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# Chromosome numbers of *Euphorbia pekinensis* complex in Far East Asia

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**ABSTRACT:** We report somatic chromosome numbers for three species belonging to the *Euphorbia pekinensis* complex distributed in Far East Asia. In *E. pekinensis* populations distributed in Korea, $2n = 28$ and 56 were found, while the Japanese native *E. lasiocaula* was also found at $2n = 28$ and 56 and the Japanese endemic *E. sinanensis* was found at $2n = 20$. Based on the number of chromosomes, *E. lasiocaula* distributed in Japan supports treatment as a variety of *E. pekinensis* rather than as a different species, while *E. sinanensis* should be recognized as a distinct species rather than as a variety of *E. pekinensis*. In the same populations of *E. pekinensis* and *E. lasiocaula*, diploid and tetraploid individuals were found, and the diversity of these chromosome numbers was consistent with the morphological diversity of these populations, suggesting the future evolutionary potential of this species.

**Keywords:** *Euphorbia pekinensis* complex, chromosome number, *Euphorbia*

The *Euphorbia pekinensis* complex, belonging to *Euphorbia* ser. *Pekinensis* (Hurusawa, 1940), consists of several species morphologically similar to *E. pekinensis* Rupr. (Park et al., 2002). They are *E. pekinensis*, *E. fauriei* Levl. et. Vant., *E. subulatifolia* Hurusawa in Korea, and *E. lasiocaula* Boiss. and *E. sinanensis* (Hurusawa) T. Kurosawa et H. Ohashi in Japan. However, these species are very similar in morphology and having a great polymorphism, they have treated as many infraspecific taxa. Recently, *E. fauriei* is treated as a distinct species in Korea (Park et al., 2002; Chung et al., 2003), but *E. subulatifolia* is morphologically very similar to *E. pekinensis*, and it treated as a *Galarhoeus pekinensis* var. *subulatifolius* (Hurusawa, 1954), or the same species as *E. lasiocaula* in Japan (Kurosawa and Ohashi, 1994b). In Japan, *E. sinanensis*, is traditionally treated as *G. lasiocaulus* var. *sinanensis* (Hurusawa, 1954) or *E. pekinensis* form. *sinanensis* (Hurusawa, 1940), and *E. lasiocaula* also as *G. lasiocaulus* form. *lasiocaulus* (Hurusawa, 1954), but recently both species have been recognized as an independent species based on morphological and phenological differences (Kurosawa and Ohashi, 1994a; Kurosawa, 1995).

Although few cytological studies of East Asian *Euphorbia* species have been conducted, two species of the *E. pekinensis* complex are known about chromosome numbers (Chung et al., 2003; Ikeda et al., 2008). In particular, *E. fauriei* was diploid with $2n = 28$, and *E. pekinensis* showed $2n = 28$ or 56.

Therefore, this study was aimed to compare the results of somatic chromosome numbers at the population level of the *E. pekinensis* complex in Korea and Japan, and to utilize them as useful information for testing species limit and their relationships in this complex.

## Materials and Methods

This study was carried out on 6 populations of 3 species belonging to the *E. pekinensis* complex distributed in Korea and Japan. The plants were collected from the natural habitats, and transplanted in the greenhouse for collecting root tips. And Voucher specimens used in this study were deposited in the herbarium of Kyungnam University (KNUH).

To investigate the number of chromosomes, chromosomes of metaphase cells were observed using the squash technique according to Chung et al. (2003) who recently observed the chromosomes of *Euphorbia* species. Five roots per plant

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cultivated in pots were cut and placed in a tube containing secondary distilled water for pretreatment at 4°C for overnight. Roots were fixed with aceto-alcohol (1:3) for 6 h. The fixed roots were washed with distilled water for 20 min, and then hydrolyzed with 1N HCl at 60°C for 4 min. After cleaning the roots with distilled water for 10 min, stained with Feulgen reagent for 5 min, and removed extra parts of root tips, and finally double-stained with 2% aceto-carmine and squashed. The squashed samples of five individuals per population were observed with light microscopy under ×1000 magnification.

## Results and Discussion

According to the results of this study, *E. pekinensis* individuals, which are collected from Danyang, Gotji Beach and Mt. Gahak, showed only a diploid with a chromosome number of $2n = 28$ (Table 1; Fig. 1f). However, in the case of the Daegu population, both the diploid ($2n = 28$) and tetraploid (2n = 56) were occurred (Fig. 1c, d), and the Japanese native species, *E. laciocaula*, also showed $2n = 28$ and 56 (Fig. 1a, b) at the same population. This result is consistent with the report of the previous *E. pekinensis* chromosome study (Chung et al., 2003). They reported that *E. pekinensis* and *E. fauriei* in Korean populations were $2n = 28$, and diploid and tetraploid individuals were only found in *E. pekinensis* population. The Japanese endemic *E. sinanensis* was the first to report chromosomal numbers with $2n = 20$ (Fig. 1e, basic chromosome number $x = 10$) in this study, which is a distinct difference from the rest of the *E. pekinensis* complex species having basic chromosome number $x = 14$.

The Japanese endemic species, *E. sinanensis*, was treated by Hurusawa (1940, 1982) as a variety or forma of *E. pekinensis*. Recently, however, a smaller capsule and the obtuse verrucae of its surface appeared in *E. sinanensis* are distinct from those of *E. pekinensis*, and was treated as a different species by Kurosawa and Ohashi (1994a). Our results show that the two species differ in basic chromosome number as $x = 14$ and 10, supporting the treatment of Kurosawa and Ohashi (1994a) with different species rather than as infraspecific taxa of *E. pekinensis* by Hurusawa (1940, 1982). Two species have different basic chromosome numbers, and *E. sinanensis* can be assumed to be not closely related to the members of the *E. pekinensis* complex, and this hypothesis is consistent with the results of recent molecular studies. Phylogenetic relationships based on *matK* + ITS data represented that the populations of *E. sinanensis* were not placed within the *E. pekinensis* complex, but they showed as a sister group of the complex (Park et al., unpub. data).

*Euphorbia pekinensis* is widely distributed in Northeast Asia and is highly variable in morphology, so it has been confusing to taxonomic treatment, and there have been reported various subspecific taxa depending on the region. Three species of *E. pekinensis*, *E. fauriei* and *E. subulatifolia* were reported in Korea, but they have recently been treated with one species of *E. pekinensis* with three varieties. Recent systematic studies have shown that the genetic identity among three taxa is very high and may have recently been differentiated from a common ancestor (Park, 2004). In Japan, *E. pekinensis* and *E. lasiocaula* are regarded as independent species based on the difference of verrucae shapes of capsules, and also Korean endemic *E. subulatifolia* is treated as a same species of *E. lasiocaula* while *E. fauriei* is treated as a subspecies of *E. pekinensis* (Hurusawa, 1954; Kurosawa and Ohashi, 1994b). Traditionally, however, *E. lasiocaula* has been treated as the same species as *E. pekinensis* (Maximowicz, 1883; Hayata, 1904; Hara, 1935).

Although there are limitations in determining whether two species are the same species with only chromosome information, the species mentioned above showed the same number of chromosomes, and the similar variation such as occurring diploid and tetraploid individuals in a population, It would be more likely to hypothesize them as the same species rather than treating them as different species. Recent phylogenetic results based on molecular data show that these groups are combined to a single clade without resolution, which

**Table 1.** Species, population, sampling location and somatic chromosome number of three species in the *Euphorbia pekinensis* complex. The number of population examined is in parentheses.

| Species | Locality | Chromosome number ($2n$) |
|---|---|---|
| *E. lasiocaula* (pop 09) | Sugou, Murata-machi, Miyagi Pref., Japan | 28, 56 |
| *E. pekinensis* (pop 01) | Mt. Gahak, Haenam, Jeonnam, Korea | 28 |
| *E. pekinensis* (pop 05) | Gotgi, Chungnam, Korea | 28 |
| *E. pekinensis* (pop 27) | Woonhung-Sa, Daegu, Korea | 28, 56 |
| *E. pekinensis* (pop 28) | Danyang, Chungbuk, Korea | 28 |
| *E. sinanensis* (pop 13) | Numayama, Tsubonuma, Sendai-shi, Miyagi Pref., Japan | 20 |

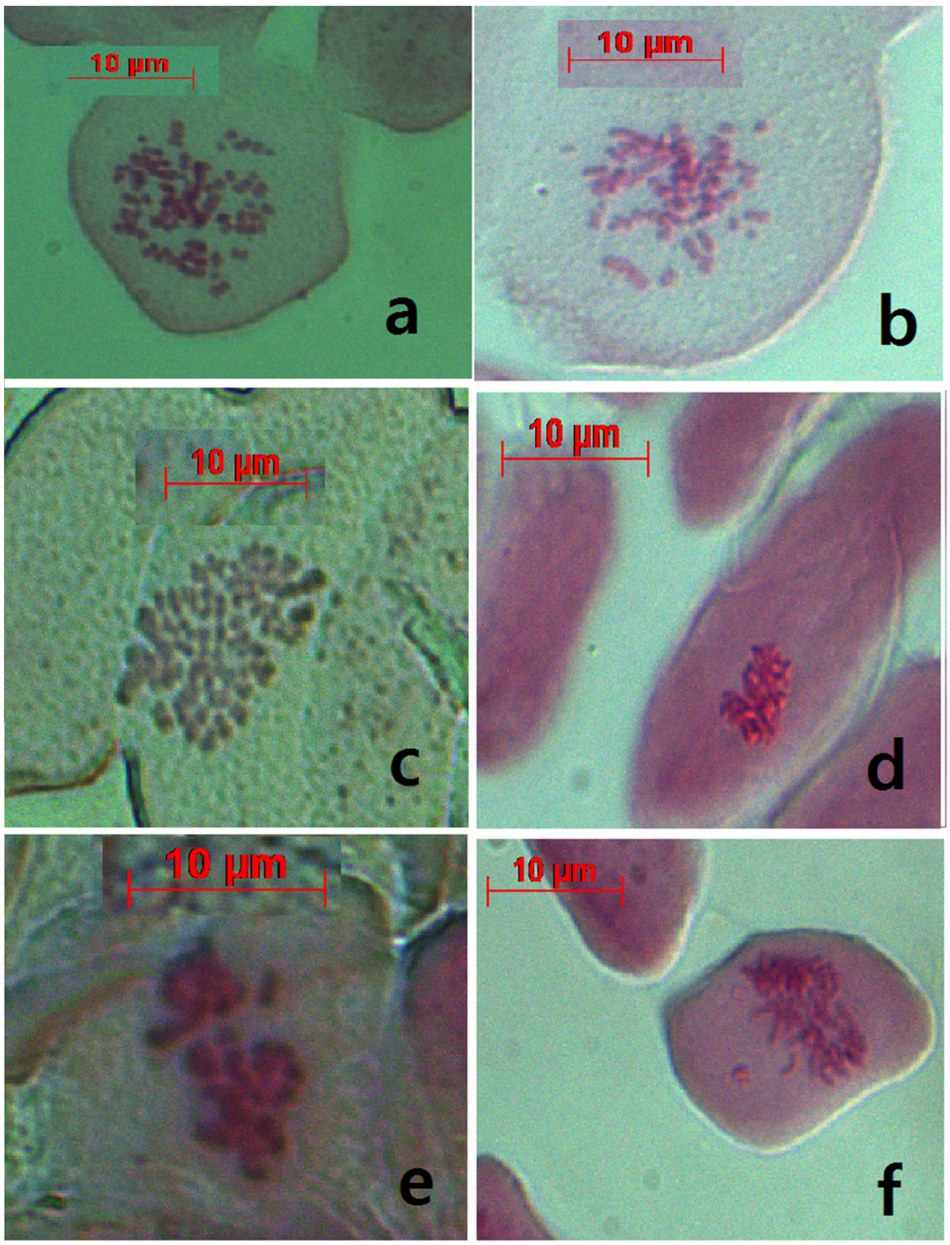


**Fig. 1.** Somatic chromosomes of the *Euphorbia pekinensis* complex species. a. *E. lasiocaula* (pop9, 2*n* = 56), b. *E. lasiocaula* (pop9, 2*n* = 28), c. *E. pekinensis* (pop27, 2*n* = 56), d. *E. pekinensis* (pop27, 2*n* = 28), e. *E. sinanensis* (pop13, 2*n* = 20), f. *E. pekinensis* (pop1, 2*n* = 28). Scale bars = 10 μm.

supports this interpretation (Park et al., unpubl. data).

The occurring of diploid and tetraploid individuals in the *E. pekinensis* complex is believed to be related to the various morphological variations within them, and they may suggest the evolutionary potential of differentiating into new species through chromosomal evolution. These similar patterns are frequently found in the Himalayan flowering plants, and are presumed to be related to the morphological variation of these plants (Wakabayashi, 2002).

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