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# New diploid populations of *Chrysanthemum indicum* L. (Asteraceae) from Korea

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**ABSTRACT:** *Chrysanthemum indicum* (Asteraceae) is a perennial plant belonging to the genus *Chrysanthemum*. The basic chromosome number of *Chrysanthemum sensu stricto* is $x = 9$, and it consists of a series of polyploids ranging from diploid to decaploid. However, *C. indicum*, which occurs in Korea, is known to consist of only tetraploids, except for two diploid populations that are sympatric with *C. zawadskii* and *C. boreale*. During the collection of plant materials as part of a study to ascertain the diversity of *Chrysanthemum* in Korea, we found new diploid populations ($2n = 18$) of *C. indicum* in the southern region of Korea and describe them here in detail.

**Keywords:** *Chrysanthemum indicum*, diploid, tetraploid, chromosome number

The genus *Chrysanthemum* (subfamily Asteroideae of Asteraceae) comprises 41 recognized species, mainly distributed across East Asia including Korea, China, and Japan (Bailey and Bailey, 1976; Ohwi, 1984; Fu et al., 2005; Oberprieler et al. 2007). It is characterized by the obovoid and generally mucilaginous cypselae without pappus and involucral bracts with dark brown margins (Bremer and Humphries, 1993; Lee, 2006; Zhao et al., 2009). Historically the genus *Chrysanthemum* distributed in Asia was especially separated from *Chrysanthemum s. l.* of the broad sense by Kiramura (1940), and was recognized as an independent genus *Dendranthema* (DC.) Des Moul. of the narrow sense (Bremer and Humphries, 1993). However, it was changed again and back to the *Chrysanthemum* (Trehane, 1995; Nicolson, 1999). *Chrysanthemum indicum* L. is a perennial plant and it has commonly yellow ray flower as well as *C. boreale*.

Kim and Tobe (2009) had reported that the leaf shape of *C. boreale* and *C. indicum* were similar to each other though the thickness were slightly different. However, Jeong (2011) and Song et al. (2012) found a wide variation of leaf shape within both species and sometimes it was difficult to distinguish each species because of overlapped variation. They suggested that an adaptation to their native habitats leads the similar external feature appearance between both species. Although it is obviously difficult to distinguish these closely related species to each other clearly, the head flower size has been regarded as one of key characters to classify two species. According to Lee (2003), the *C. boreale*'s head flower is 1.5 cm and the *C. indicum* is 2.5 cm in diameter. Kim et al. (2003) described that *C. boreale*' head flowers was less than 1.5 cm and *C. indicum* was 1.5–2.5 cm in diameter. Therefore, for a long time, they have been accepted the diagnostic characters for classifying both species.

Polyploidization has played an important role in plant evolution and speciation, and it is prevalent in the genus *Chrysanthemum* (Dowrick, 1952; Shimotomai et al., 1956; Tanaka, 1959, 1960; Nakata and Tanaka, 1987; Tsukaya, 2002; Kim et al., 2003). From the accumulated data of cytological studies for long time, it was clear that the basic chromosome number of the genus *Chrysanthemum* is $x = 9$ and it has a successive ploids in the species level (Grant, 1981; Oberprieler et al. 2007). For the example, *C. zawadskii* has 2*x*, 4*x*, 6*x*, 8*x*, and 10*x* (Nakata and Kumagai, 1999; Kim et al., 2004), *C. indicum* has 2*x*, 4*x*, and 6*x* (Lee and Oh, 1976; Nakata et al. 1987; Taniguchi 1987; Du et al., 1989). Since Lee and Oh (1976) found two cytotypes of yellow-flowered wild chrysanthemum in Korea, one is *C. boreale* which has numerous small heads and 18 chromosomes and the other is

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*C. indicum* which has a few larger heads of 1.5–2.3 cm in diameter and 36 chromosomes in the mitotic cells. The information of the *C. indicum* ($2n$ = 36) population reported by Lee and Oh (1976) is not detailed and is known as a total of 59 populations. *C. indicum* had been known to be composed of only tetraploid population in Korea until the sympatric diploid populations (Fig. 1, Table 1) were reported by Kim et al. (2003).

For the purpose of understanding the diversity in the genus *Chrysanthemum*, we re-investigated the ploidy of *C. indicum* populations and found new diploid populations are widely distributed in Korea. Therefore, we described the details of the population information in the present study for the advanced research.

## Materials and Methods

### Plant materials

A total of 167 living individuals were collected from seventeen populations of *C. indicum* (Fig. 1, Table 1) which are native and composed of over 20 individuals, and identified by external morphological characters of the head size and color. After collecting from the national habitats, they were managed for sufficient water supply in the greenhouse of Chungbuk

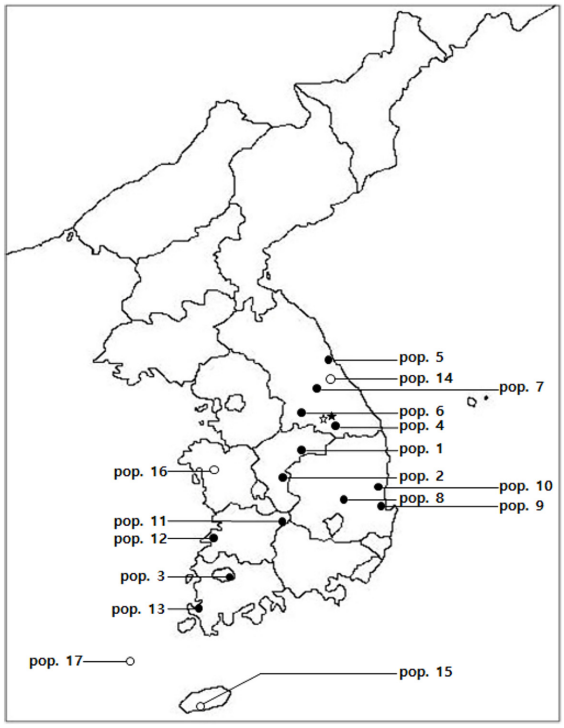


**Fig. 1.** Map showing the localities of the populations which were described in the Table 1. ●, The diploid population ($2n$ = 18); ○, the tetraploid population ($2n$ = 36); ★ & ☆, The previously reported diploid populations.

**Table 1.** Collection sites information and chromosome number of *Chrysanthemum indicum* populations investigated in the study.

| Pop. No. | Locality | Coordinate | Elevation (m) | Voucher specimen | Chromosome number |
|---|---|---|---|---|---|
| 1 | Songgye-ri, Hansu-myeon, Jecheon-si, Chungcheongbuk-do | 36.86°N, 128.08°E | 360 | CBNU2018-0267 | 2x, $2n$ = 18 |
| 2 | Songnisan-myeon, Boeun-gun, Chungcheongbuk-do | 36.53°N, 127.82°E | 338 | CBNU2019-0225 | 2x, $2n$ = 18 |
| 3 | Mudengsan, Geumgok-dong, Gwangju, Jeollanam-do | 35.13°N, 126.98°E | 520 | CBNU2019-0226 | 2x, $2n$ = 18 |
| 4 | Beagisan Mt., Nam-myeon, Jeongseon-gun, Gangwon-do | 37.17°N, 128.42°E | 380 | CBNU2019-0227 | 2x, $2n$ = 18 |
| 5 | Gwangjin-ri, Hyeonnam-myeon, Yangyang-gun, Gangwon-do | 37.96°N, 128.76°E | 65 | CBNU2019-0209 | 2x, $2n$ = 18 |
| 6 | Baekdal-ri, Ucheon-myeon, Hoengseong-gun, Gangwon-do | 37.44°N, 128.06°E | 220 | CBNU2018-0206 | 2x, $2n$ = 18 |
| 7 | Cheokcheon-ri, Jinbu-myeon, Pyeongchang-gun, Gangwon-do | 37.69°N, 128.50°E | 970 | CBNU2018-0220 | 2x, $2n$ = 18 |
| 8 | Binggyegyegok, Chunsan-myeon, Uiseong-gun, Gyeongsangbuk-do | 36.22°N, 128.75°E | 120 | CBNU2019-0216 | 2x, $2n$ = 18 |
| 9 | Josa-ri ,Songna-myeon, Buk-gu, Pohang-si, Gyeongsangbuk-do | 36.22°N, 129.38°E | 28 | CBNU2019-0214 | 2x, $2n$ = 18 |
| 10 | Docheon-ri, Namjeong-myeon, Yeongdeok-gun, Gyeongsangbuk-do | 36.31°N, 129.35°E | 34 | CBNU2019-0213 | 2x, $2n$ = 18 |
| 11 | Deogyusan Mt., Seolcheon-myeon, Muju-gun, Jeollabuk-do | 35.88°N, 127.78°E | 717 | CBNU2019-0221 | 2x, $2n$ = 18 |
| 12 | Mapo-ri, Byeonsan-myeon, Buan-gun, Jeollabuk-do | 35.64°N, 126.47°E | 20 | CBNU2019-0220 | 2x, $2n$ = 18 |
| 13 | Yudalsan Mt., Mokwon-dong, Mokpo-si, Jeollanam-do | 34.78°N, 126.36°E | 32 | CBNU2018-0396 | 2x, $2n$ = 18 |
| 14 | Sangjinbu-ri, Jinbu-myeon, Pyeongchang-gun, Gangwon-do | 37.65°N, 128.57°E | 540 | CBNU2018-0221 | 4x, $2n$ = 36 |
| 15 | Cheonjeyeon, Jungmun-dong, Seogwipo-si, Jeju-do | 33.24°N, 126.42°E | 17 | CBNU2018-0398 | 4x, $2n$ = 36 |
| 16 | Yongbongsan Mt., Sangha-ri, Hongbuk-myeon, Hongseong-gun, Chungcheongnam-do | 36.64°N, 126.64°E | 139 | CBNU2019-0197 | 4x, $2n$ = 36 |
| 17 | Gageodo-gil, Heuksan-myeon, Sinan-gun, Jeollanam-do | 34.03°N, 125.07°E | 215 | CBNU2019-0228 | 4x, $2n$ = 36 |
| ★ | Kwangha-ri, Jeongseon-eup, Jeongseon-gun, Gangwon-do | Previously reported diploid (2x, $2n$ = 18) population (Kim et al., 2003) | | | |
| ☆ | Baekun-ri, Mitan-myeon, Pyungchang-gun, Gangwon-do | | | | |

National University. The voucher specimens were deposited in the herbarium of Department of Forest Science, Chungbuk National University (CBNU).

### Root fixation

According to Kim et al. (2003), fresh root tips were collected from the planted living materials and pre-treated with 0.002 M 8-hydroxyquinolin solution for 2 h at 20°C. It was then washed with distilled water, and fixed with Carnoy's solution (45% acetic acid:99% ethanol = 1:3) for 30 min on ice. And they were stored in 70% cold ethanol at -20°C for the mitotic chromosome observation.

### Mitotic chromosome observations

To observe the mitotic metaphase chromosomes, the squash method was performed with the same condition of Kim et al.'s study (2003). The root tips were dissociated at 65°C for 1 min pre-heated 1 N HCl solution and stained by 1% aceto-orcein solution.

It was repeated at least five times per individual, and the chromosome numbers were observed under the optical microscope (Olympus BX50, Tokyo, Japan) to verify their chromosome number.

## Results and Discussion

We found that *C. indicum* showed various leaf shapes (Fig. 2) even though they were identified as *C. indicum* based on the head flower size and leaf shape.

Based on the observation of mitotic metaphase chromosome, we found that new diploid populations of *C. indicum* are distributed in Korea. Out of 17 populations which were investigated in the present study, 13 populations were diploid of $2n = 18$ (pop No. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13) and 4 populations were tetraploid of $2n = 36$ (pop No. 14, 15, 16, and 17) (Fig. 3, Table 1).

These newly reported diploid populations of *C. indicum* could be used as an important material for understanding the

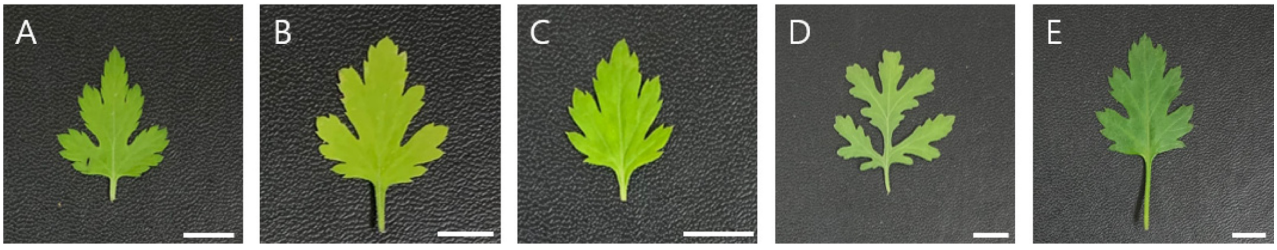


**Fig. 2.** Representative leaf shapes of five populations of *Chrysanthemum indicum*. **A.** Pop. 1. **B.** Pop. 2. **C.** Pop. 3. **D.** Pop. 14. **E.** Pop. 15. Scale bars = 1 cm.

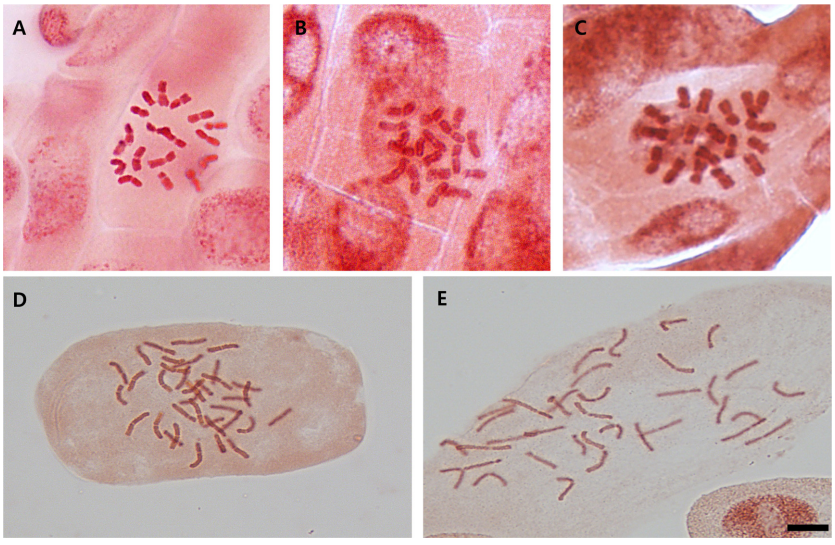


**Fig. 3.** Metaphase chromosomes during the mitosis of *Chrysanthemum indicum*. **A.** Pop. 1 ($2n = 18$). **B.** Pop. 2 ($2n = 18$). **C.** Pop. 3 ($2n = 18$). **D.** Pop. 14 ($2n = 36$). **E.** Pop. 15 ($2n = 36$). Scale bar = 10 μm.

evolution patterns of *C. indicum* in Korea. The most important mechanisms for understanding plant evolution are hybridization and polyploidization (Dowrick, 1952; Shimotomai et al., 1956; Tanaka, 1959, 1960; Lee, 1975; Nakata and Tanaka, 1987; Nakata et al., 1987; Hotta et al., 1996; Tsukaya, 2002; Kim et al., 2003). *C. zawadskii* complex has been reported from 2*x* (2*n* = 18) to 10*x* (2*n* = 90), whereas *C. indicum* is composed of diploid and tetraploid, and *C. boreale* is specifically known only diploid (Lee and Oh, 1976; Nakata et al. 1987; Taniguchi 1987; Du et al. 1989; Nakata and Kumagai 1999; Kim et al. 2004; Kim et al. 2008). From the result, *C. indicum* should be recognized as a species complex as well as *C. zawadskii* complex composed of different ploid level of diploid and tetraploid for the further study. It needs extensive cytological studies of *C. indicum* for clarifying the diversification mechanism and relationship among the populations. Also it is suggested the probability that the expression of polyploidization is differ depend on the species. In addition, the presence of intermediate type of both species, *C. indicum* and *C. boreale,* revealed the interspecific hybrids have naturally and repeatedly generated among the species and it makes to difficult and complicate to understand the evolutionary history in the genus.

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## Conflict of Interest

The authors declare that there are no conflicts of interests.

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