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Karyological Studies in Two Natural Populations of *Boissiera squarossa*

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ABSTRACT

Boissiera squarossa is an annual, diploid (2n = 2x = 14) grass species native to central and southwest Asia and can be used as a genetic resource for desirable genes in cereal breeding programs. The karyotype of this species was studied in two natural populations from northwest and center of Iran using aceto-iron-hematoxilin staining method. Chromosome characteristics including long-arm and short-arms and chromosome lengths, arm ratio index and relative chromosome lengths were measured using Micro-measure software. Results revealed that *B. squarossa* has a symmetric karyotype with six pairs of metacentric and one pair of sub-metacentric chromosomes. This species has only one SAT chromosome. The nucloelar organizing region and the satellite are located on the short arm of chromosome 5.

Key Words: *Boissiera squarossa;* Bromeae; Iran; Karyotype

INTRODUCTION

Many wild annual grasses belong to a highly important gene pool for cereal breeding. They all possess a tremendous richness of genes and gene complexes useful in agricultural research and breeding. *Boissiera squarossa* is an annual, diploid (2n = 2x = 14) grass species native to mesophytic to xerophytic regions in open habitats and dry stony soils of central, western and southwest Asia (Clayton *et al.,* 2006). This species belongs to Poaceae family, Pooideae subfamily and *Bromeae* tribe. It is a bisexual plant with hermaphrodite florets. Inflorescence is paniculate disarticulating above the glumes and not disarticulating between the florets (Watson & Dallwitz, 1994). It is important as a native pasture species and can be used as a genetic resource in cereal breeding programs. However, no published report could be found on detailed karyotype analysis of chromosomes in *B. squarossa*. In the present studies an attempt was made to develop detailed karyotypes of this species and compare its caryological characters with *Bromus* species in the same tribe.

MATERIALS AND METHODS

This study was conducted at cytogenetic laboratory of Mohaghegh Ardabili University, Iran in 2006. Seeds of two natural populations of *B. squarossa,* collected from East Azarbayjan and Esfahan provinces, northwest and central regions of Iran, were germinated on moist blotting paper and the root tips were pretreated in 0.05% solution of colchicine for 2.5 h at room temperature. Staining method (Asghari-Zakaria *et al.,* 2002) was used for preparation of metaphase spreads. Chromosome measurements including long arm, short arm, total length of chromosome set, arm ratio index and relative chromosome length were made from 15 enlarged well-spread metaphase cells of each population, using Micro-measure software developed by the Department of Biology, Colorado State University, USA (http://www.colostate.edu/Depts/Biology/Micromeasure). The nomenclature of chromosomes was followed as described by Levan *et al.* (1964), and chromosomes were named as 1, 2, 3, 4, 5, 6 and 7 in descending order of length.

RESULTS AND DISCUSSION

Mitotic chromosomes of the two *B. squarossa* populations are shown in Fig. 1 and Karyotypic characters of the seven mitotic chromosomes for each population are shown in Table I. There were minor differences for karyological characters between the two populations of this species. The chromosome lengths in population 2 were greater than those in population 1. This may be from different condensation of chromosomes at mitosis in these two populations. Hence, the arm ratio indices and relative lengths of chromosomes were mostly similar (Table I). The analysis of karyotype showed that *B. squarossa* had 2n = 2x = 14 chromosomes (Fig. 1), which was in agreement with Watson and Dallwitz (1994). Chromosome length in *B. squarossa* ranged from 3.95 µm in chromosome 7 to 4.91 µm in chromosome 1. On the other hand, arm ratio index values ranged from 1.14 in chromosome 2 to 1.70 in chromosome 6 (Table I). Based on arm ratio indices the karyotype of this species consisted of six pairs of metacentric and one pair of sub-metacentric chromosomes. The ratio between the largest and the smallest chromosome was 1.24. Since *B. squarossa* has chromosomes with median centromers and mostly equal arms, it showed a symmetric karyotype and placed in 1A category of Stebbins

Table I. Karyotypic characteristics of seven mitotic chromosomes in *B. squarossa*

Population	Character	Chromosome						
	(µm)	1	2	3	4	5	6	7
1	Length	4.56 ± 0.20	4.48 ± 0.22	4.21 ± 0.18	4.08 ± 0.21	4.00 ± 0.16	3.78 ± 0.13	3.78 ± 0.16
	RL	15.78 ± 0.24	15.42 ± 0.21	14.50 ± 0.21	14.12 ± 0.28	13.91 ± 0.27	13.15 ± 0.19	13.12 ± 0.32
	L	2.52 ± 0.13	2.38 ± 0.11	2.40 ± 0.10	2.23 ± 0.14	2.26 ± 0.10	2.37 ± 0.09	2.19 ± 0.10
	S	2.05 ± 0.08	2.09 ± 0.11	1.81 ± 0.09	1.85 ± 0.08	1.75 ± 0.07	1.41 ± 0.04	1.58 ± 0.07
	L/S	1.23 ± 0.04	1.14 ± 0.02	1.32 ± 0.04	1.22 ± 0.04	1.29 ± 0.03	1.68 ± 0.05	1.39 ± 0.04
2	Length	5.25 ± 0.12	5.04 ± 0.12	4.85 ± 0.10	4.77 ± 0.12	4.75 ± 0.14	4.32 ± 0.12	4.11 ± 0.12
	RL	15.85 ± 0.14	15.25 ± 0.13	14.66 ± 0.14	14.43 ± 0.21	14.33 ± 0.21	13.06 ± 0.20	12.42 ± 0.22
	L	2.81 ± 0.08	2.68 ± 0.07	2.62 ± 0.06	2.77 ± 0.07	2.58 ± 0.07	2.72 ± 0.07	2.34 ± 0.08
	S	2.44 ± 0.05	2.37 ± 0.06	2.22 ± 0.05	2.01 ± 0.05	2.16 ± 0.07	1.60 ± 0.06	1.78 ± 0.05
	L/S	1.15 ± 0.02	1.13 ± 0.03	1.18 ± 0.02	1.38 ± 0.03	1.20 ± 0.02	1.72 ± 0.05	1.32 ± 0.04
Mean	Length	4.91 ± 0.16	4.76 ± 0.17	4.58 ± 0.14	4.49 ± 0.16	4.38 ± 0.15	4.05 ± 0.14	3.95 ± 0.12
	RL	15.80 ± 0.19	15.30 ± 0.17	14.55 ± 0.18	14.40 ± 0.24	14.10 ± 0.24	13.00 ± 0.26	12.70 ± 0.21
	L	2.66 ± 0.10	2.53 ± 0.09	2.43 ± 0.08	2.58 ± 0.11	2.42 ± 0.08	2.52 ± 0.08	2.27 ± 0.08
	S	2.24 ± 0.07	2.23 ± 0.09	2.04 ± 0.07	1.91 ± 0.06	1.96 ± 0.07	1.53 ± 0.06	1.68 ± 0.05
	L/S	1.19 ± 0.03	1.14 ± 0.02	1.20 ± 0.03	1.35 ± 0.04	1.25 ± 0.02	1.66 ± 0.05	1.35 ± 0.04
	type	m	m	m	m	m	sm	m

RL=Chromosome relative length (%), L=Long arm length, S=Short arm length, L/S=Arm ratio index, m= metacentric, and sm=submetacentric, ± (mean ± standard error)

Table II. Comparative characteristics of two *B. squarossa* populations and *Bromus sterilis*

Population	KF	TCL	CL	DRL %	TF %	S %	R	SAT	ST
Boissiera squarossa (1)	6m+1sm	28.89	4.13	2.6	43.58	13.1	1.21	0.57 ± 0.05	1A
B. squarossa (2)	6m+1sm	33.09	4.72	3.4	41.07	12.4	1.28	0.79 ± 0.06	1A
mean	6m+1sm	31.12	4.42	2.91	42.33	12.75	1.24	0.68 ± 0.06	1A
Bromus sterilis	7m	30.8	4.4	2.93	44.86	11.7	1.49	-	1A

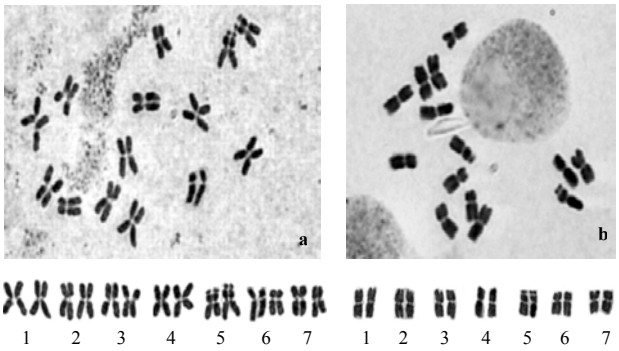
Karyotype formulae (KF), Total length of the haploid complement (µm) (TCL), Mean chromosome length (CL), Difference between relative length of the longest and the shortest chromosomes (DRL), Total form percentage (TF), Relative length of the shortest chromosome (S%), Ratio of the longest to the shortest chromosome (R), Satellite length (µm) (SAT), and Stebbin's asymmetry category (ST).

(1971) asymmetry categories. Total length of chromosomes in this species was 62 ± 1.06 µm (Table I). *B. squarossa* has only one satellite chromosome. The nucloelar organizing region and the satellite (with length of 0.68 µm) are located on the short arm of chromosome 5.

The Bromeae tribe of Poaceae family consisted of *Bromus, Littledalea* and *Boissiera* genera. The chromosome morphology and karyological characters of *Bromus* species in this tribe have been studied previously (Lövkvist & Hultgård, 1999; Tuna *et al.,* 2001; Oja & Laarmann, 2002; Martinello & Schifino-Wittmann, 2003; Sheidai & Fadaei, 2005; Mirzaie-Nodoushan *et al.,* 2006) in which the chromosome numbers varied from 2n = 14 to 2n = 84. The available literature dealing with cytogenetics of *Bromus* indicates the importance of cytological studies for understanding the evolution of this genus. Such karyological studies are also needed for considering the evolution and phylogenetic relationships within and between the two other genera in this tribe.

Comparing of *B. squarossa* with *Bromus sterilis* (2n = 2x = 14) for some karyological characters (Table II) showed that total length of the haploid complement, mean chromosome length, difference between maximum and minimum relative length of chromosomes, total form percentage, relative length of the shortest chromosome, ratio of the longest to the shortest chromosome and Stebbin's asymmetry category of these two species are approximately equal, indicating the close evolutionary relationships

Fig. 1. Metaphase chromosomes of *B. squarossa* stained with aceto-iron- hematoxilin, (a) Population 1 (from northwest of Iran) and (b) population 2 (from central region of Iran)



between these two species. However, the ploidy levels in *Bromus* vary from diploid (*Bromus sterilis*, 2n = 14) to didecaploid (*Bromus tomentellus*, 2n = 84), the *Boissiera* is mono-species genus. In this study the two populations of *Boissiera squarrosa* had 14 chromosomes, while, according to Watson and Dallwitz (1994) two cytotypes of this species with 2n = 14 and 2n = 28 chromosomes exists. The results of present study are useful in phylogenetic understanding and taxonomic studies of *Boissiera squarrosa* species.

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