Pak. J. Bot., 49(4): 1345-1350, 2017.

KARYOMORPHOLOGICAL CONTRIBUTION TO THE GENUS *BELLEVALIA* (ASPARAGACEAE) IN TURKEY

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Abstract

As part of the karyological studies of the Asparagaceae grow in the Kahramanmaraş province, chromosome number and chromosomes morphology of three *Bellevalia* species have been determined: *B. macrobotrys* 2*n*= 8; *B. gracilis* 2*n*= 8, 16, *B. tauri* 2*n*= 8. This is the first karyotype reconstruction for *B. macrobotrys*. *B. tauri* is an endemic species to Turkey. Photographs of the mitotic metaphase plates and drawings with the idiograms are given. Additionally, idiograms and karyotypes of the species were performed by the use of Cameram© software.

Key words: *Bellevalia, B. tauri, B. gracilis, B. macrobotrys,* Karyotype, Southern Anatolia, Turkey.

Introduction

The genus *Bellevalia* Lapeyr. is currently placed within Asparagaceae, Asparagales, according to APG III classification based on phylogenetic studies (APGIII, 2009; Chase *et al*., 2009). The genus is represented by 75 taxa (Feinbrun, 1940; Wendelbo, 1984; Bareka *et al*., 2008; Jafari *et al*., 2008; Borzatti *et al*., 2013). It has a wide distribution in Mediterranean basin as far as to Caucasus, temperate Europe, North Africa and South West Asia.

In Turkey, *Bellevalia* is represented by 28 taxa, 17 of which are endemic to the country (Wendelbo, 1984, Davis *et al*., 1988; Özhatay & Kultur, 2006; Özhatay *et al*., 2009, 2011; Güner *et al*., 2012; Uzunhisarcıklı *et al*., 2013; Gürdal *et al*., 2014; Karabacak *et al*., 2014, 2015).

Morphologically, the genus *Bellevalia* is closely related to the genus *Muscari*. However, it is very distinct karyologically, with large chromosomes and a basic chromosome number *x* = 4 (Pfosser & Speta, 1999).

The aim of this paper was to provide good karyotypes data to clearly define chromosome numbers and morphology for three *Bellevalia* species including *B. macrobotrys* for which no information about chromosomes is available.

Material and Methods

Plant material: In 2011-2014, living plants were collected from Kahramanmaraş and voucher specimens were prepared and kept in ISTE Herbarium (Herbarium of Istanbul University, Faculty of Pharmacy). The plants were all grown and maintained in the Geophyte Garden in Yalova Atatürk Central Horticultural Research Institute. The localities, populations and herbarium numbers are given in Table 1 and Fig. 1.

Chromosome analyses: The specimens were collected from their natural habitat in Kahramanmaraş province cited in Southern Turkey and potted in the bulb Garden in Yalova. Root tips were collected on warm sunny mornings in June, and pre-treated with ABN (alpha bromonaphtalene) for 24 hours at a temperature of -4°C. Before staining, the material was fixed in Carnoy's solution and hydrolyzed in 1N HCl for 13 min at 60ºC. The material was stained with basic fuchsin and then squashed in a drop of 2% acetic orcein onto glass microscope slides. At least five metaphase plates were examined from different individuals for all the counts. Preparations were examined using an Olympus BX53 light microscope, equipped with a digital camera. At least five metaphase plates were examined from different individuals for all the counts.Also measurements of somatic chromosomes were taken by CAMERAM© software, they were calculated with a formula of the relative variation in chromosome length (CV_{CL}), mean centromeric asymmetry (M_{CA}) (Zuo & Yuan, 2011; Peruzzi & Eroğlu, 2013). The classification of chromosomes as having centromeres in metacentric (m), submetacentric (sm), subtelocentric (st), and telocentric (t) was used, according to Levan *et al.* (1964).Chromosomal data of the specimens is summarized in Table 2.

Results

The karyotypes and idiograms of eight different populations of three *Bellevalia* species are summarized in Figures 2-4. The chromosome length ranges, total haploid chromosome length, intrachromosomal asymmetry and the interchromosomal asymmetry estimationss of eight different populations of Bellevalia taxa are given in detail in Table 2. Chromosome heteromorphism is rather common at the population level. No B chromosomes were found.

B. gracilis Lapeyr.

The chromosome number counted 2*n* = 8 in two populations and 2*n* = 16 in one population. Diploid karyotypes consist of 1 metacentric, 2 submetacentric and 1 subtelocentric chromosome pairs. No satellite was observed (Fig. 2). The intrachromosomal asymmetry index (M_{CA}) varies from 23.85 to 31.80. The interchromosomal asymmetry index (CV_{CL}) varies from 16.38 to 21.35 (Table 2). The karyotype is 3A.

Table 1. Localities of taxa used in research area.

Taxa	Population number	Locality	Herbarium number (ISTE)
B. gracilis	1-3	B6 Kahramanmaraş: Ahır mountain, 1480 m, 19.03.2013; K.maras: Berit mountain, Ahmetcik village, 1400 m, 23.03.2013; Caglayancerit, Engizek mountain, rocky places, 1536 m, 29.05.2013.	100251; 100247; 100250
B. macrobotrys	4-5	C6 K. maras: Turkoglu, İmali river, grassland, 626 m, 13.04.2012; Kılavuzlu, in field, 540 m, 23.03.2013	100244; 100245
B. tauri	6-8	C6 K. maras: Andirin, Cigsar, 1500 m, 12.05.2012; Geben village, field, 1400 m, 23.03.2013; Goksun, 1400 m, 23.03.2013.	100238;100240-100241

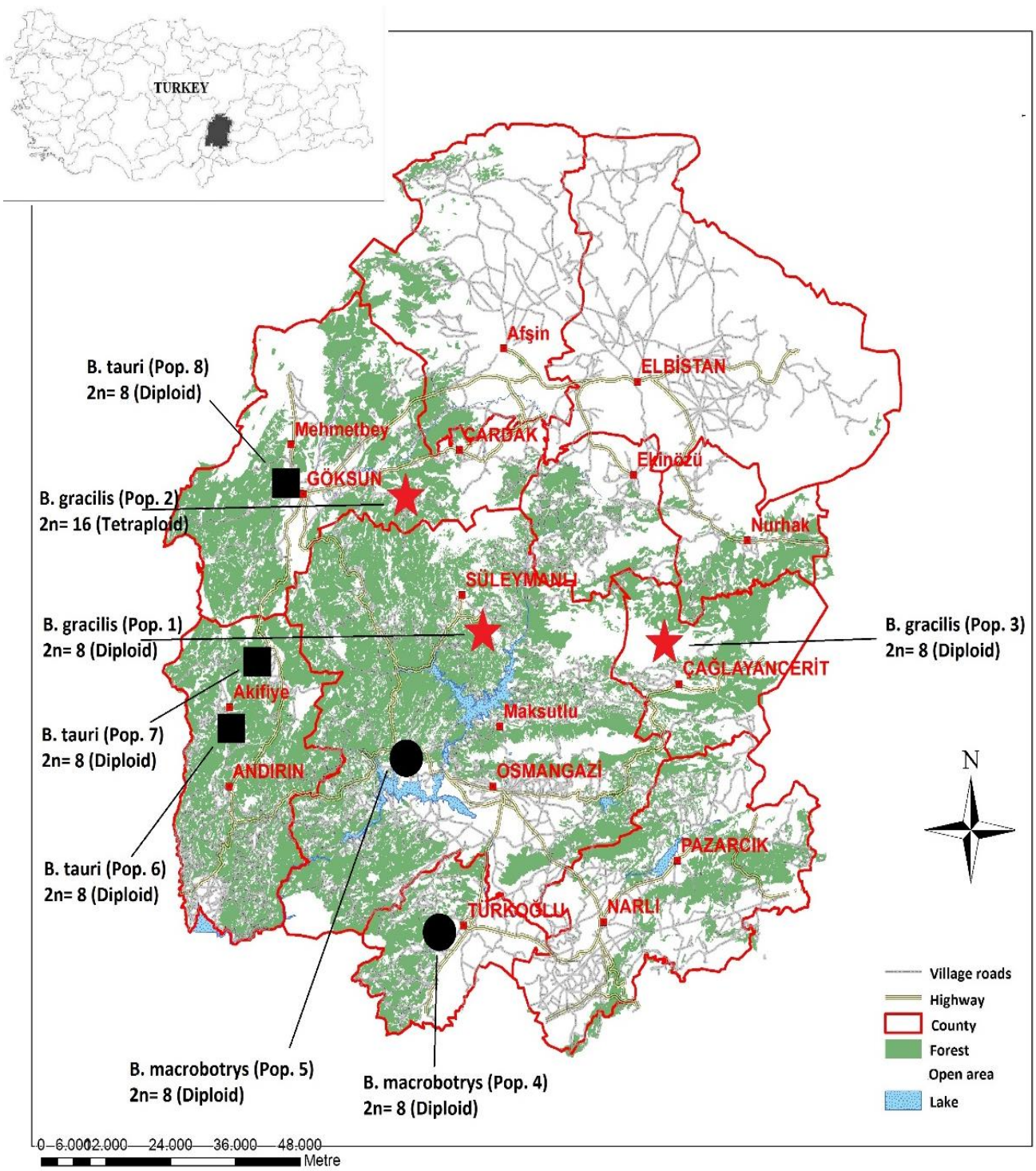

Fig. 1. Chromosome number of studied *Bellevalia* species in the research area, Kahramanmaraş (Turkey).

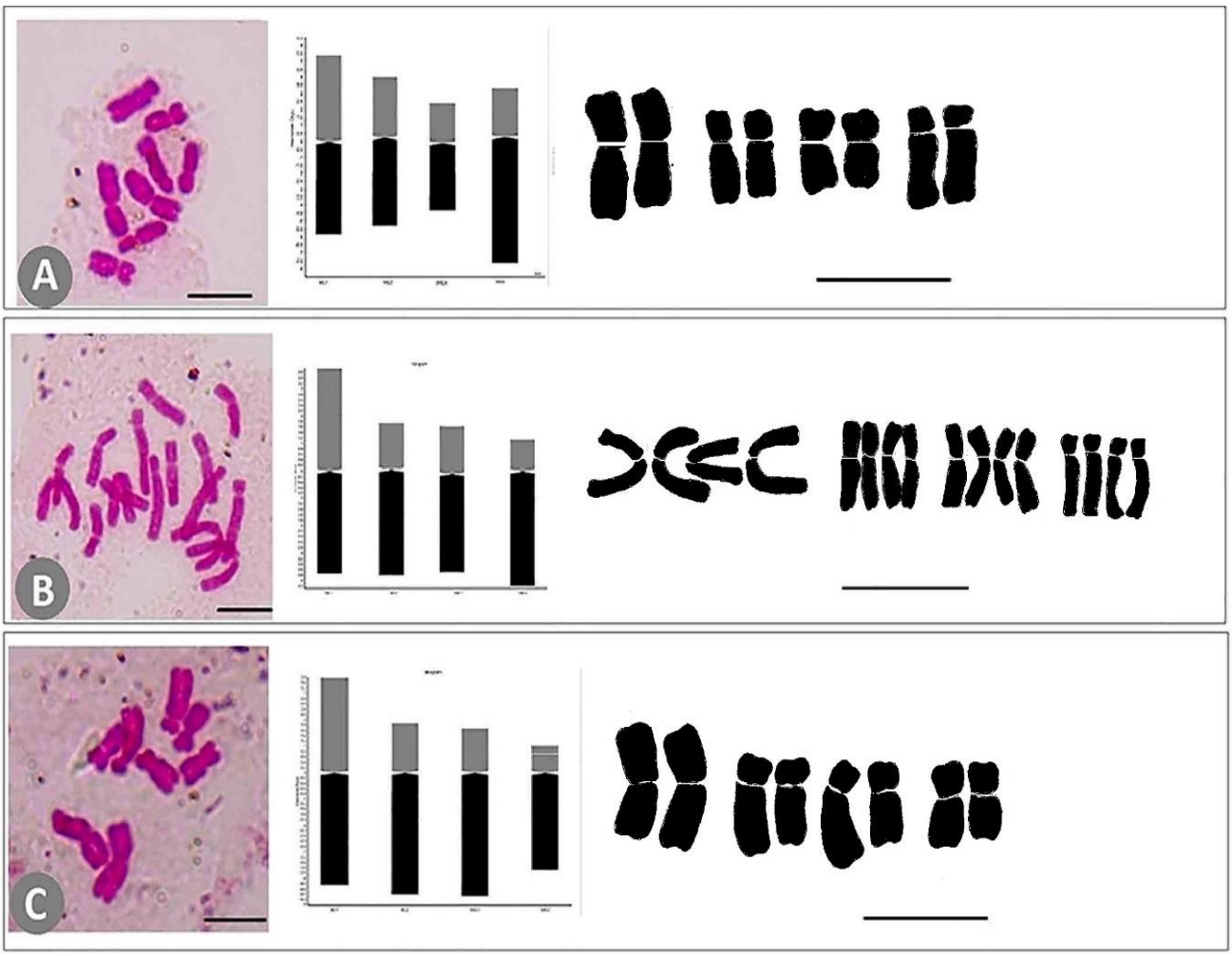

Fig. 2. Metaphase chromosomes in root tip cells and idiograms of *B. gracilis* in three populations (A: Population 1; B: Population 2; C: Population 3) (Scale bars: 10 µm).

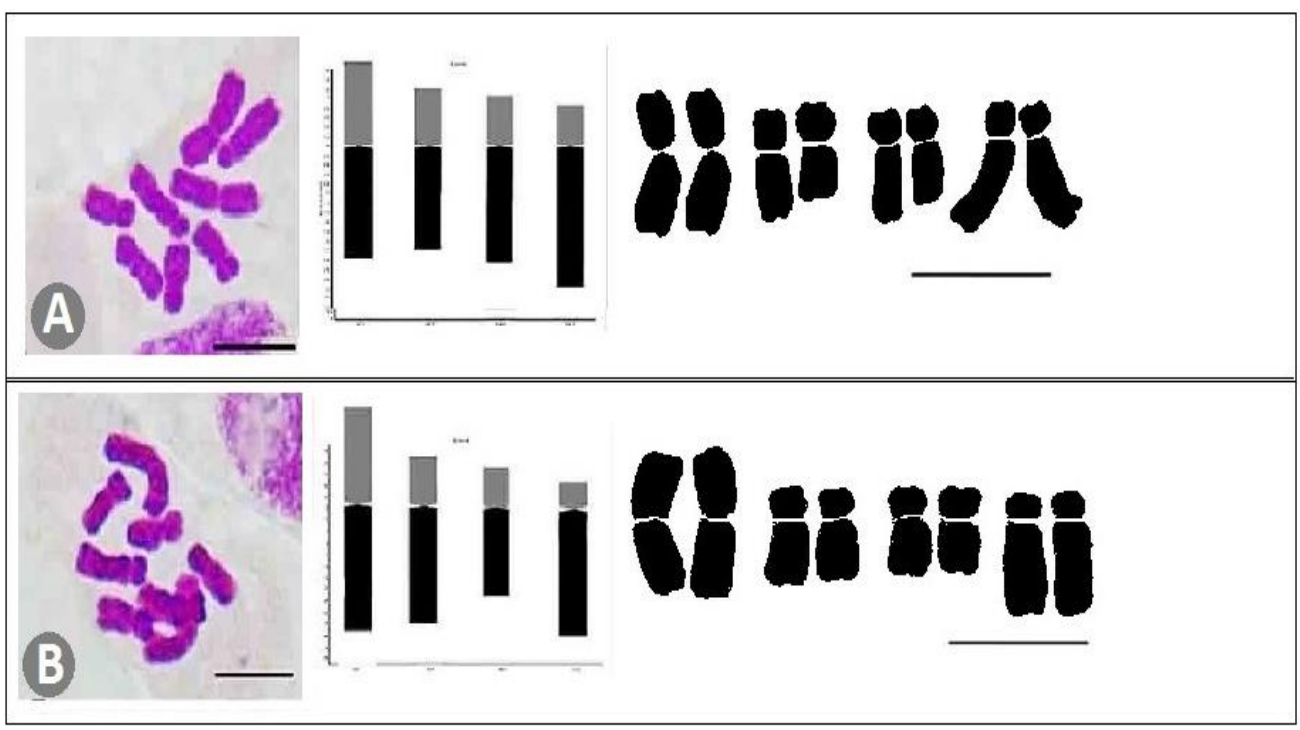

Fig. 3. Metaphase chromosomes in root tip cells and idiograms of *B. macrobotrys* in two populations (A: Population 4; B: Population 5) (Scale bars: 10 µm).

Table 2. Chromosomal comparison of the eight populations of *Bellevalia* species.

Taxa	Collection number	2*n*	Chromosome lenght ranges (µm)	Total haploid chromosome length (µm)	Intrachromosomal asymmetrys index (M_{CA})	Interchromosomal asymmetry index (CV_{CL})	Karyotype formula
B. gracilis	100251 (Pop. 1)	8	5.03 to 7.85	22.94	27.01	16.38	2m + 4sm+ 2st
	100247 (Pop. 2)	16	7.28 to 14.07	35.91	31.80	21.35	4m + 8sm+ 4st
	100250 (Pop. 3)	8	4.76 to 7.47	21.75	23.85	16.63	2m + 4sm+ 2st
B. macrobotrys	100244 (Pop. 4)	8	2.96 to 4.92	15.76	34.55	18.88	2m + 4sm + 2st
	100245 (Pop. 5)	8	2.98 to 5.15	16.34	29.59	21.91	2m + 4sm + 2st
B. tauri	100238 (Pop. 6)	8	4.27 to 9.64	27.82	38.60	26.34	1m + $1m^{SAT}$ + 4sm + 2st
	100241 (Pop. 7)	8	2.59 to 4.75	14.68	25.66	23.01	2m + 4sm+ 2st
	100240 (Pop. 8)	8	4.25 to 6.31	21.12	30.93	16.18	1m + $1m^{SAT}$ + 4sm+ 2st

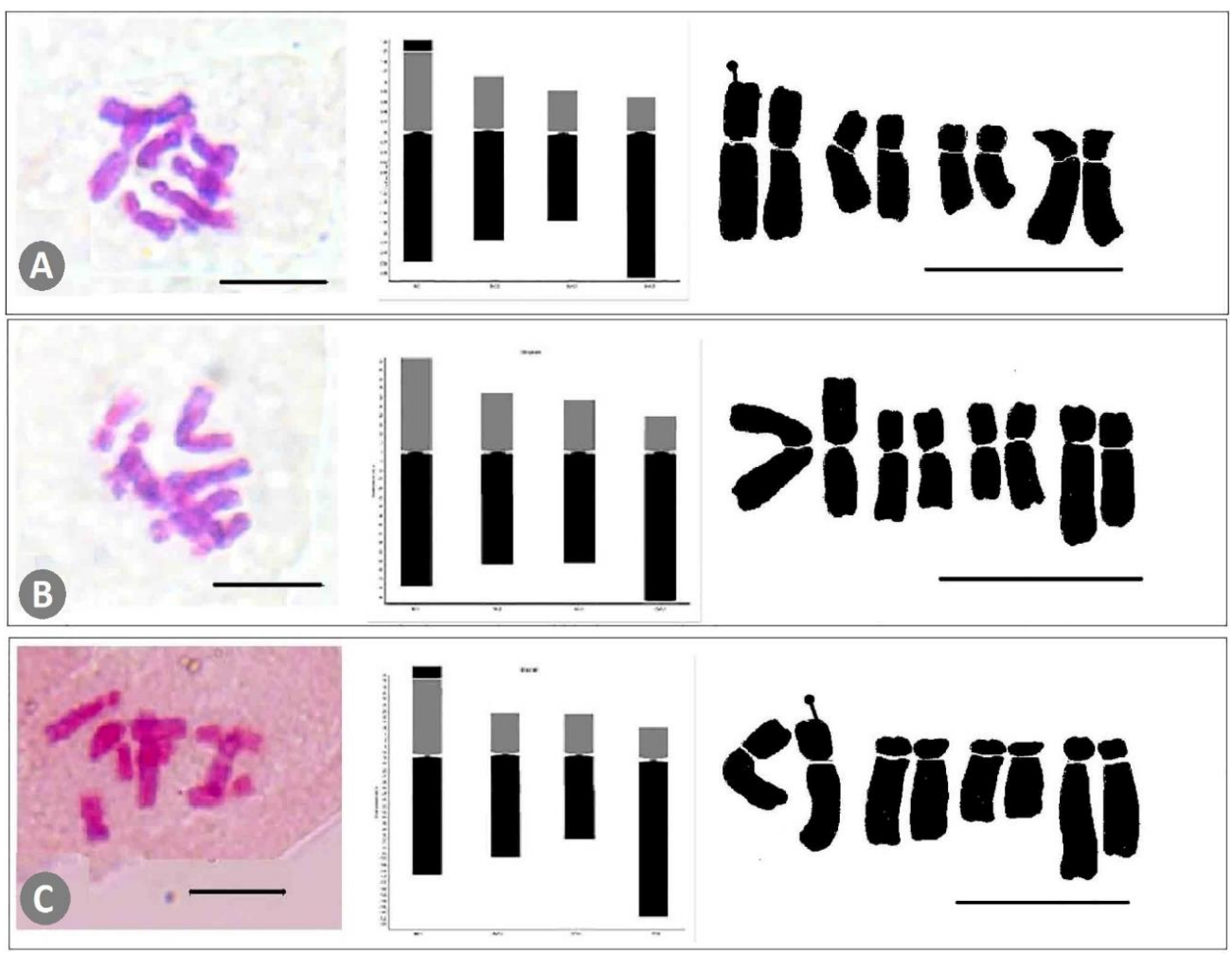

Fig. 4. Metaphase chromosomes in root tip cells and idiograms of *B. tauri* in three populations (A: Population 6; B: Population 7; C: Population 8). (Scale bars: 10 µm).

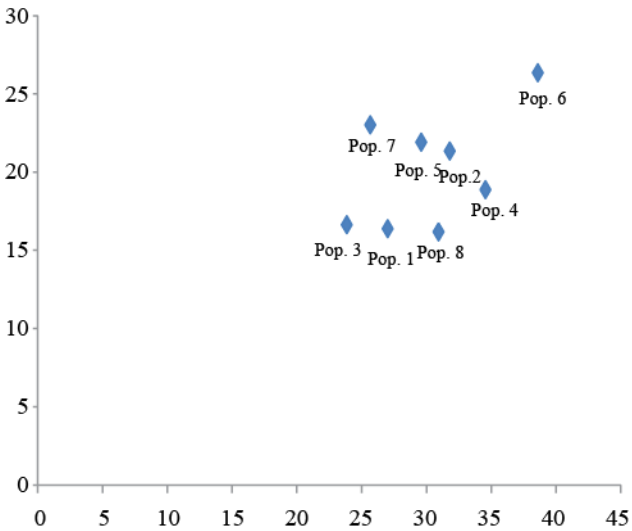

Fig. 5. Scatter plot of interchromosomal and intrachromosomal asymmetryis (CVCL vs. MCA) of populations of 3 *Bellevalia* species: *B. gracilis* (Pop. 1-3), *B. macrobotrys* (Pop. 4-5), *B. tauri* (Pop. 6-8).

***B. macrobotrys* Boiss.**

The chromosome number counted 2*n* = 8. The karyotype consists of 1 metacentric, 2 submetacentric and 1 subtelocentric chromosome pairs. No satellite was observed (Fig. 3). The intrachromosomal asymmetry index (M_{CA}) varies from 29.59 to 34.55. The interchromosomal asymmetry index (CV_{CL}) varies from 18.8 to 21.96 (Table 2). The karyotype is 3A.

***B. tauri* Feinbrun**

The chromosome number counted 2*n* = 8 in all examined populations. The karyotype consists of 1 metacentric, 2 submetacentric and 1 subtelocentric chromosome pairs. Satellites were observed (Fig. 4). The intrachromosomal asymmetry index (M_{CA}) varies from 25.66 to 48.58. The interchromosomal asymmetry index (CV_{CL}) from to 16.18 to 26.34 (Table 2). The karyotype is 3B.

Discussion

The number, size and shape of chromosome can be used to characterize the karyotype of plants (Jahan *et al*., 2014). The genus *Bellevalia* is characterized by a basic chromosome number *x* = 4, most representatives of the genus have a set 2*n* = 2*x* = 8 chromosomes in the diploid complement. It is evident from earlier studies that the chromosome complement in *Bellevalia* is more or less stable, having a "typical" karyotype occurring in almost all species studied (see e.g. Feinbrun, 1940; Levan ***et al.,*** 1964; Bothmer & Wendelbo, 1981; Özhatay *et al*., 1991; Johnson & Brandham, 1997). The haploid karyotype sets can be described as follows: Pair no.1: a metacentric (m) chromosome, this is the longest chromosomes in the complement. Pairs no: 2-3: two submetacentric (sm) chromosomes of about equal size. Pair no. 4: a subtelocentric (st) chromosome with a length of ca. 10-14 µm. Small satellites are often but not always found on chromosome pair no. 1 and /or on the long arm of the chromosome pairs 2-3.

In this investigation *B. macrobotrys*, *B. gracilis* and *B.tauri* have been examined from eight populations. In this studies, the chromosome number and the karyotype formulae of *B. gracilis* from three populations were counted as 2*n* = 2*x* = 8 = 2m + 4sm + 2st (diploid) in two populations, 2*n*= 4*x*= 16 = 4m + 8sm + 4st (tetraploid) in a population. Özhatay *et al*. (1991) determined the chromosome number of the species as 2*n*= 2*x*= 8 + 0–3B. The chromosome number of the species was recorded as 2*n*= 2*x*= 8+3B (diploid) (Johnson & Brandham, 1997) and 2*n* = 2*x*= 16 (tetraploid) (Mirici & Arslan, 1994). The chromosome number of *B. macrobotrys* from two populations is 2*n* = 2*x*= 8 (diploid), the first record for the species. The chromosome number of *B. tauri* from three populations are 2*n* = 2*x* = 8 (diploid), while Bothmer & Wendelbo (1981); Özhatay & Johnson (1996); Johnson & Brandham (1997) reported its chromosome number as 2n = 4x = 16 (tetraploid) (Fig. 4).

The karyological investigation from different populations exhibited mostly the same karyotype formulae and chromosome numbers of *Bellevalia* species. Karyotype asymmetry indices have been widely used to make assumptions about the mechanism of chromosomal evolution in plants (Pazsko, 2006). Karyotype asymmetry was based on different methods. According to the Stebbins system, the karyotypes of the studied species are classified into two groups, namely 3A and 3B. Species from 3A group include *B. gracilis* and *B. macrobotrys*. Group 3B includes *B. tauri*. *Bellevalia tauri, B. macrobotrys, B. gracilis* showed high levels of karyotype asymmetry (Fig. 5). The interchromosomal asymmetry index average (CVCL) of *B. tauri* is 26.34 and the species have the most asymmetrical karyotypes. The asymmetry index, based on M_{CA} values, showed up the highest asymmetry of the karyotype for all *Bellavalia* taxa and this is separated remarkably in relation to chromosomal evolutionary processes. Mostly *Bellevalia* species are diploid with a chromosome number 2*n*= 2*x*= 8. However, some studied populations also showed polyploidy. *B. gracilis* has only tetraploid population in the studied populations. Polyploidy is one of the main evolutionary mechanisms promoting genetic diversity and speciation in plants (Stebbins, 1950; Grant, 1971).

Acknowledgements

This study was part of Serpil Demirci's Ph.D. thesis is titled "Pharmaceutical Botanical Studies on the family Hyacinthaceae (Kahramanmaraş)". Financially supported by Scientific Project Research Unit of İstanbul University (BAP) (Project No: 26188). Bulbs for karyological studies planted in the Geophyte Garden in the Yalova Atatürk Central Horticultural Research Institute.

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(Received for publication 2 May 2016)