Karyotype of *Brachanthemum krylovii* Serg.

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ABSTRACT. Seeds of *Brachanthemum krylovii* Serg., a member of *Chrysanthemum sensu lato* collected in 50°23'38", 26°40'39" on the limestone area near the confluence of the Katun River and Chuya River, Mts. Altai, Altai Republic, Russian Federation, were sown and germinated to perform seedlings of which root tips were used to observe somatic chromosomes. The karyotype was K(2n=18)=14m+2sm+2st. This diploid karyotype was commonly observed in the members of the Anthemideae or *Chrysanthemum sensu lato*.

KEYWORDS: *Brachanthemum krylovii*, Chromosomes, Karyotype, Mts. Altai

Brachanthemum is distributed from Altai Republic, Russian Federation to Mongolia, down to Kazakhstan and Gansu Province, the People's Republic of China and forms small, more or less procumbent shrublets, woody at the base (Bremer and Humphries 1993). All of the species of *Brachanthemum* seem to be quite closely related to each other, except *B. baranovii* is different and placed in the monotypic sect. *Dendranthemopsis* (Bremer and Humphries 1993). They form woody at base, and their inflorescences are quite closely similar to those of the former *Dendranthema* (Bremer and Humphries 1993) but the present *Chrysanthemum* (Trehane 1995).

Brachanthemum krylovii Serg. grows in the limestone area near the confluence of the Katun River and Chuya River, Mts. Altai (50°23'38", 26°40'39"), Altai Republic, Russian Federation (Smirnov 2000). Smirnov (2000) discovered that *B. baranovii* was a natural hybrid between *B. krylovii* and *Chrysanthemum sinuatum* (= *Dendranthema sinuatum*) according to the data of internal transcribed spacer (ITS) and chloroplast DNA. Moreover, Vechernina *et al.* (2004). has in fact synthesized *B. baranovii* by artificial cross-pollination between *B. krylovii* and *Chrysanthemum sinuatum* and the ovule culture *in vitro*. The chromosome number of *B. krylovii* was 2n=18 (Smirnov 2000) while *C. sinuatum* was 2n=18 (Zhmyleva and Kondo 2006). Thus, some introgressive hybridization and gene flow might be occurred between *B. krylovii* and *C. sinuatum*.

Thus, the karyotype of *Brachanthemum krylovii* is here reported for the first time.

MATERIALS AND METHODS

Seeds of *Brachanthemum krylovii* Serg. were collected near the confluence of the Katun River Chuya River, Mt. Altai (50°23'38", 26°40'39"), Altai Republic, Russian Federation. They were sown in wet vermiculite in pots for germination. As soon as they got germination, they were

harvested and placed in 0.002M 8-hydroxyquinoline at 18°C for 2h. Then, they were fixed in the mixture of absolute ethanol and glacial acetic acid of 3:1. They were macerated in the 2:1 mixture of 1N hydrochloric acid and 45% acetic acid at 60°C for 10 sec, and then, stained in aceto-orcein for 30 min before squashed.

The karyotype description followed Levan *et al.* (1964): m=median-centromeric chromosome (Arm ratio 1.0-1.7), sm=submedian-centromeric chromosome (Arm ratio 1.8-3.0) and st=subterminal-centromeric chromosome (Arm ratio 7.1 and more).

RESULTS AND DISCUSSION

The karyotype of *Brachanthemum krylovii* Serg. at metaphase was 2n=18=14m+2sm+2st described here for the first time (Fig. 2). The largest chromosome at metaphase was 7.66 µm while the smallest chromosome was 3.72 µm. The karyomorphology of the resting chromosomes was the complex chromocenter type and that of the prophase chromosomes was the interstitial type.

The karyotype of *Chrysanthemum* (previously *Dendranthema*) *sinuatum* was described as 2n=18=14m+2sm+2st (Zhmyleva and Kondo 2006). The chromosome number of *Brachanthemum krylovii* was previously described as 2n=18 (Smirnov 2000). *Brachanthemum baranovii* was synthesized by intergeneric hybridization within all the diploid background in laboratory between *Brachanthemum krylovii* and *Chrysanthemum sinuatum* by Vechernina *et al.* (2004). Thus, the karyotypes of the diploid species of *Chrysanthemum sensu lato* studied seemed to be quite similar to each other (Kondo *et al.* 1995; Kondo *et al.* 2003; Kondo *et al.* 2012).

Close relationship of *Brachanthemum krylovii*, *B. baranovii* and *Chrysanthemum sinuatum*, all grown in Mts. Altai have been analyzed by using ITS and chloroplast DNA of molecular genetics by Smirnov (2000). Thus, it

would make sense if those three taxa of *Chrysanthemum sensu lato* studied here could be all diploid ($2n=18$). These chromosome data and relationship within three taxa can be made the sense of the relationship proposed by Smirnov (2000). Further chromosome studies within the three taxa such as *Brachanthemum krylovii*, *B. baranovii* and *Chrysanthemum sinuatum* would be very much necessary to clarify their interspecific relationships.

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Fig. 1. *Brachanthemum krylovii* Serg. The research site near the confluence of the Katun River and Chuya River, Mt. Altai (50°23'38", 26°40'39").

Fig. 2. The karyotype of $2n=18=14m+2sm+2st$ in *Brachanthemum krylovii* Serg. Bar = 10 μ m.