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Karyotype Analysis of Eight Korean Native Species in the Genus *Iris*

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ABSTRACT : Karyotypes were established in the eight Korean native species of the genus *Iris*. Chromosome numbers were 2n=50 in *I. koreana* and 2n=42 in *I. uniflora* var. *carinata* and their karyotype formulas were K = 2n = 50 = 14m + 28sm + 8st and K = 2n = 42 = 16m + 26sm, respectively. *I. dichotoma* and *I. pseudoacorus* were diploids of 2n=34. However, they showed different karyotype formulas: K = 2n = 34 = 26m + 6sm + 2st in *I. dichotoma* and K = 2n = 34 = 8m + 24sm + 2st in *I. pseudoacorus*. *I. setosa*, and *I. pallasii* var. *chinensis* carried the same chromosome numbers of 2n=40, but they showed different patterns of karyotype formula: K = 2n = 40 = 22m + 14sm + 4st in *I. setosa* and K = 2n = 40 = 26m + 12sm + 2st in *I. pallasii* var. *chinensis*. *I. sanguinea* was a diploid of 2n=28 and the karyotype formula was K = 2n = 28 = 14m + 14sm. *I. ensata* var. *spontanea* was a diploid of 2n=24 and the karyotype formula was K = 2n = 24 = 10m + 14sm. Each species showed characteristic chromosome composition with a pair of satellite chromosome except *I. koreana* with three pairs of satellite chromosomes. The chromosomes of *I. dichotoma* and *I. uniflora* were comparatively short, while the chromosomes of *I. ensata* were remarkably bigger than those of other species. These cytological data will give a useful information for the identification and breeding program of the *Iris* plants.

Key words : *Iris* species, karyotype formula, satellite chromosome

INTRODUCTION

The genus *Iris* plants in the family Iridaceae, mostly perennial rhizomous plants, have more than two hundred species and are distributed in the temporal regions of northern hemisphere (Park *et al.*, 2002). More than eleven species of *Iris* are known in Korean peninsula (Lee, 1985; Lee, 1996; Sim, 1988; Ko & Jun, 2003; Park, 2004). Recently, native plants are recognized as the valuable genetic resources, natural compound source and ornamental material. *Iris* is a high-valued ornamental plant with breeding potential and the rhizomes of some species are used as medicinal purpose (Purev *et al.*, 2002; Rahman *et al.*, 2002, 2003, 2004).

The classification of this genus was only based on phenotypic and morphological characters, which is still in argument. To clarify the relationships among the species, cytological, molecular and genetic approaches are needed (Artiukova *et al.*, 2001; Kentner *et al.*, 2003). Molecular phylogenetic methods were also applied to this genus (park *et al.*, 2002; Sim *et al.*, 2002).

Cytogenetic study is a very useful tool for the identification of species, since each species has characteristic cytological informations such as number, composition and morphology of chromosomes (Bang, 2004). The basic chromosome numbers of *Iris* plants are quite variable, ranging from x = 7 to 25 (Darlington & Wylie, 1955). The chromosome compositions of *Iris* species are polymorphic among species and their large numbers and tiny size of the

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chromosomes were the barrier for cytological study (Bolkhovskikh *et al.*, 1969).

Chromosome numbers were reported in the limited species: 2n=26, 28 in *I. sanguinea* (Lee, 1967), 2n = 34 in *I. rossii* and 2n = 24+2B in *I. savatieri* (Lee, 1969). A few karyological study is also available to date (Wang *et al.*, 1998; Kim & Yoon, 2002a, b; Park *et al.*, 2003). In the present study, the chromosome numbers and karyotypes of eight Korean native *Iris* plants were investigated to provide cytogenetic informations for clarifying the relationships among the species and practical use in breeding program.

MATERIALS AND METHODS

Iris plants were collected from the Chuncheon Experimental Station in Kangwon Provincial Agricultural Research and Extension Services (*I. Koreana* Nakai, *I. uniflora* var. *carinata* Kitagawa, *I. setosa* Pallas, *I. dichotoma* Pallas, *I. pseudoacorus L.*, *I. sanguinea* Hornem, *I. ensata* var. spontanea (Makino) Nakai and Andong Agricultural Technology Center in Kyungpook Province (*I. pallasii* var. *chinensis* Fisch). Plants were grown in artificial soil (peatmoss 1: vermiculite 1) and actively growing root tips were sampled for chromosome analysis.

The root tips were pretreated in 2 mM 8-hydroxyquinoline for 5~6 hrs at 12~16℃ and fixed in acetic acid ethanol (1:3, v/v). Root tips were hydrolyzed in 1N HCl (60℃) for 15 sec and preparations were made using squash method in a drop of 1% aceto-orcein. Cells of good smatic metaphase spread were photographed with Fuji HR-20 negative microfilm (ASA 25, 1,000x, Green filter). Chromosome types were designated as metacentric (m) for arm ratio value (R) of 1.00~1.70, submetacentric (sm) for 1.70~3.00, subtelocentric (st) for 3.00~7.00, and telocentric (t) for >7.00 (Levan et al, 1964).

RESULTS AND DISCUSSION

The somatic metaphase chromosomes and the karyotype idiograms from eight *Iris* species are presented in Fig. 1 and Fig. 2. *Iris* plants classified into two groups (bulbous and rhizomous) and their basic chromosome numbers were extremely variable from x=7 to x=25 (Darlington & Wylie, 1955).

I. koreana was a diploid of 2n = 2x = 50 and had the largest chromosome numbers among the genus *Iris*. The chromosome complement consisted of 7 pairs of metacentrics (chromosomes 6, 7, 10, 12, 15, 21 and 25), 14 pairs of submetacentrics (1, 2, 3, 4, 5, 11, 13, 14, 17, 18, 19, 20, 22 and 24) and 4 pairs of subtelocentrics (8, 9, 16, and 23). The sizes of the chromosomes were in 1.6~5.5 ㎛. Three pairs of satellite chromosomes were found: chromosomes 6, 13, and 23 (Fig. 2).

I. uniflora was a diploid of 2n = 2x = 42 and had satellites on the chromosme 5. The chromosme complement consisted of 8 pairs of metacentrics (chromosomes 1, 4, 7, 8, 18, 19, 20, and 21) and 13 pairs of submetacentrics (2, 3, 5, 6, 9, 10, 11, 12, 13, 14, 15, 16 and 17). No subtelocentrics were found. This plant carried short and tiny chromosomes, ranging from 1.0 to 2.0 ㎛.

I. dichotoma and *I. pseudoacorus* were diploids of 2n = 2x = 34. However, their karyotype formulas based on the method of Levan *et al.* (1964) showed different patterns: K = (2n) = 32 = 26m + 6sm + 2st in *I. dichotoma* and K = (2n) = 32 = 8m + 24sm + 2st in *I. pseudoacorus* (Fig. 2). The chromosme complement of *I. dichotoma* consisted of 13 pairs of metacentrics (chromosomes 1, 3, 4, 6, 7, 8, 9, 10, 12, 13, 14, 15 and 17), 3 pairs of submetacentrics (2, 11, and 16) and 1 pair of subtelocentrics (5). *I. pseudoacorus* had 4 pairs of metacentrics (chromosomes 1, 6, 8 and 15), 12 pairs of submetacentrics (2, 3, 4, 5, 9, 10, 11, 12, 13, 14, 16 and 17) and 1 pair of subtelocentrics (7). The chromosome 7 had satellites. The chromosome sizes of *I. dichotoma* were relatively short, ranging from 1.0 to 3.4 ㎛, while the chromosomes of *I. pseudoacorus* were in 2.6~6.0 ㎛. The karyotype of *I. dichotoma*, which is still in taxonomic problem, was similar to *I. uniflora*. *I. ensata* had long and thick chromosomes compare to other species.

The chromosome numbers of *I. setosa* and *I. pallasii* were diploids of 2n = 2x = 40, but their karyotype formulas based on the methods of Levan *et al.* (1964) showed quite different patterns: K = (2n) = 40 = 22m + 14sm + 4st in *I. setosa* and K = (2n)

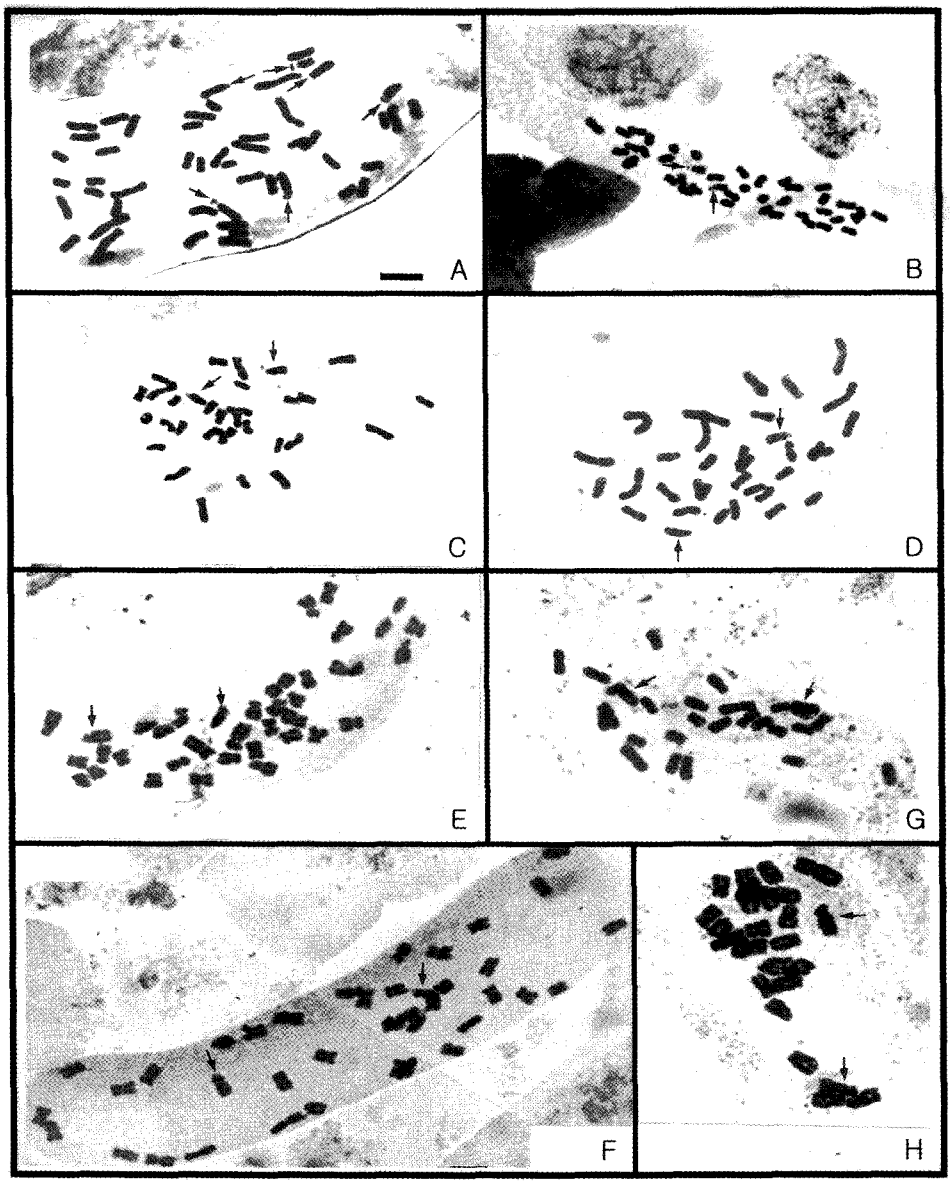

Fig. 1. Somatic metaphase chromosome spreads of *Iris* plants. A; *koreana* (2n=50), B; *I. uniflora* var. *carinata* (2n=42), C; *I. dichotoma* (2n=34), D; *I. pseudoacorus* (2n=34), E; *I. setosa* (2n=40), F; *I. pallasii* var. *chinensis* (2n=40), G; *I. sanguinea* (2n=28), H; *I. ensata* var. *spontanea* (2n=24). Scale bar, 5 ㎛. Arrows indicate satellite chromosomes.

= 40 = 26m + 12sm + 2st in *I. pallasii* (Fig. 2). The chromosome complement of the *I. setosa* consisted of 11 pairs of metacentrics (chromosomes 1, 2, 3, 5, 7, 8, 9, 15, 18, 19 and 20), 7 pairs of submetacentrics (4, 6, 10, 11, 12, 13 and 14) and 2 pairs of subtelocentrics (16 and 17). The chromosome sizes of this species were in 2.0~4.2 ㎛. The chromosome complement of the *I. pallasii* was consisted of 13 pairs of metacentrics (chromosomes 1, 2, 5, 6, 8, 10, 12, 13, 14, 16, 17, 19 and 20), 6 pairs of submetacentrics (3, 4, 7, 9, 15, and 18) and one pair of subtelocentrics (11). The chromosome sizes of this species were in 2.6~4.8 ㎛. Satellites were found on the chromosome 4 in both species (Fig. 2).

I. sanguinea was a diploid of 2n = 2x = 28 and the chromosome complement consisted of 7 pairs of metacentrics (chromosomes 1, 2, 3, 8, 9, 10 and 11) and 7 pairs of submetacentrics (4, 5, 6, 7, 12, 13 and

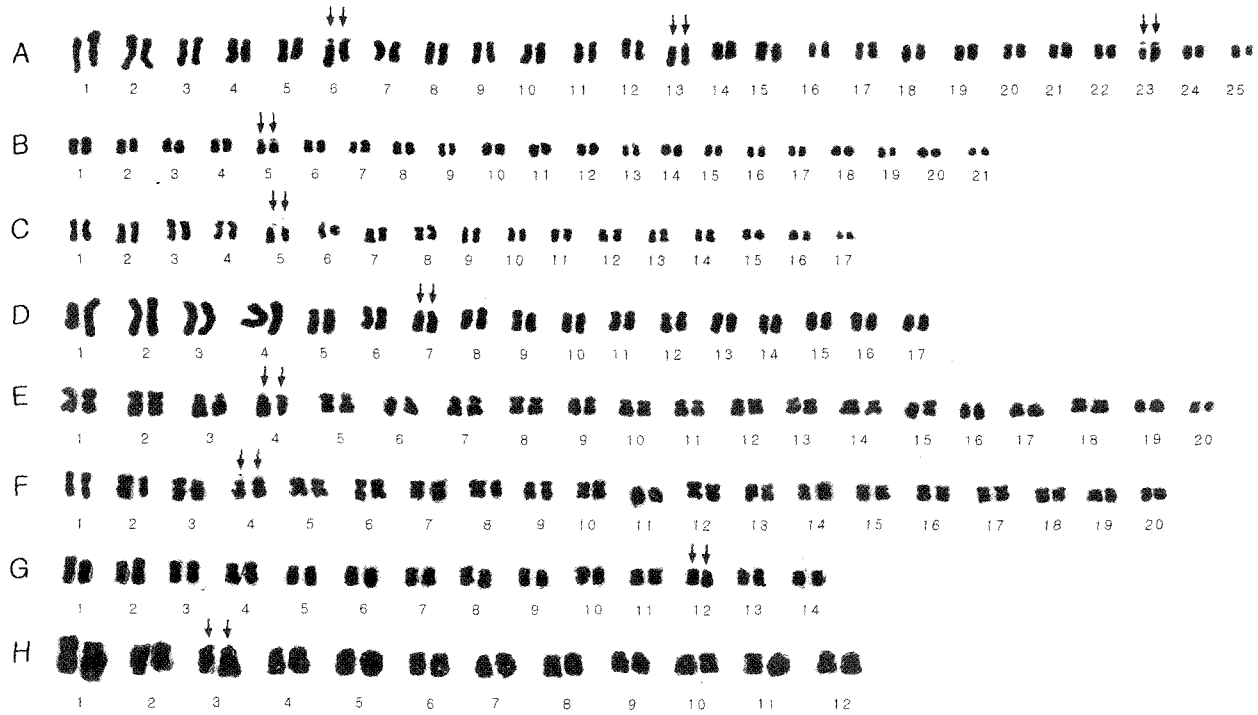

Fig. 2. Karyotype idiograms of *Iris* plants. A; *koreana* (K = 2n = 50 = 14m + 28sm + 8st), B; *I. uniflora* var. *carinata* (K = 2n = 42 = 16m + 26sm), C; *I. dichotoma* (K = 2n = 34= 26m + 6sm + 2st), D; *I. pseudoacorus* (K = 2n = 34 = 8m + 24sm + 2st), E; *I. setosa* (K = 2n = 40 = 22m + 14sm + 4st), F; *I. pallasii* var. *chinensis* (K = 2n = 40 = 26m + 12sm + 2st), G; *I. sanguinea* (K = 2n = 28 = 14m + 14sm), H; *I. ensata* Thunberg var. *spontanea* (K = 2n = 24 = 10m + 14sm). m, metacentric for R = 1.00-1.70); sm, submetacnntric for R = 1.70-3.00; st, subtelrocentric for R = 3.00-7.00 (Levan *et al.*, 1964). Arrows indicate satellite chromosomes.

14). The chromosome sizes of this species were in 2.3~3.9 ㎛ (Fig. 2). Satellites were found on the chromosome 12. The chromosome number of this species was reported as 2n = 26, 28 (Lee, 1967).

I. ensata was a diploid of 2n = 2x = 24 and the chromosome complement consisted of 5 pairs of metacentrics (chromosomes 1, 2, 4, 9 and 10) and 7 pairs of submetacentrics (3, 5, 6, 7, 8, 11 and 12). The chromosome 3 had nucleolar organizer regions. The chromosome size of this species was bigger than other species investigated.

As a result, eight *Iris* species examined had all diploids, ranging from 2n=2x=24 to 2n=2x=50 and showed polymorphic karyotypes. This karyological data could be useful for the identification of *Iris* species. Molecular methods using RAPD markers and ITS sequences were introduced to the phylogenetics of this genus to overcome the limits of phenotypic characters (Park *et al.*, 2002; Sim *et al.*, 2002). However, our cytogenetic results were not consistent with the molecular data. An integrated data, such as morphological, cytological and molecular traits should be considered to elucidate inter-specific relationships in this genus. Our results will also be very informative in providing basic cytogenetic data for the breeding program of the *Iris* plants.

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