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Full Length Research Paper

A comparative study of chromosome morphology among some accessions of *Aegilops crassa*

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In this study karyotype and chromosome characteristics of the nine accessions of *Aegilops crassa* species obtained from gene bank of Seed and Plant Improvement Research Institute (SPII) of Iran and one accession collected by authors were inspected. Aceto-iron-hematoxilin staining method was used to stain chromosomes. For each accession, chromosome characteristics including long and short arms, chromosome lengths, arm ratio index and relative chromosome lengths were measured using micro measure 3.3 software. Results revealed that all of the studied accessions were tetraploid (2n = 4x = 28) and consisted of 13 pairs of metacentric and one pair of submetacentric chromosomes, of which two pairs were satellite chromosomes. Karyotype formula for these accessions were 13 m + 1 sm. Arm ratio index value of chromosomes ranged from 1.11 in chromosome number 6 to 1.77 in chromosome number 12. The B chromosomes were not seen in any of the accessions. Karyological characteristics of these accessions were similar to each other. However, some differences were observed between the accessions in some chromosome characteristics.

Key words: *Aegilops Crassa*, karyotype, aceto-iron-hematoxilin staining, satellite chromosome.

INTRODUCTION

Aegilops crassa has large degree of morphological variation and a wide area of distribution including Turkey, Israel, Lebanon, Syria, Iraq, Iran, Afghanistan, Transcaucasia, Southern Turkmenistan and the Pamirs and Altai mountains (Witcombe, 1983; Kimber and Feldman, 1987; Slageren, 1994). *A. crassa* belongs to vertebrata section of *Aegilops* and has two cytotypes, hexaploid (2n = 6x = 42) with $X^{cr}D^{cr1}D^{cr2}$ genomes and tetraploid (2n = 4x = 28) with $X^{cr}D^{cr1}$ genomes (Zhukovsky, 1928; Witcombe, 1983; Kimber and Feldman, 1987).

Because of importance of genus *Aegilops* as a wild genetic source of wheat, some cytogenetic analysis reported (Chennaveeraiah, 1960; Badaeva et al., 1998, 2001) and indicated that all *A. crassa* chromosomes can be identified by their morphology and C-banding patterns. Cytogenetical studies have been carried out on *A. crassa* but a comparative study of chromosome morphology among accessions in *A. crassa* is not well documented. Hence, cytogenetic study among its accessions could cover its slight cytogenetic data. In the present work, karyotype characteristics between 10 accessions of *A. crassa* were analysed by Aceto-Iron-hematoxilin staining method.

MATERIAL AND METHODS

The study was conducted at laboratory of cytogenetic of Agriculture Faculty of Guilan University, Iran. Nine accessions of *A. crassa* species obtained from gene bank of Seed and Plant Improvement Research Institute (SPII) of Iran and one accession collected by authors were used in this study (Table 1). Seeds were germinated on moist filter paper in petri dishes, kept at room temperature 20 - 25°C in the dark. Actively growing roots of about 1 - 1.5 cm in length were excised, pretreated with a 0.002 M aqueous solution of 8-hydroxyquinoline for 4 h at 4°C, washed in distilled water for 10 - 15 min and fixed in Lewitsky fixative at 4°C For 30 - 36 h. The fixative was prepared by mixing equal parts (in volume) of 1% chromic acid and 4% formaldehyde (10% formalin) just before using (Agayev, 2002).

The root tips were washed and hydrolyzed in 1 N NaOH for 7 min at 60°C and stained with aceto-iron-hematoxylin 4% for 12 h at

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Table 1. List of studied accessions of *A. crassa* for karyotype analysis.

Name	Code	Region	Province
A	TN-01-757	Dehloran 2	Ilam
B	TN-01-778	Dehloran1	Ilam
C	TN-01-944	Kermanshah 1	Kermanshah
D	TN-01-947	Kermanshah 2	Kermanshah
E	TN-01-976	Kermanshah 3	Kermanshah
F	TN-01-1107	Kermanshah 4	Kermanshah
G	TN-01-1166	Kermanshah 5	Kermanshah
H	TN-01-1511	Kermanshah 6	Kermanshah
I	so114	Bojnurd	North khorasan
J	New accession collected by authors	Malekan region (north west of Iran)	East Azerbaijan

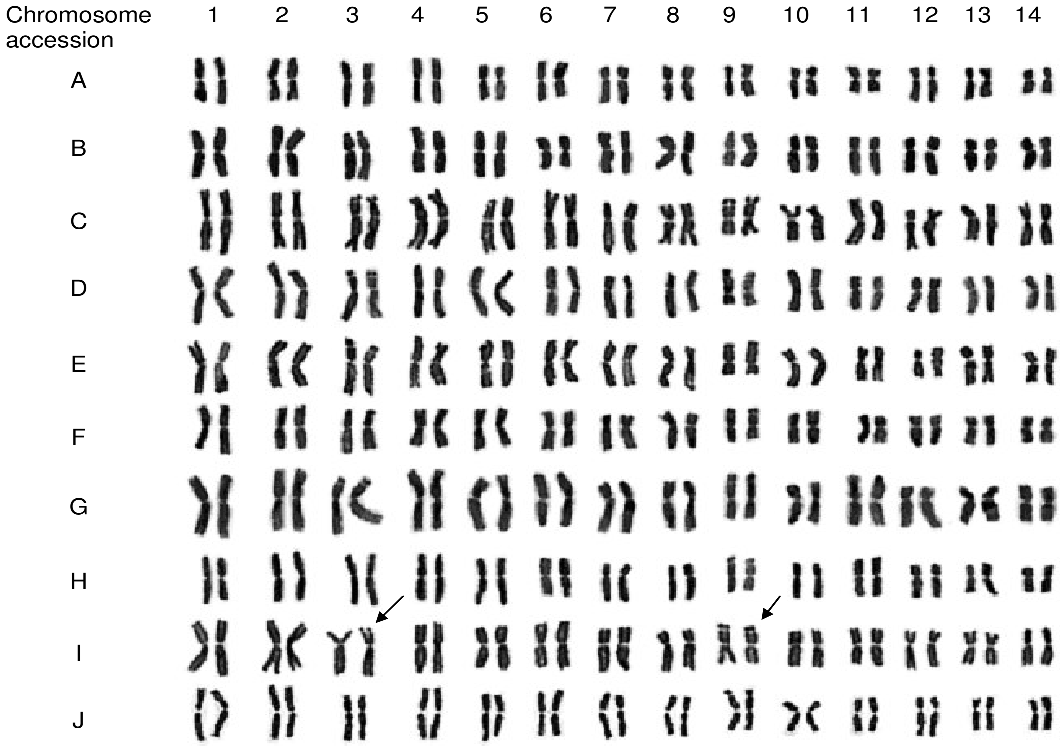

Figure 1. Karyotype of the 10 accessions of *A. crassa.* Arrow shows the satellite chromosome.

25°C. Stained roots were washed in distilled water for at least 10 min, then 1 - 1.2 mm the root of tips were cut and macerated in cytase for 1.5 h. Each of the root tips was carefully transferred on a drop of 45% acetic acid on a slide for 3 - 5 min, covered by cover slip and gently squashed. A number of 10 cells with well spread chromosomes were studied and photographed.

Chromosome measurements including long arm, short arm, chromosome lengths, arm ratio index and relative chromosome length were made with micro measure 3.3 software (Reeves, 2001). Chromosomes were designated according to Levan et al. (1964) and chromosomes were named as 1, 2, 3…13 and 14 in descending order of length. karyotype asymmetry was calculated according to Stebbins (1971). Statistical analysis performed by SPSS version 14 software.

RESULTS AND DISCUSSION

All the 10 accessions of *A. crassa* showed a tetraploid somatic chromosome number of 2n = 4x = 28 and this generally agree with the previously reported karyotype (Badaeva et al., 1998; 2001). Size of long and short arms, relative lengths, L/S ratios and total chromosome length for each accessions are presented in Table 2. Means of karyotypic characters of fourteen mitotic chromosomes in 10 accessions are available in Table 3. These accessions had 13 m + 1 sm karyotype formula (Table 3). Karyotype for 10 accessions of *A. crassa* is

Table 2. The karyotypic characters of fourteen mitotic chromosomes in 10 accessions of *Ae. crassa*.

Ch.	Ac.	Character					Ch.	Ac.	Character				
		length	RL	L	S	L/S			length	RL	L	S	L/S
Chromosome 1	A	9.99±0.48	9.05±0.17	5.49±0.26	4.5±0.26	1.24±0.06	Chromosome 8	A	7.27±0.56	6.5±0.22	4.49±0.42	2.79±0.17	1.6±0.1
	B	8.59±0.26	9.08±0.24	4.8±0.18	3.8±0.13	1.27±0.05		B	6.79±0.15	7.17±0.12	4.19±0.11	2.6±0.1	1.64±0.08
	C	9.39±0.47	8.68±0.17	5.2±0.28	4.18±0.21	1.25±0.04		C	7.17±0.19	6.69±0.16	4.36±0.13	2.81±0.1	1.57±0.06
	D	9.75±0.56	9.05±0.23	5.45±0.29	4.3±0.3	1.29±0.06		D	7.53±0.54	6.93±0.13	4.69±0.31	2.84±0.26	1.7±0.09
	E	9.05±0.31	8.69±0.15	5.09±0.19	3.96±0.14	1.29±0.03		E	7.19±0.25	6.92±0.16	4.47±0.19	2.72±0.11	1.66±0.08
	F	8.56±0.27	9.07±0.24	4.79±0.2	3.77±0.11	1.27±0.05		F	6.72±0.17	7.12±0.13	4.13±0.12	2.59±0.11	1.62±0.08
	G	10.7±0.21	8.96±0.13	5.92±0.18	4.75±0.08	1.25±0.04		G	8.15±0.27	6.85±0.23	4.77±0.14	3.38±0.22	1.46±0.1
	H	9.3±0.34	8.74±0.19	4.84±0.16	4.45±0.19	1.09±0.03		H	7.36±0.26	6.91±0.11	4.64±0.17	2.72±0.12	1.71±0.06
	I	11.4±0.31	8.9±0.11	6.43±0.2	4.98±0.14	1.29±0.03		I	8.82±0.19	6.89±0.09	5.79±0.13	3.03±0.08	1.92±0.05
	J	11.9±0.16	8.89±0.09	6.45±0.15	5.41±0.12	1.2±0.04		J	9.2±0.14	6.9±0.08	5.4±0.14	3.8±0.12	1.44±0.07
Chromosome 2	A	9.91±0.69	8.88±0.24	5.22±0.31	4.68±0.4	1.17±0.09	Chromosome 9	A	7.62±0.42	6.92±0.26	4.05±0.29	3.57±0.16	1.13±0.06
	B	8.29±0.18	8.77±0.19	4.4±0.12	3.89±0.07	1.13±0.02		B	5.77±0.18	6.12±0.26	3.21±0.13	2.56±0.08	1.26±0.04
	C	9.15±0.34	8.5±0.14	4.85±0.16	4.3±0.19	1.13±0.03		C	6.87±0.26	6.41±0.24	3.96±0.17	2.91±0.11	1.36±0.05
	D	9.59±0.81	8.76±0.13	5.08±0.42	4.5±0.41	1.14±0.04		D	6.42±0.59	5.84±0.15	3.82±0.36	2.59±0.25	1.49±0.07
	E	8.96±0.32	8.61±0.22	4.65±0.16	4.31±0.17	1.08±0.02		E	6.18±0.17	5.95±0.13	3.53±0.07	2.65±0.12	1.35±0.05
	F	8.32±0.19	8.84±0.22	4.43±0.14	3.89±0.07	1.14±0.03		F	5.84±0.2	6.22±0.27	3.24±0.14	2.6±0.07	1.25±0.05
	G	10.4±0.28	8.68±0.15	5.55±0.17	4.81±0.14	1.16±0.03		G	7.78±0.35	6.49±0.19	4.39±0.24	3.38±0.12	1.29±0.03
	H	9.68±0.31	9.09±0.09	5.02±0.13	4.66±0.18	1.08±0.02		H	6.85±0.34	6.41±0.15	3.86±0.22	2.99±0.13	1.29±0.04
	I	11.5±0.33	8.99±0.14	6.11±0.2	5.41±0.16	1.13±0.03		I	9.38±0.39	7.3±0.18	4.97±0.21	4.41±0.2	1.13±0.03
	J	11.8±0.3	8.82±0.17	6.02±0.14	5.73±0.16	1.05±0.01		J	9.48±0.31	7.11±0.32	5.06±0.16	4.42±0.16	1.15±0.03
Chromosome 3	A	8.94±0.66	7.98±0.28	5.2±0.35	3.74±0.32	1.42±0.06	Chromosome 10	A	7.36±0.47	6.6±0.09	4.2±0.27	3.16±0.21	1.33±0.03
	B	7.71±0.21	8.13±0.15	4.48±0.18	3.23±0.06	1.39±0.05		B	6.07±0.17	6.4±0.14	3.46±0.13	2.6±0.09	1.35±0.07
	C	9.2±0.26	8.57±0.13	5.54±0.19	3.67±0.1	1.51±0.05		C	6.61±0.16	6.19±0.22	3.93±0.1	2.68±0.1	1.48±0.06
	D	8.85±0.83	8.04±0.15	5.14±0.5	3.71±0.33	1.38±0.05		D	6.99±0.58	6.43±0.22	4.15±0.4	2.84±0.19	1.44±0.05
	E	8.97±0.37	8.64±0.3	5.4±0.25	3.58±0.16	1.52±0.07		E	6.81±0.21	6.55±0.15	4.33±0.17	2.48±0.09	1.76±0.08
	F	7.68±0.18	8.14±0.14	4.52±0.11	3.16±0.11	1.44±0.05		F	6±0.2	6.35±0.17	3.52±0.16	2.47±0.11	1.44±0.08
	G	9.43±0.38	7.88±0.17	5.69±0.23	3.74±0.18	1.54±0.05		G	7.39±0.17	6.21±0.17	4.46±0.09	2.93±0.11	1.54±0.06
	H	8.74±0.43	8.18±0.22	5.16±0.28	3.58±0.17	1.45±0.05		H	6.72±0.26	6.3±0.07	4.17±0.17	2.55±0.13	1.67±0.09
	I	10.5±0.39	8.22±0.21	6.43±0.24	4.1±0.18	1.58±0.05		I	8.25±0.3	6.44±0.17	4.85±0.17	3.4±0.16	1.44±0.05
	J	10.5±0.11	7.91±0.08	6.65±0.07	3.9±0.07	1.71±0.03		J	8.03±0.39	6.02±0.52	4.92±0.34	3.11±0.13	1.59±0.12
Chromosome 4	A	9±0.47	8.16±0.22	4.71±0.21	4.29±0.28	1.12±0.06	Chromosome 11	A	6.24±0.24	5.7±0.18	3.51±0.12	2.74±0.16	1.31±0.07
	B	7.55±0.25	7.98±0.22	3.99±0.14	3.56±0.12	1.12±0.02		B	5.69±0.12	6.01±0.14	3.13±0.06	2.55±0.1	1.25±0.06
	C	8.79±0.41	8.16±0.25	4.62±0.22	4.17±0.2	1.11±0.03		C	6.89±0.36	6.39±0.22	3.72±0.19	3.17±0.18	1.18±0.04
	D	8.82±0.7	8.08±0.11	4.68±0.33	4.14±0.38	1.15±0.05		D	6.73±0.4	6.24±0.17	3.67±0.25	3.06±0.17	1.2±0.05
	E	8.53±0.23	8.21±0.16	4.44±0.15	4.09±0.1	1.09±0.03		E	6.82±0.35	6.57±0.31	3.71±0.18	3.12±0.2	1.21±0.06
	F	7.55±0.25	8±0.2	3.98±0.16	3.57±0.1	1.11±0.03		F	5.56±0.12	5.9±0.11	3±0.06	2.56±0.1	1.19±0.04
	G	10.1±0.3	8.41±0.09	5.17±0.14	4.88±0.17	1.06±0.02		G	7.41±0.51	6.15±0.28	3.96±0.21	3.44±0.31	1.19±0.05
	H	8.67±0.41	8.11±0.15	4.69±0.26	3.98±0.17	1.18±0.04		H	6.88±0.37	6.43±0.18	3.73±0.17	3.15±0.21	1.2±0.04
	I	10.5±0.24	8.23±0.15	5.67±0.1	4.86±0.19	1.18±0.04		I	8.45±0.36	6.58±0.18	4.54±0.18	3.9±0.21	1.18±0.04
	J	11±0.38	8.27±0.27	5.85±0.18	5.17±0.26	1.14±0.05		J	7.89±0.21	5.92±0.14	4.3±0.12	3.59±0.11	1.2±0.03

Table 2. Contd.

Chromosome 5	A	8.71±0.62	7.79±0.24	5.05±0.38	3.66±0.26	1.38±0.04	Chromosome 12	A	7.23±0.31	6.6±0.22	4.7±0.24	2.53±0.08	1.86±0.06
	B	7.59±0.16	8.02±0.13	4.14±0.1	3.45±0.11	1.21±0.04		B	5.94±0.2	6.26±0.15	3.81±0.14	2.13±0.08	1.8±0.07
	C	8.56±0.36	7.94±0.17	4.92±0.27	3.64±0.12	1.35±0.05		C	6.21±0.26	5.76±0.08	3.97±0.24	2.25±0.1	1.79±0.12
	D	8.85±0.54	8.2±0.15	5±0.27	3.86±0.28	1.31±0.04		D	6.48±0.61	5.9±0.16	4.11±0.45	2.36±0.17	1.73±0.09
	E	8.06±0.24	7.75±0.13	4.43±0.18	3.63±0.11	1.23±0.05		E	5.71±0.18	5.51±0.16	3.57±0.16	2.14±0.05	1.68±0.08
	F	7.6±0.2	8.06±0.17	4.14±0.12	3.46±0.1	1.2±0.03		F	5.86±0.19	6.2±0.14	3.76±0.15	2.09±0.06	1.8±0.07
	G	9.61±0.2	8.06±0.11	5.56±0.19	4.05±0.11	1.38±0.07		G	6.8±0.3	5.71±0.23	4.45±0.25	2.35±0.07	1.9±0.09
	H	8.99±0.34	8.43±0.13	5.36±0.19	3.62±0.16	1.49±0.04		H	6.08±0.26	5.7±0.13	3.83±0.17	2.25±0.1	1.71±0.06
	I	9.62±0.38	7.51±0.22	5.51±0.27	4.11±0.16	1.35±0.05		I	7.39±0.27	5.8±0.26	4.43±0.13	2.95±0.19	1.54±0.07
	J	9.03±0.49	6.77±0.49	5.37±0.3	3.66±0.22	1.48±0.06		J	8.7±0.23	6.52±0.27	5.74±0.19	2.96±0.07	1.94±0.06
Chromosome 6	A	8.5±0.54	7.66±0.2	4.58±0.32	3.92±0.24	1.17±0.04	Chromosome 13	A	6.17±0.17	5.67±0.24	3.65±0.14	2.52±0.11	1.48±0.08
	B	6.74±0.18	7.12±0.13	3.59±0.12	3.15±0.07	1.14±0.02		B	5.67±0.23	5.99±0.2	3.36±0.15	2.31±0.09	1.46±0.06
	C	8.13±0.45	7.51±0.18	4.25±0.23	3.89±0.23	1.1±0.03		C	6.63±0.19	6.18±0.1	4.02±0.17	2.61±0.05	1.54±0.06
	D	8.33±0.61	7.66±0.13	4.36±0.34	3.97±0.28	1.1±0.03		D	6.45±0.43	5.96±0.17	3.86±0.25	2.58±0.22	1.53±0.07
	E	8.08±0.26	7.77±0.12	4.16±0.15	3.92±0.12	1.06±0.02		E	6.23±0.23	5.98±0.13	3.81±0.16	2.41±0.09	1.59±0.05
	F	6.83±0.18	7.24±0.13	3.63±0.12	3.2±0.08	1.13±0.03		F	5.57±0.2	5.91±0.18	3.26±0.14	2.31±0.07	1.42±0.05
	G	8.99±0.31	7.52±0.17	4.67±0.15	4.32±0.18	1.09±0.03		G	6.81±0.18	5.72±0.16	4.09±0.11	2.72±0.1	1.52±0.06
	H	7.84±0.37	7.34±0.17	4.14±0.18	3.69±0.2	1.13±0.03		H	5.8±0.3	5.42±0.11	3.4±0.2	2.4±0.12	1.42±0.04
	I	9.48±0.25	7.42±0.18	5.01±0.19	4.48±0.1	1.12±0.04		I	6.9±0.26	5.38±0.15	3.93±0.17	2.96±0.12	1.33±0.04
	J	10.2±0.22	7.61±0.26	5.2±0.11	4.95±0.13	1.05±0.02		J	7.69±0.13	5.76±0.08	4.59±0.14	3.09±0.06	1.49±0.06
Chromosome 7	A	7.96±0.63	7.08±0.21	4.81±0.39	3.14±0.26	1.55±0.06	Chromosome 14	A	6.8±0.35	6.18±0.18	3.87±0.17	2.93±0.21	1.35±0.05
	B	7.32±0.16	7.74±0.15	4.47±0.11	2.85±0.15	1.61±0.09		B	4.92±0.07	5.2±0.08	2.78±0.05	2.14±0.04	1.31±0.03
	C	8.06±0.33	7.47±0.11	5.18±0.25	2.87±0.12	1.81±0.08		C	6.01±0.35	5.56±0.16	3.48±0.19	2.53±0.17	1.39±0.04
	D	7.87±0.62	7.22±0.15	4.81±0.39	3.06±0.27	1.6±0.09		D	6.3±0.59	5.72±0.11	3.55±0.35	2.75±0.26	1.3±0.05
	E	7.66±0.3	7.36±0.18	4.66±0.15	3.01±0.21	1.6±0.1		E	5.71±0.16	5.5±0.12	3.31±0.15	2.4±0.05	1.38±0.07
	F	7.26±0.18	7.71±0.19	4.43±0.12	2.83±0.15	1.61±0.09		F	4.93±0.1	5.23±0.09	2.86±0.08	2.06±0.06	1.4±0.05
	G	8.96±0.19	7.54±0.19	5.74±0.13	3.23±0.12	1.8±0.08		G	6.96±0.44	5.81±0.29	3.96±0.23	2.99±0.25	1.36±0.09
	H	8.03±0.31	7.55±0.2	4.98±0.18	3.05±0.15	1.65±0.07		H	5.74±0.27	5.38±0.17	3.28±0.15	2.45±0.15	1.37±0.08
	I	9.16±0.23	7.16±0.12	5.5±0.18	3.66±0.22	1.56±0.12		I	7.77±0.18	6.07±0.09	4.36±0.15	3.42±0.12	1.29±0.07
	J	10.6±0.14	7.94±0.1	6.29±0.11	4.29±0.1	1.48±0.05		J	7.41±0.17	5.56±0.32	4.38±0.14	3.04±0.13	1.46±0.09

RL= chromosome relative length, L= Long arm length, S= Short arm length and L/S= Arm ratio Index.

presented in Figure 1. Arrangement of chromosomes in karyotype are based on mean of relative lengths of each chromosome in all accessions (Table 3).

Chromosomes number 3 and 9 are two chromosomes that consistently have satellite (Figure 1) and this is in agreement with previous studies (Chen-naveeraiah, 1960; Badaeva et al. 1998). Chromosome number 3 had a satellite in

Table 3. The means of karyotypic characters of fourteen mitotic chromosomes in *A. crasssa*.

Chr.	Type	Chromosome (µm)	Relative length (%)	Short arm (µm)	Long arm (µm)	Arm ratio
1	m	1.28±9.86	0.01±8.91	0.46±5.45	0.32±4.41	0.02±1.24
2	m	1.8±9.75	0.03±8.79	0.46±5.13	0.51±4.62	0.02±1.12
3*	m	1.88±9.06	0.01±8.17	0.71±5.42	0.37±3.64	0.03±1.49
4	m	1.51±9.05	0.07±8.16	0.4±4.78	0.46±4.27	0.02±1.13
5	m	1.47±8.66	0.24±7.85	0.57±4.95	0.31±3.71	0.02±1.34
6	m	1.35±8.31	0.07±7.49	0.42±4.36	0.31±3.95	0.01±1.11
7	m	1.23±8.29	0.01±7.48	0.51±5.09	0.33±3.2	0.07±1.63
8	m	0.94±7.62	0.01±6.89	0.43±4.69	0.22±2.93	0.06±1.63
9*	m	1.18±7.22	0.1±6.48	0.46±4.01	0.23±3.21	0.02±1.27
10	m	1.04±7.02	0.27±6.35	0.5±4.2	0.19±2.82	0.05±1.5
11	m	1.07±6.86	0.02±6.19	0.27±3.73	0.34±3.13	0.02±1.21
12	sm	0.92±6.64	0.07±6	0.53±4.24	0.12±2.4	0.06±1.77
13	m	0.61±6.39	0.01±5.8	0.28±3.8	0.13±2.59	0.04±1.48
14	m	0.96±6.25	0.1±5.62	0.34±3.58	0.26±2.67	0.04±1.36

*Satellite chromosome.

short arm, that is, in agreement with Badaeva et al. (1998) and chromosome number 9 had a satellite in the long arm, but Badaeva et al. (1998) reported that secondary constriction in this chromosome is located in the short arm.

Some differences were observed among accesssions, for example, length of the individual chromosomes and arm ratio, which could be due to variety of reasons such as methods of measurement or use of different accessions. Results revealed that all chromosomes could be distinguishable by their morphology characteristics and this is in agreement with Badaeva et al. (2001). In these accessions, chromosome modifications such as variation in chromosome number or B chromosome was not seen.

All the accessions showed the symmetric karyo-type and were placed in 1A category of stebbines (1971) asymmetry categories. This shows that *A. crassa* acessions can be considered as relatively primitive acessions. However, some polymorphisms were observed among 10 accessions for karyotype characters.

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