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Chromosome studies of Korean *Orostachys* species (Crassulaceae)

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Chromosomes of three Korean *Orostachys* species were studied at meiosis. Examined species were *O. japonicus*, *O. malacophyllus*, and *O. sikokianus*. Their meiotic chromosome number was n=12 in all species. Chromosome pairing at metaphase I was normal with 12 bivalents that formed either open rings or rods. Several nucleolar organizing regions were frequently observed with prophase I chromosomes in microsporocytes. No B chromosome was detected. Pollen fertility was relatively high in all species exhibiting no less than 90%. Chromosomal variation of *Orostachys* species in Korea appears rather simple on the basis of the chromosome data presented in the current study. This is the first report on meiotic chromosome information for the genus *Orostachys*.

Keywords: *Orostachys* species, chromosome number, meiotic behavior

Orostachys is a succulent genus of monocarpic perennials that possess spine-tipped rosette leaves. Approximately 10 species are included in the genus which occur mostly in temperate regions of the northeastern Asia (Park, 1974; Lee, 1979; Ohwi, 1984; Makino, 1989; Suk, 1993a, b). They are distinct from species in other genera of the family in their reproductive features. About three species are known in Korea (Lee, 1979).

The genus *Orostachys* has not drawn any attention until recently, whereas closely related *Sedum* has been studied extensively in many scopes, including cytogenetics (Goldblatt, 1988) and biosystematics (Amano and Ohba, 1992). The only recent information available for *Orostachys* species are found in works of Kim *et al.* (1995a) that particularly dealt with structural aspects. Although chromosome count of *Orostachys* was first recorded as *Sedum* species in 1935 (Toyohuku, 1935) and as *Orostachys* species in 1983 (Probatova

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Table 1. Collection data of three Korean *Orostachys* species. All collections were from Kyungbuk Province. ISK, I. S. Kim; YJB, Y. J. Baek.

Species	Voucher	Collection site and date
O. japonicus	ISK & YJB 9596	Mt. Palkong, Sep. 22, 1995
O. malacophyllus	ISK & YJB 9591	Gampo, Kyungju, Sep. 7, 1995
O. sikokianus	ISK & YJB 9571	Mt. Palkong, July 15, 1995

and Skolovoskaya, 1983), no detailed chromosome study has been carried out for the genus since. This implies no chromosome counts for the Korea *Orostachys* species and they are yet to be studied. Hence, the present study was attempted to reveal the meiotic features of chromosomes in three Korean *Orostachys* species and reports various information on chromosome numbers, meiotic behavior, and pollen fertility.

Materials and Methods

Several hundred florets of *Orostachys japonicus* A. Berger and *O. malacophyllus* Fisch. and less than 50 of *O. sikokianus* Ohwi were collected and fixed in the field. The fixed materials were transferred to the laboratory, placed 5 to 7 days at room temperature, and stored in a freezer (-4℃) until further examinations. Inflorescences and/or florets were collected at Mt. Palkong, Taegu, in September for *O. japonicus* and in July for *O. sikokianus*. Materials of *O. malacophyllus* were sampled at coastal area of Gampo-kun, Kyungju in September (Table 1). Vouchers of samples collected by the author and others are deposited at Keimyung University. Immature floral buds were fixed in Carnoy's fixative (chloroform-absolute ethanol-glacial acetic acid, 6:3:1, v/v/v). Anthers were squashed, stained with acetocarmine, and mouted in a drop of Hoyer's medium and followed according to the methods of Beeks (1955). More than 200 microsporocytes were examined for the chromosome counts and meiotic behavior, except *O. sikokianus* where fixed florets were less than 50 due to insufficient materials. Percent pollen fertility by stainability, based on 500 grain samples stained in aniline blue in lactophenol, was calculated for each species. Fertile pollen was filled with cytoplasm and showed nucleus, whereas abortive ones had less cytoplasm or other abnormalities and empty grains. Selected cells were photographed with a Zeiss Jenalumar phase contrast microscope.

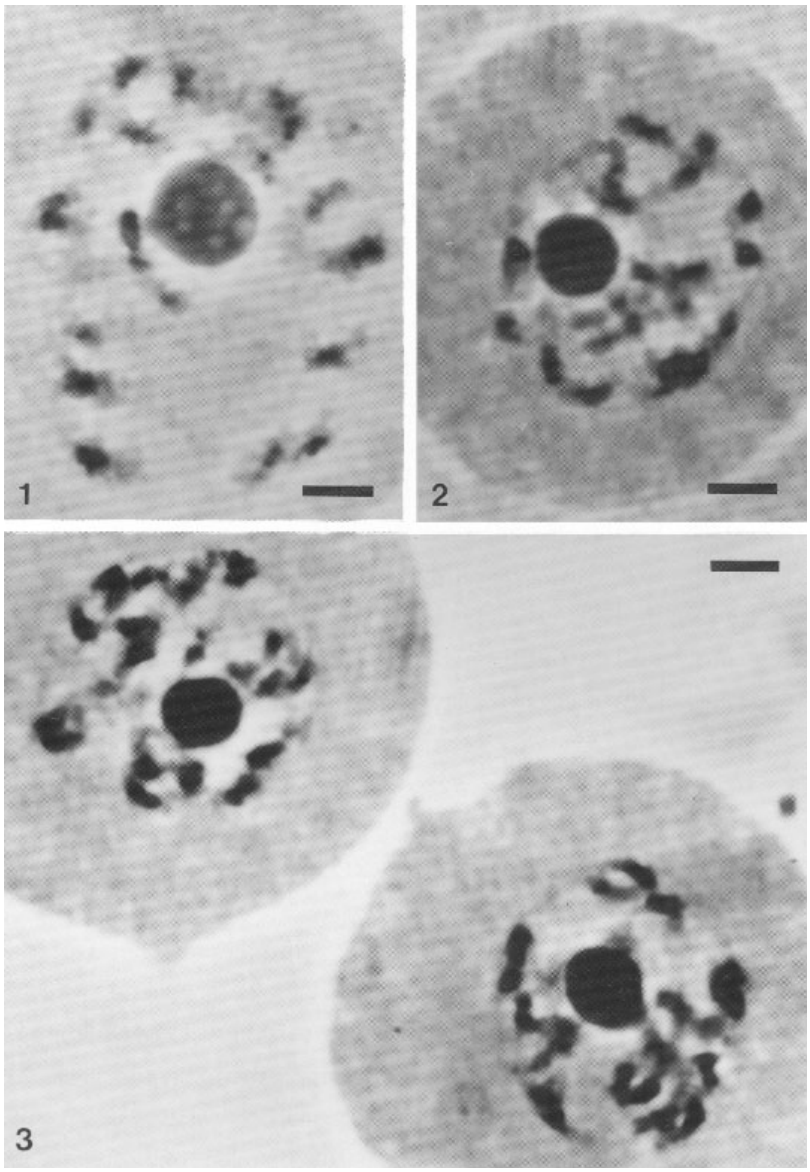
Results and Discussion

Meiotic chromosome numbers and behavior, and pollen fertility were studied in three Korean *Orostachys* species. *Orostachys japonicus*, *O. malacophyllus*, and *O. sikokianus* had same chromosome number of n=12. Association of nucleolus and chromosomes at prophase were frequently observed (Figs. 1-4). Several nucleolar organizing regions were more frequently observed in prophase I chromosomes, especially in microsporocytes of *O. malacophyllus* (Fig. 3). No meiotic irregularities were observed among the microsporocytes of *Orostachys* species. Chromosome pairing at metaphase I was normal with 12 bivalents that formed either open rings or rods (Fig. 5). *Orostachys sikokianus* had very small anthers with rather small microsporocytes among studied materials. Except *O. sikokianus* that had limited microsporocytes, analysis of 30 cells per species at anaphase I did not reveal the presence of bridges, fragments, or lagging chromosomes (Figs. 6, 7). No B chromosome was either observed during the study. However, meiotic chromosome irregularities causing considerablely various chromosome numbers has been reported in closely related genus *Sedum* (Amano and Ohba, 1992), which exhibits remarkable variation in chromosome number (Goldblatt, 1981, 1984, 1985, 1988). According to Amano and Ohba (1992), irregularities obviously lead to the formation of microsporocytes with various chromosome numbers causing aneuploidy and polyploidy chromosome evolution in this group.

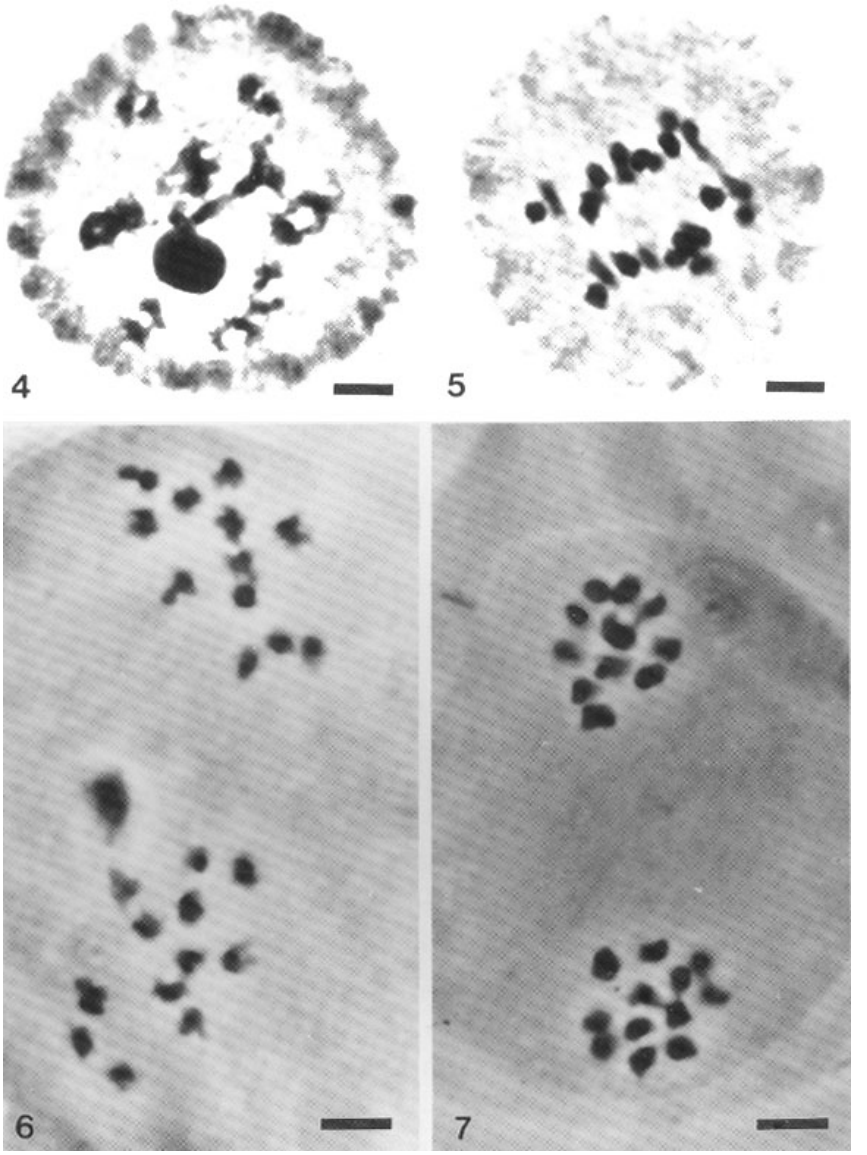
Pollen fertility in *Orostachys* was relatively high and was not significantly different among species. *Orostachys japonicus*, *O. malacophyllus*, and *O. sikokianus* exhibited relatively high pollen stainability, showing over 90% in most cases. The highest percentage, ca. 95%, was shown in *O. japonicus*. In *O. malacophyllus* and *O. sikokianus*, it was about 91% and 92%, respectively.

It may be presumtuous to mention a basic chromosome number for the genus *Orostachys*, since the sample size is rather small to indicate a general trend for the genus. However, using counts from this study and reports of others (Toyohuku, 1935; Probatova and Skolovoskaya, 1983; Malakhova, 1990), n=12 appears to be the best candidate for a base number in *Orostachys*. More chromosome counts of *Orostachys* species from other geographical areas are especially needed before the detailed phylogenetic significance of these data can be assessed.

Chromosomal variation of *Orostachys* species in Korea appears rather simple on the basis of the chromosome data presented in the current study. Since meiotic chromosome information usually offers strong potential for elucidating phylogeny (John, 1990) among plant taxa, findings of this study may provide an intriguing consequence for future studies



Figs. 1-3. Association of nucleolus and chromosomes at prophase in *Orostachys* species. All scale bar=3 ㎛. 1. A couple of nucleolar organizing regions (NORs) were shown in *O. malacohyllus*. Vesicle-like structures were also observed within the nucleolus. 2. Many NORs seen in *O. japonicus*. 3. Two microsporocytes showing several NORs in *O. malacophyllus*.



Figs. 4-7. First meiotic division in three *Orostachys* species. All scale bar=3 ㎛. 4. Late prophase chromosomes in *O. sikokianus*. 5. Twelve pairs of late metaphase chromosomes in *O. malacophyllus*. 6. Chromosomes being separated and are moving toward opposite poles at early anaphase in *O. malacophyllus*. 7. Two sets of chromosomes grouped at each pole in late anaphase of *O. japonicus*.

attempting crosses among the examined species or other *Orostachys* from geographically different regions.

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바위솔속 식물 3종의 염색체 연구

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바위솔, 둥근바위솔, 난장이바위솔의 화분 형성시 감수분열상에 나타나는 염색체의 특성을 연구하였다. 위 바위솔속 종들은 모두 12쌍의 2가 염색체를 지니고 있으며 정상적인 감수분열을 수행하였다. 제1 감수분열의 전기에서 여러 개의 인형성 염색체(nucleolar organizing chromosome)가 흔히 관찰되었다. 부수염색체(B chromosome)는 관찰되지 않았으며, 3종 모두 90% 이상의 높은 화분 염성도를 보였다. 위 특성에 비추어 볼 때 한국에 분포하는 바위솔속 3종의 염색체는 비교적 초기 단계의 분화 상태에 있는 것으로 사료된다.

주요어: 바위솔속, 염색체수, 감수분열상

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