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Infraspecific Variability of *Juniperus* L. (Cupressaceae) Based on Monoterpenoid Compositions

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The compositions of leaf monoterpenoids from 11 species in the Juniperus section (*Juniperus chinensis* var. chinensis, *J. virginiana*, *J. communis* var. montana, *J. rigida*, *J. chinensis* var. globosa, *J. chinensis* var. sargentii, *J. chinensis* 'Kaizuka Variegata', *J. squamata* 'Wilsonii', *J. x media* 'Shimpake', *J. x media* 'Plamosa Aurea', and *J. squamata* 'Aloderi') were comparatively analyzed by GC-MS. Of the 24 compounds identified, α-pinene, myrcene, limonene, terpinolene, and bornyl acetate were common to all, but particular combinations differed remarkably among taxa. The simplest composition (eight compounds) was found in *J. chinensis var.* chinensis; the most complex (19 compounds), in *J. x media* 'Shimpake'. Cluster analysis generated four distinctive clades within the Juniperus section. The minimum spanning network revealed that *J. squamata* 'Wilsonii' and *J. x media* 'Shimpake' were most similar in their chemical makeup.

Keywords: Cupressaceae, GC-MS, *Juniperus*, monoterpenoids, PAUP (Phylogenetic Analysis Using Parsimony)

The genus *Juniperus*, a member of the family Cupressaceae, consists of approximately 70 species (Adams et al., 2002), all of which grow in the Northern Hemisphere. This genus is divided into three sections: Caryocedrus, Juniperus, and Sabina (Adams, 1999, 2000). Section Juniperus is represented in eastern Asia (Japan, Korea, and Taiwan), with four species and four varieties being native to Korea (Lee, 1986). However, new forms and cultivars have recently been introduced from other countries. Most Juniperus species are aromatic and yield volatile oils with important commercial value. Moreover, plants in that section are evergreen and tolerant of cold temperature, diseases, and environmental pollution (Kim, 1988), making them easily adaptable to Korean soils and climates.

The leaf components of Juniperus plants have been investigated by Adams (1998, 2000) and Cavaleiro et al. (2001). In contrast to those within section Sabina (Adams, 1998), essential oils from section Juniperus are generally much simpler, being predominantly monoterpenoid hydrocarbons (approximately 70 to 90%). Their main constituents are limonene, sabinene, and α-pinene. In studying the evolutionary history of this genus, Adams and Demeke (1993) have claimed that members of section Sabina are relatively advanced compared with Juniperus with regard to their oil compositions.

Because approximately 50 species within Sabina occur in Northern Hemisphere, this may have led to increased selection in their various habitats, thereby increasing the diversity in leaf essential oils from that section. Adams et al. (2002) have also used random amplified polymorphic DNA (RAPD) analyses to demonstrate a major trend in the separation of *J. chinensis* from both *J. procumbens* of Japan and *J. squamata* Buch. Nevertheless, although leaf monoterpenoids have been examined from some taxa of *J. conferta*, *J. rigida*, *J. chinensis*, and *J. formosana*, no data have been presented on those from Korean plants of *J. virginiana*, *J. rigida*, *J. squamata*, and *J.* x *media*. Therefore, the objective of this research was to provide a report on the compounds found in leaf monoterpenoids from *Juniperus* species in Korea, and to compare their compositions among several members of section Juniperus.

MATERIALS AND METHODS

Plants of *Juniperus chinensis* var. chinensis, *J. chinensis* var. globosa, *J. virginiana*, *J. communis* var. montana, *J. chinensis* var. sargentii, *J. chinensis* 'Kaizuka Variegata', *J. rigida*, *J. squamata* 'Wilsonii', *J.* x *media* 'Shimpake', *J.* x *media* 'Plamosa Aurea', and *J. squamata* 'Aloderi' were collected from Chollipo Arboretum (Chungnam Province, Korea) and Kyungnam Arboretum (Kyungnam Province, Korea) in 2003.

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Leaves from each plant were sealed in plastic bags for transport. In the laboratory, 3-g samples were ground with pure sand, then treated with n-pentane and 1 ml of 1% tetradecane as an internal standard. The extracts were filtered with sodium sulfate and concentrated to a final volume of 1 ml by evaporation with a gentle stream of nitrogen gas (Kim and Langenheim, 1994).

Samples were analyzed by gas chromatography-mass spectrometry (GC-MS; Hewlett Packard 5890, USA), in a 30-m HP5 capillary column (0.25 mm i.d.) with a flame ionization detector. Helium gas was used as the carrier. The temperature program for terpenes was initially 37°C for 5 min, followed by an increase to 180°C (rate of 5°C min^{-1}), then to a final temperature of 320°C (20°C min^{-1}). A final 1-μl extract was analysed by GC-MS. Individual terpenes were identified by comparing their fractions with data from the internal spectral library for that instrument (Wiley library 7.0) as well as with their retention times, based on references. Concentrations at selected retention times were estimated from peak areas, using the internal standard curve for tetradecane.

The minimum spanning network was based on 24 monoterpenoids, with similarities computed by Phylogenetic Analysis Using Parsimony (PAUP, ver. 4.0; Swofford, 2002). The composition of monoterpenoids from *Pinus rigida* served as an out-group for the hierarchical clustering analysis. Only constituents with concentrations higher than 0.05% were included.

RESULTS AND DISCUSSION

The compositions of leaf monoterpenoids from 11

Table 1. Components (percentages) of leaf monoterpenoids in 11 *Juniperus* species: *J. chinensis* var. chinensis (1), *J. rigida* (2), *J. virginiana* (3), *J. chinensis* var. globosa (4), *J. chinensis* var. sargentii (5), *J. communis* var. montana (6), *J. chinensis* 'Kaizuka Variegata' (7), *J. squamata* 'Wilsonii' (8), *J.* x *media* 'Shimpake' (9), *J.* x *media* 'Plamosa Aurea' (10), and *J. squamata* 'Aloderi' (11). Only compounds found at levels >0.05% are included.

Monoterpenoid	1	2	3	4	5	6	7	8	9	10	11
Tricyclene	0.00	0.00	0.00	0.16	0.26	0.00	0.91	0.05	0.57	0.05	0.00
α-Thujene	0.20	0.00	0.83	0.24	0.37	0.00	0.63	0.00	0.39	0.21	0.50
α-Pinene	7.82	44.52	9.71	13.83	24.63	26.05	11.02	3.56	8.96	5.89	5.14
β-Pinene	0.00	2.56	0.00	0.00	0.00	1.30	0.00	0.00	0.00	0.00	0.00
Camphene	0.00	0.00	0.00	0.27	0.46	0.16	1.13	0.00	0.68	0.00	0.05
Sabinene	10.08	0.00	34.76	12.93	21.23	0.18	12.39	9.56	10.07	7.65	10.83
Myrcene	1.11	4.21	2.80	1.93	3.54	1.25	4.72	4.50	2.94	1.21	6.43
Phellandrene	0.00	1.70	0.00	0.00	0.00	0.00	0.00	0.14	0.03	0.00	0.00
δ-4-Carene	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.24	0.05	0.05	0.08
para-Cymene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05
Limonene	0.60	3.79	1.86	1.46	2.71	2.05	5.56	5.27	3.44	0.52	1.11
γ-Terpinene	0.00	0.00	0.18	0.08	0.00	0.00	0.24	2.35	0.14	0.07	0.14
Sabinene hydrate	0.00	0.00	0.00	0.00	0.20	0.00	0.32	0.85	0.23	0.05	0.07
Terpinolene	0.19	0.82	0.63	0.31	0.52	0.48	0.82	1.10	0.62	0.21	0.40
Linalool	0.00	0.00	0.20	0.00	0.50	0.00	0.20	0.05	0.00	0.05	0.15
Allo-ocimene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00
Exo-camphenilol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.06	0.00	0.00
Endo-borneol	0.00	0.00	0.00	0.00	0.00	0.06	0.10	0.25	0.05	0.00	0.00
Terpineol-4-ol	0.00	0.11	0.00	0.00	0.00	0.00	0.05	0.07	0.00	0.05	0.05
1-Methanol	0.00	0.00	0.00	0.00	0.00	0.26	0.00	0.84	0.00	0.00	0.00
Citronellol	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.05	0.05	0.05
Hexyl isovalerate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10
Bornyl acetate	0.07	0.99	0.09	2.81	4.80	0.57	0.30	4.79	7.95	0.44	0.05
α-Terpinene	0.00	0.07	0.00	0.07	0.33	0.59	0.38	0.15	0.65	0.15	0.24
Total (%)	20.06	58.77	51.06	34.09	59.64	32.94	38.84	34.16	36.94	16.65	25.46
Compound no.	8	10	9	11	14	11	17	17	19	16	18

species of *Juniperus* (section Juniperus) are presented in Table 1. Their yields varied from 16.65% to 59.64%. Out of 24 monoterpenoids, α-pinene, myrcene, limonene, terpinolene, and bornyl acetate were common to all species. *J. chinensis* var. chinensis had the simplest composition (8 monoterpenoids), while *J.* x *media* 'Shimpake' was the most complex (19 monoterpenoids).

The monoterpenoids of *J. chinensis* var. chinensis were dominated by α-pinene (7.82%) and sabinene (10.08%), with a moderate amount of myrcene (1.11%). In contrast, *J. virginiana*, *J. chinensis* var. globosa, and *J. chinensis* 'Kaizuka Variegata' had considerable amounts of α-pinene (9.71 to 13.83%) and sabinene (12.39 to 34.76%), moderate levels of limonene (1.46 to 5.56%), and a little terpinolene. The leaf extracts from *J. rigida* and *J. chinensis* var. montana were distinctly different from the others, being dominated by high amounts of α-pinene (44.52% and 26.05%, respectively), with a moderate level of myrcene, some β-pinene (2.56% and 1.30%, respectively), but little or no sabinene (0.18%). This composition for *J. rigida* is quite similar to the one described for that species in Japan and Taiwan (Adams, 1998), i.e., 39.7% α-pinene, 11.2% myrcene, 1.9% β-pinene, and only a trace of sabinene.

The natural distribution of *J. rigida* is limited to eastern Asia, and includes Korea. Adams (1998) previously reported, albeit without data for its essential oils, that this species belongs to a group with *J. conferta* and *J. formosana*. Likewise, our results suggest that the *J. rigida* of Korea can be clustered with *J. conferta* and *J. formosana*, based on our monoterpenoid analysis.

In this study, the major compounds of *J. chinensis* 'Kaizuka Variegata', *J. squamata* 'Wilsonii', *J.* x *media* 'Shimpake', *J.* x *media* 'Plamosa Aurea', and *J. squamata* 'Aloderi' were α-pinene, sabinene, and limonene, with only minor amounts of δ-4-carene. Distinct from our other species, *J. squamata* 'Wilsonii' and *J. x media* 'Shimpake' also showed moderate levels of bornyl acetate (4.79% and 7.95%, respectively).

Adams et al. (2002) have used RAPD analyses to demonstrate that five taxa are present within *J. chinensis* from Japan and Taiwan. They have also suggested that *J. chinensis* and *J. squamata* are distinct at specific levels, based on both RAPD and essential oil determinations. Our results also support their claim that *J. chinensis* differs from other Juniperus species.

Our delineation was also confirmed through a dendrogram (Fig. 1), which was based on PAUP analysis. Therefore, those 11 species can be classified into four groups, according to their monoterpenoid compositions. Group I is represented by *J. chinensis* var. chinensis, whose combination of compounds (pinene plus sabinene, with only a moderate amount of myrcene), as well as the length, number, and type of its chromosomes (Kim, 1988), makes this species quite different from the others. Group II also includes a single species, *J. virginiana*, which has a high amount of sabinene. In contrast, the two members of Group III -- *J. chinensis* var. montana and *J. rigida* -- have high levels of α-pinene but little or no sabinene. Group IV contains the remaining seven species, with *J. squamata* 'Wilsonii' and *J.* x *media* 'Shimpake' having the most similar combinations of compounds.

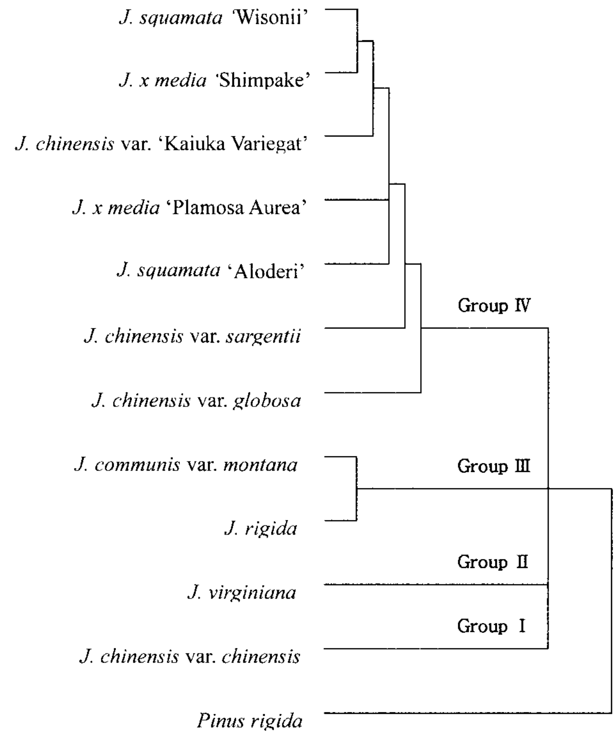

Figure 1. The cluster dendrogram accoding to the difference of monoterpenoid in *Juniperus*.

Morphological studies of these members of the Juniperus section have been based on their chromosomes and pollen. Whereas *J. chinensis* var. chinensis is tetraploid, both *J. chinensis* var. globosa and *J. rigida* are diploid (Kim, 1988). The pollen grains of *J. rigida* are prolate-spheroidal monads of medium size, while those of *J. chinensis* var. globosa, *J. chinensis* var. sargentii, and *J. chinensis* var. chinensis are subprolate (Kim et al., 1997). Although these morphological data are not associated with particular leaf monoterpenoids, these taxa may be treated as distinct species based on either type of examination. Therefore, if environmental factors were also considered, the analysis of quantitative variations in monoterpenoids would be quite useful in assaying infraspecific trends, as has been suggested by Cavaleiro et al. (2001).

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