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Full Length Research Paper

Chromosome numbers of two *Colchicum* L. species, *C. burttii* and *C. balansae*, from Turkey

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Chromosome numbers and morphologies for C*olchicum burttii* Meikle and *Colchicum balansae* Planchon showing distribution in western Anatolia were studied. Squashing preparation method was used. The chromosome counting and morphologies of the species were determined by examining the mitosis prepared from root tips of plants. Chromosome number of *C. burttii* is 2n = 60 + 2B, showing that this species is hexaploid (x = 10). On the other hand, chromosome number of *C. balansae* is found as 2n = 90. Formerly, chromosome number of this species was reported as 2n = 32, 54 and 108. In conclusion, chromosome number of the sample obtained with this investigation is different from previous studies. 2n = 90 chromosome number is a basic polyploid (hexaploid) with x_2 = 15 (15*6 = 90), involving the summation of the primary basic numbers, 7 and 8.

Key words: Chromosome number, chromosome morphology, *Colchicum* spp., Turkey.

INTRODUCTION

The genus *Colchicum* L. (Liliaceae) is represented by about 100 species (incl. *Merendera* Ram. and *Bulbocodium* L.) in the world (Stefanoff, 1926; Persson, 1999a, 1999b, 2000, 2001, 2005). Eurasia and North Africa in terms of species distribution is remarkable. There are almost 36 taxa of the genus *Colchicum* in Turkey. 15 of them are endemic (Brickell, 1984; Persson, 2000; 2005; Akan and Eker, 2005).

Taxonomically a problematical genus, is partly due to the paucity of adequate well documented material available and partly to the difficulties in assessing the status of hysteranthous species which have, in most instances, been described from flowering material alone (Brickell, 1980, 1984). No contemporary, overall investigation has been undertaken and only the monographs of Baker (1879) and Stefanoff (1926) exist, which are not only old but also based only on Feinbrun (1958). In addition, Persson (1993) stated that chromosome numbers are very important in understanding *Colchicum* evolution. Nevertheless, *Colchicum* is known to be a difficult material for chromosome studies. In some species, chromosomes stained faintly, but in others they failed to scatter, in still others they failed to show their primary constrictions, etc. (Levan, 1940; Levan and Steinegger, 1947; D'Amato, 1955; Feinbrun, 1958; Fernandes and França, 1977).

In the literature no report has been found about chromosome numbers of *C. burttii* which is the material of the study. However, we have some studies about on some other species of *Colchicum*. Cytological investigations on *Colchicum* species started with Furlani (1904)'s and Heimann-Winawer (1919)'s studies.

Colchicum was investigated in a comprehensive manner as cytological by Levan (1940). He reported some chromosome numbers for some *Colchicum* species for the first time: *C. bivonae* Guss. (2n = 36), *C. neapolitanum* Ten. (2n = 38), *C. speciosum* Stev. (2n = 38), *C. byzantinum* Ten. (2n = 40), *C. giganteum* Hort. (2n = 40), *C. bornmuelleri* Freyn. (2n = 42), *C. variegatum* L. (2n = 44), *C. latifolium* S.S. (synonym: *C. bivonae*) (2n = 54) and *C. montanum* L. (2n = 54).

Sato (1942) gave out chromosome numbers of some species (*C. sibthorpii* Bak. (Synonym: *C. bivonae*) (2n = 36), *C. variegatum* L. (2n = 44) and *C. fimbriatum* (2n = 36)).

Feinbrun (1953, 1958) gave chromosome numbers and morphology in some species which were collected from

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Palestine (7 species), Cyprus (1 species) and Sinai Peninsula (1 species) (*C. schimperi* Janka (2n = 14), *C. ritchii* R.Br. (2n = 14), *C. tuviae* Feinbr. (2n = 14), *C. guess*feldtianum Asch. and Schw. (2n = 14), *C. hiemale* Freyn (2n = 54), *C. stevenii* Kth. (2n = 54), *C. hierosolymitanum* Feinbr. (2n = 18), *C. decaisnei* Boiss. (Synonym: *C. troodii*) (2n = 54), *C. tunicatum* Feinbr. (2n = 54)).

D'Amato (1955) examined C. *autumnale* L. (2n = 38), *C. lusitanum* Brot. (2n = 106) and *C. neapolitanum* Ten. (2n = 140) taxa in respect of their cytotaxonomy. D'Amato (1956) determined chromosome numbers of *C. bieberstеinii*, *C. arenarium*, *C. cupanii* and *C. alpinum,* firstly.

Camarda (1978) found chromosome numbers of C. *gonarei* (2n = 182), *C. neapolitanum* Ten. (2n = 146) and *C. cupanii* Guss. (2n = 54).

Küçüker (1985) determined chromosome numbers of *C. chalcedonicum* (2n = 50), *C. turcicum* (2n = 52), *C. micranthum* (2n = 54).

Küçüker (1990) studied about karyomorphological peculiarities of *C. lingulatum* Boiss. and Spruner. He pointed out that chromosome number of *C. lingulatum* is 2n = 48.

Şık and Küçüker (1998), made a chromosomal investigation on *C. bivonae* Guss. (2n = 36), *C. boissieri* Orph. (2n=46), *C. triphyllum* G. Kunze (2n = 42), *C. variegatum* L. (2n = 44).

A major evolutionary trend in *Colchicum* is towards polyploidization and the primary chromosome base number is most probably x = 9 (Persson, 1993; Nordenstam, 1998). Chromosome numbers determined for taxa showing distribution in Turkey are as 2n = 20, 22, 24, 36, 38, 42, 44, 46, 50, 52 and 108 (Özhatay, 2002).

Genome size has been studied for the first time in the *Colchicum* genus by Fridlender et al. (2002). In their conclusion, they found that values obtained by flow cytometry in relation to the investigated taxa were quite stable and specific to each taxon.

C. burttii Meikle is endemic to Turkey and it grows in Çanakkale, Kütahya, Denizli, Mugla and Antalya as local. No literature finding exists for chromosome number of *C. burttii*. *C. balansae* Planchon is also endemic to Turkey. *C. balansae* is confused in literature and herbaria with *C. kotschyi* and it grows in Mugla, Antalya and Izmir as a local (Brickell, 1984). The numbers of chromosomes of species was given in 3 different ways (2n = 54 and 108; 2n = 32, respectively) in the 2 different studies (Persson, 1999b; Özkum et al., 1999, respectively).

This work has been conducted to use knowledge of chromosome of species to classify species in addition to morphological properties.

MATERIALS AND METHODS

As for the cytological study, the examined species were collected from the localities in west Anatolia of Turkey. *C. burttii* Meikle was obtained from Denizli, Honaz mountain, 2403 m., 37°42′ N, 37° 42′ E, 30.04.2004. *C. balansae* Planchon was obtained from Mugla, Bodrum, Karaova village, 167 m. 37°04′ N, 27°32′ E, 14.10.2004.

The plants were collected during the periods in which they blossomed. For each species, 10 samples were collected in different localities. Samples dried out following herbarium rules were converted to herbarium material and species determination was made according to "Flora of Turkey and the East Aegean Islands" (Brickell, 1984). The samples are currently stored in herbarium of Ege university.

Some of the samples were taken from the field where they were planted in the flowerpots and they were allowed to keep alive. Chromosome counting was made and morphological characteristics of their chromosomes were established from cells in root tips of corms.

About 75 root tips were karyologically examined in each studied species. Root tips taken from the plants for this purpose were pretreated in 0.002 M 8-hydroxyquinoline solution in water for 3 h. Then, root tips were washed in pure water and placed in Carnoy fixative (glacial acetic acid + pure alcohol, 1:3 v/v). Parts of 2 mm were cut from root tips and their mitosis preparations were made according to powdering method following dyeing with acetoorceine dye. 50 slides were prepared and at least 10 well spread metaphase plates were photographed in a Carl Zeiss Jena and drawn from slides.

RESULTS AND DISCUSSION

Of the two *Colchicum* species, the number of chromosome in *C. burttii* counted for the first time in this study and its chromosome count was found to be 2n = 60 + 2B (Figure 1A). Chromosome number of the species *C. balansae* was determined to be 2n = 90 (Figure 1B).

Chromosomes number and morphology is valuable in view of taxonomy. However, chromosome studies on *Colchicum* are very limited. From the cytological as well as taxonomic points of view, *Colchicum* presents a difficult subject. Levan (1940), Levan and Steinegger (1947), D'Amato (1955), Fernandes and França (1977) reported that in some species chromosomes stained faintly, in others they failed to scatter, in still others they failed to show their primary constrictions, etc.

There was no chromosomal number introduction found in the other *Colchicum* taxa except *C. burttii*'s chromosome number as 2n = 90 and *C. balansae*'s chromosome number as 2n = 60 + 2B. Nevertheless, it is known that variabilities could be seen on chromosomal numbers of the *Colchicum* taxa (Levan, 1940; D'Amato, 1956; Feinbrun, 1958; Darlington and Wylie, 1965; Federov, 1974; Camarda, 1978; Persson, 1992; Özhatay, 2002).

Chromosomes of *C. burttii* are larger than those of *C. balansae* although both species have very small chromosomes. Levan (1940) reported that metaphase chromosomes were usually metacentric in the *Colchicum* species. In regard to chromosome morphology, it has been seen that chromosomes of *C. burttii* are usually metacentric and this species has 2B chromosomes.

On the other hand, Stebbins (1971) reported that the species with karyograms showing metacentric chromosomes might be regarded as primitive. Most chromosomes are metacentric in both of the species studied in

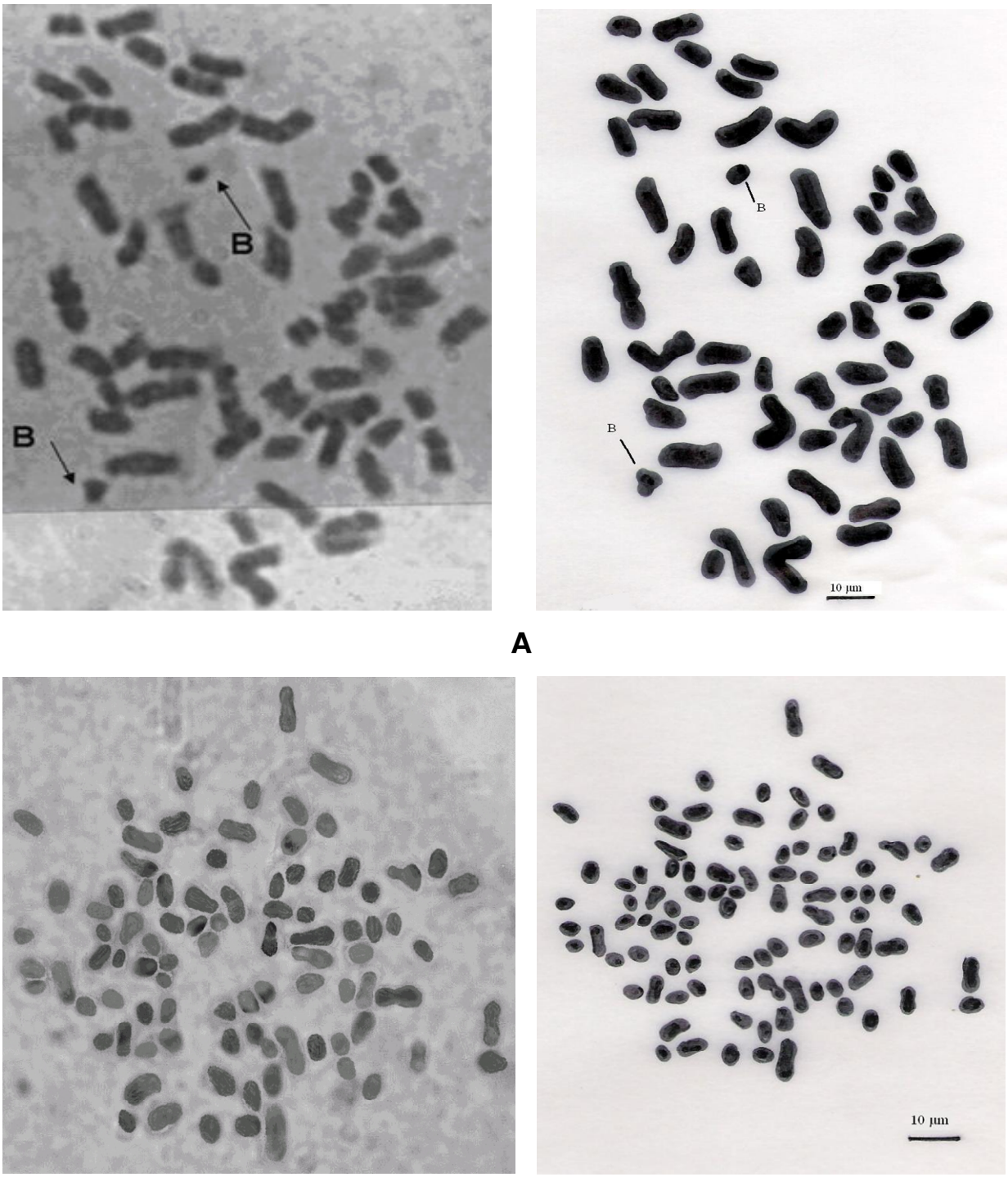

Figure 1. Mitotic metaphase plates of *C. burttii* (A), *C. balansae* (B) [B: B chromosome] Bar = 10 µm.

the current study, suggesting that these species might be old evolutionally.

It is seen that both species of interest has high polyploidy values. A number of different chromosome numbers were found in the *Colchicum* species such as 2n = 14, 18, 20, 22, 24, 36, 38, 40, 42, 44, 46, 50, 52, 54, 72,

76, 102, 106, 108, 146 and 182 (Levan, 1940; D'Amato, 1956; Feinbrun, 1958; Darlington and Wylie, 1965; Federov, 1974; Camarda, 1978; Persson, 1992; Özhatay, 2002). These determined chromosome numbers indicate chromosomal evolutional direction of the genus and is toward polyploidy. Just as Persson (1993) reported that 75% of *Colchicum* species of which 90% was examined had polyploidy and 67% of the species with polyploidy had chromosome numbers derived from basic chromosome numbers of x = 7 and x = 9.

Dibasic polyploidy is suggested by D'Amato (1956) in his summarizing account on the cytotaxonomy of *Colchicum*. D'Amato (1956) gives chromosome numbers 18 for *Colchicum* species. D'Amato (1956) comprises the following numbers: 2n = 16, 18, 20 and 20 + 1 supern. 36, 38, 40, 42, 44, 52, 54, 106 and 140. The discovery of *Colchicum* species with low chromosome numbers such as 16, 18 and 20 permits for the first time to trace in x = 8, 9, 10 the basic, or some of the basic, chromosome numbers of the genus *Colchicum*. According to D'Amato, starting with initial species with low chromosome numbers, new species formation has developed through allopolyploidy. D'Amato (1956) stated that the chromosome number of certain species exactly the same as the other 2 chromosome numbers in the genus (that is, 36 = 16 + 20; 52 = 16 + 36; 54 = 16 + 38 or 18 + 36; 106 = 52 + 54).

Darlington and Wylie (1965) give chromosome numbers of the *Colchicum* as 2n = 14, 18, 36, 38, 40, 42, 44, 54 and 102. The basic numbers cited by them are: x_1 = 7, 9, 10 and x_2 = 17, 19.

Feinbrun (1958) thought that while 2n = 14 and 2n = 18 are diploids, 2n = 36 and 2n = 54 are tetraploids and hexaploids respectively, basic number of x = 9. The basic number x1 = 10 on the other hand seems to have been derived from 2n = 40, which has to be regarded as tetraploid. 2n = 38 is a dibasic polyploid with x2 = 19, involving the summation of the primary basic numbers 9 and 10. 2n = 102 of *C. lusitanum* is a dibasic polyploid (hexaploid) with x2 = 17 and derived from the primary basic 7 and 10.

The fact that chromosome number of the species *C. burttii* studied in the current investigation is 2n = 60 + 2B shows that this species is hexaploid (x = 10). This result is in accordance with Feinbrun's (1958) explanation regarding basic chromosome number as 10 in some taxa. As well known, when low in number, B chromosome provides the plant with chance of a better adaptation. It can be thought that B chromosomes that belong to *C. burttii* give some advantages in this aspect.

In this study, chromosome number of *C. balansae* is found as 2n = 90. Formerly, chromosome number of the species was reported as 2n = 54 and 2n = 108 (Persson, 1999b), 2n = 32 (Özkum et al., 1999). Chromosome number obtained in this investigation is different from previous studies as it is seen. It is difficult to state this chromosome number obtained in this study with D'Amato (1956)'s dibasic polyploidy explanation. If it is explained according to Feinbrun (1958), chromosome number of *C. balansae* was found to be 2n = 90 in this study, is a dibasic polyploid (hexaploid) with x_2 = 15 (15*6 = 90), involving the summation of the primary basic numbers 7 and 8.

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