moench) 167. 1794.

Korean name: 황기 [Hwang-gi]

Distribution: China, Russia, Japan, and Korea.

Korea: Growing on high mountains of North Korea and cultivated in North and South Korea.

Taxonomic note: South Korean *A. mongholicus* cultivar has been known as *A. membranaceus*, nom. illeg., in Korean flora (Lee, 2003; Lee, 2006). However its legitimate and validly

published name was *A. mongholicus* var. *dahuricus*. Plants of this cultivar differ from those of *A. mongholicus* var. *mongholicus* based on the number of leaflets, lengths of leaf axes and leaflets, and the presence of hairs on the ovary and legume. Because the South Korean cultivar and *A. mongholicus* var. *dahuricus* do not differ significantly in their morphology and ITS sequences, we suggest that the cultivar should be treated as *A. mongholicus* var. *dahuricus*.

var. *nakaianus* (Y.N. Lee) I.S. Choi & B.H. Choi, com. nov.
A. nakaianus Y.N. Lee, Kor. J. Bot. 24: 28. 1981. *A. membranaceus* var. *nakaianus* Y.N. Lee, New Flora of Korea, 738. 2006, nom. illeg. **Type:** Jeju Island, Korea, Aug. 9, 1957, Lee, Y.N. *s.n.* (Lectotype, designated here, EWH!, EWUA200909201123, see Fig. 3); Jeju Island, Korea, Aug. 9, 1957, Lee, Y.N. *s.n.* (Isolectotype, designated here, EWH!, ENHA200508012079, see Fig. 3).

Korean name: 제주황기 Je-ju-hwang-gi

Perennial, Stems 15-40 (70) cm tall; tufted and ascending or decumbent, sulcate, angled, sparsely covered with appressed white hairs; **Leaves** (2) 3-6 (10) cm long, petiole short; stipules free or adnate, cauline, green, foliaceous, triangular-ovate, subacute, 3-14 mm long, sparsely on the outside with white hairs; leaflets oblong-obovate, oval or oblong-oval, round tipped or subobtuse, 7-10 pairs, 4-8 (9) mm long, thick, glabrous

above, beneath with white appressed hairs; **Inflorescences** racemes loose, oblong-ovoid to ovoid, 3-5 cm long (4-8 cm in fruit), 7-13 flowered; peduncles firm, stiffly upright, axillary or solitary, divergent, as long as or slightly longer than subtending leaves, 4-7 cm long, sparsely covered with short appressed white hairs (rarely with isolated black hairs at inflorescence base), sulcate; bracts lanceolate, 3-8 mm long, white-membranous, sparsely covered with white hairs on the outside; pedicels 2-4 mm long, beset with white hairs; **Calyx** 5-7 mm long, campanulate, strongly oblique, saccate at base, hispidulous with very short appressed black or white hairs, the tube 4 times as long as the linear-subulate teeth; **Corolla** yellow 13-15 (17) mm; standard 14-16 mm long, the limb obovate, retuse gradually attenuate and 3-4 times as long as the claw; wings 13-15 mm long, the claw 1.5 times as long as the oblong obtuse limb; keel slightly short or equaling to the wings, the claw 1.5 times as long as the acute limb; ovary hairy on stipe 1.5 times to twice its length; **Legumes** pendulous from a slender stipe ca. 1.5 times the length of calyx, suborbicular-oval in outline, short-mucronate, 28-33 mm long, ca. 10 mm broad, slightly compressed laterally, inflated, thinly membranous, sparsely pubescent with very short appressed black and white hair, unilocular. **Flowering** July (later)-August; **Fruit** August-September.

Distribution: Alpine meadow at Mt. Halla on Jeju Island, Korea.

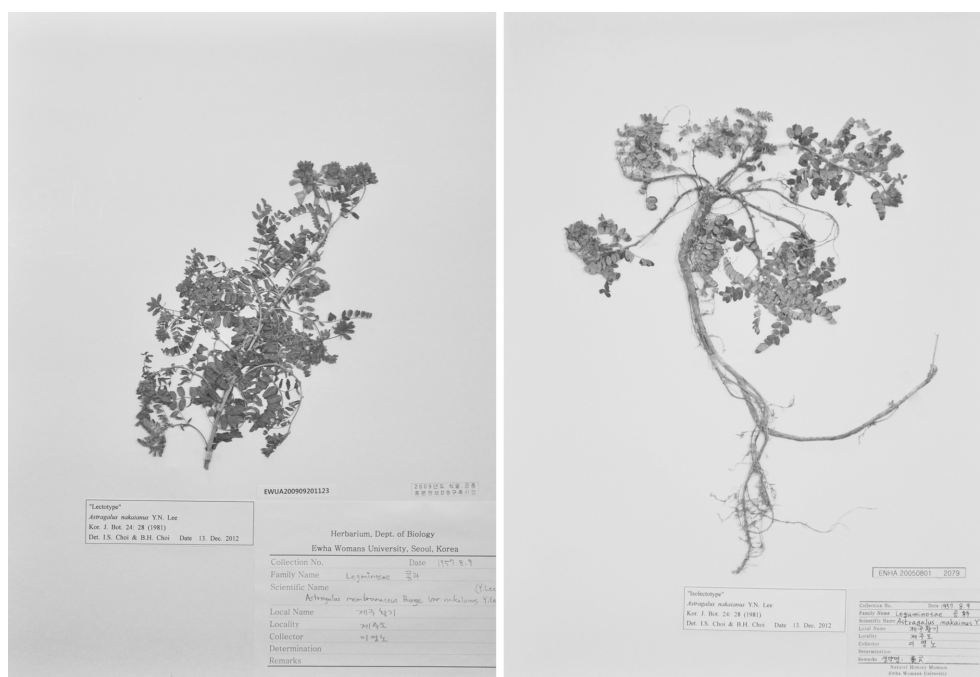


Fig. 3. Type specimens of *Astragalus mongholicus* Bunge var. *nakaianus* (Y.N. Lee) I.S. Choi & B.H. Choi. Left: Lectotype, Right: Isolectotype.

Typification: In the protologue, Lee (1981) indicated the type and the deposited herbarium (Ewha Womans University) without collection number. However the protologue includes the information of the locality, collector, and date of collection. So, we searched the specimens from herbarium of Ewha Womans University (EWH) and found two sheets of *A. nakaianus* (without collection number, EWUA2009201123 and ENHA200508012079) that matches the type information indicated in protologue. Thus, the two sheets are regarded as the original material (ICN Art. 9.3, McNeill et al., 2012). Since Lee (1981) did not designate a holotype, we considered these two specimens as syntypes and selected sheet EWUA2009201123 (with flowering stem) as lectotype and sheet ENHA200508012079 as isolectotype (ICN Art. 9.2, 9.5, 9.12, McNeill et al., 2012).

Taxonomic note: *Astragalus nakaianus* has been separated from *A. membranaceus* by its tufted stems and very small leaflets (Lee, 1981). In our morphological comparisons, the former was characterized by its tufted and ascending or decumbent stems and relatively small vegetative features, including plant height and lengths of axes and leaflets. However, we found no clear morphological discontinuities between it and *A. mongholicus*. Thus, these characters might represent a geographic variation, especially when one considers the wide distributional area of *A. mongholicus*. Likewise, *A. nakaianus* could not be distinguished from *A. mongholicus* when we examined the DNA sequences for ITS regions of the nuclear and five non-coding regions in the chloroplast. Differences have been reported with regard to histological characters (Lee and Tho, 1959) and karyotype analysis of chromosomes (Tho, 1971; Kim et al., 2006). Our structure analysis of nine microsatellites revealed that *A. nakaianus* is clearly divided from *A. mongholicus* var. *dahuricus*.

Based on these results, we argue that *A. nakaianus* should be merged into *A. mongholicus*. Although it should retain the variety rank as formerly treated (Lee, 2006), the species name must be changed from *A. membranaceus*, nom. illeg., to *A. mongholicus*. Furthermore, *A. mongholicus* var. *nakaianus* should be regarded as relict on high mountains because of interglacial warming.

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Appendix: Specimens examined for this study

Astragalus mongholicus Bunge var. *dahuricus* (DC.) Podlech

Korea cultivar, Gyeonggi-do: Gapyeong-gun, Mt. Hwaya, 16 Aug. 1996, ? 86477 (SNU); Gapyeong-gun, Mt. Hwaya, 16 Aug. 1996, *S.T. Kim* 278 (SNU); **Gangwon-do:** Jeongseon-gun, Buk-myeon, Gujeon-ri, 10 Aug. 1997, *K.H. Chung* s.n. (IUI); Jeongseon-gun, Dong-myeon, Hwaamyaksu, 28 Sep. 2002, *S.Y. Kim et al.* s.n. (IUI); Jeongseon-gun, Imgye-myeon, Bancheon-ri, 10 Aug. 1997, *K.H. Chung* s.n. (IUI); Jeongseon-gun, Imgye-myeon, Golji-ri, 2 Aug. 2002, *S.Y. Kim et al.* 308021 (IUI); Jeongseon-gun, Jeongseon-eup, Mt. Gariwang, 9 Aug. 1997, *K.H. Chung* s.n. (IUI); Jeongseon-gun, Jeongseon-eup, Yongtan-ri, 19 Jun. 2003, *J.S. Lee et al.* s.n. (IUI); Jeonseon-gun, Hwaam-myeon, Bukdong-ri, 2 Oct. 2011, *I.S. Choi* 1110001-1110013 (IUI); Pyeongchang-gun, Daegwallyeong, 18 Sept. 1965, *T.B. Lee & J.S. Bak* 27299, 27300 (SNUA); Pyeongchang-gun, Jinbu-myeon, Mt. Odae, 14 Aug. 1966, *T.B. Lee & M.Y. Cho* 27301 (SNUA); Pyeongchang-gun, Jinbu-myeon, Mt. Odae, 14 Aug. 1966, *T.B. Lee*, 27308 (SNUA); Taebaek-si, Cheoram-dong, 3 Oct. 2011, *I.S. Choi* 1110014-1110031 (IUI); Taebaek-si, Mt. Taebaek, 9 Aug. 1997, *K.H. Chung* s.n. (IUI); Gangneung-si, Mandeok-bong, 2 Sept. 1996, *W.C. Lee* 9204, 9205 (KWNNU); Gangneung-si, Mt. Jabyeong, 28 Aug. 1998, *W.C. Lee* 9206 (KWNNU). **Chungcheongbuk-do:** Boeun-gun, Mt. Songni, ? 1960, *T.B. Lee* 27302-27307 (SNUA); Bakbyeok, 24 May 1935, *B.S. Do* 6256, 6257 (SNU). **Chungcheongnam-do:** Cheonan-si, Dong-myeon, Sunam-ri, 19 Aug. 1996, *B.H. Choi* s.n. (IUI); Gyeryong-si, Mt. Gyeryong, 7 Sept. 1969, *T.B. Lee & B.G. Lee* 27295 (SNUA). **Gyeongsangbuk-do:** Ulleung-gun, Isl. Ulleung, Naribunji, 11 Aug. 1989, *Y.J. Chung* b-10215,

29446 (SKK); Ulleung-gun, Isl. Ulleungdo, Naridong, 1 Oct. 1981, *W.C. Lee* 9203 (KWNNU). **Jeollabuk-do:** Jinan-gun, 26 Jul. 1970, *T.B. Lee* 27292, 27293 (SNUA). **Jeollanam-do:** Sancheong-gun, Mt. Jirisan, Piagol, Banya-bong, 3 Jul. 1982, *T.B. Lee & J.S. Chang* 27296-27298 (SNUA). **Incheon:** Jung-gu, Isl. Yongyu, Mt. Oseong, 21 Aug. 1993, *J.S. Lee* 1008-1010 (IUI).

Japan: Nagano, Jul. 1927, *Nakai* s.n., 53955 (TUS); Yamanashi, 18 Aug. 1976, *Sato* s.n., 77815 (TUS); Yamanashi, 10 Aug. 1937, *Sugaya* s.n., 23686 (TUS); Yamanashi, 27 Jul. 1990, *Iketani* 2544, 145107 (TUS).

China: Shansi, Huo Hsien, 10 June 1929, *T. Tang* 1013 (NY); ?, Sichuan, 29 Jul. 1963, *Kang ding xian* 511 (PE).

Astragalus mongholicus Bunge var. *nakaianus* (Y.N. Lee) I.S. Choi & B.H. Choi

Korea, Jeju-do: Mt. Halla, 2 Aug. 1989, *J.H. Lee et al.* b-10213, b-10214, 29448, 29447 (SKK); Mt. Halla, 5 Aug. 1960, *W.C. Lee* b-10229, b-10231, 29464, 29463 (SKK); Mt. Halla, Baengnokdam, 4 Aug. 2004, *C.S. Kim & M.O. Moon* s.n. (WTFRC); Mt. Halla, Trail of Seobukbyeok, 30 Oct. 2008, *C.S. Kim et al.* 26510 (WTFRC); Mt. Halla, Witseoreum, 27 Jul. 2003, *J.S. Lee & S.Y. Kim* s.n. (IUI); Mt. Halla, Witseoreum, 5 Aug. 2011, *I.S. Choi* 1108010-1108049 (IUI); Mt. Halla, Yeongsil, 13 Oct. 2011, *I.S. Choi* 1110118-1110136 (IUI); Mt. Halla, Yeongsil, 9 Aug. 2008, *C.S. Kim* 26238 (WTFRC); Mt. Halla, Yeongsil-Witseoreum, 30 Aug. 2003, *S.Y. Kim* s.n. (IUI); Mt. Halla, Yeongsil-Witseoreum shelter, 18 Sept. 1990, *B.H. Choi & S.U. Kang* 5197, 5209, 5862, 5863, 5865, 5876, 5877, 5880 (IUI); ?, 9 Aug. 1958, *C.H. Gang* b-10230, 29465 (SKK).