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Karyological Study of Four Species of Wheat Grass (*Agropyron* sp.)

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Abstract: Karyological study of four Agropyron species (*A. desertorum*, *A. cristatum*, *A. pectinoforum* and *A. imbricatum*) showed that these species were tetraploid (2n = 4x = 28) with the base number, x = 7. In the studied cells of *A. desertrum*, 28 chromosomes with 1 B chromosome and satellite on chromosomes 2 and 4 were exist. The number of chromosomes in studied cells of *A. cristatum* ranged between 28 and 31 without B chromosome and with satellite on chromosome 3 and 5. In the cells of *A. imbricatum* that studied, 28 to 33 chromosomes with 1 to 3 B chromosomes and satellite on chromosome 7 were exist. The number of chromosomes in studied cells of *A. pectinoforum* ranged between 28 and 29 and 1 B chromosome and satellite on chromosome 3 were exist. Finally in the studded cells of four species 28-33 chromosomes were exist (the various forms of aneuploid cells). One to three B chromosomes and chromosomes with satellites also observed in these species.

Key words: *Agropyron* sp., B chromosomes, karyology, karyotype

INTRODUCTION

Traditionally, *Agropyron* has been the largest genus in the Triticeae tribe containing more than 100 species worldwide (Sakamoto, 1964). *Agropyron* in its bread traditional sense contains almost all of the perennial species of the tribe with single spikelets per node. Although the component species have certain spike characteristics in common, they differ widely in biologically important aspects such as mode of reproduction, chromosome constitution and ecological adaptation (Dewey, 1983). Nevski (1933) a soviet taxonomist, partitioned *Agropyron* S. lat. into three genera-*Agropyron*, *Roegneria* and *Elytrigia*-with each genus containing a relatively homogeneous group of species. Under Nevski's treatment, Agropyron encompassed only the crested wheatgrasses (*A. desertorom*, A. cristatum *et al.*). Cytological data also support this narrow concept of Agropyron (Dewey, 1983). This genus consists of 10 to 13 species and same number of subspecies. Three ploidy levels-diploid (2n = 14) autotetraploid (2n = 28) and autohexaploid (2n = 42)-occur in these species, with tetraploids accounting for about 90% of the natural populations (Dewey, 1969). Chromosome pairing in interploidy hybrids indicates that all of crested wheat grasses contain the same basic genome C (Dewey, 1983; Asay and Dewey, 1979).

Supernumerary chromosomes have been reported in tetraploid Iranian materials (2n = 29, 35 and 36). In *A. pectinoforum*, different meiotic cells within the same anther had chromosome numbers which ranged from 28 to 33 and the present of cells with 33 chromosomes were 72% (Dewey and Asay, 1975). Dewey (1974) reported that hybrids between tetraploids and hexaploids gave rise to cytologically unstable pentaploids. The present plants, which presumably had the somatic chromosome numbers 2n = 32 and 33, were probably a derivative of such a hybrids. The variable chromosome number in metaphase I may be caused by partial elimination of supernumerary chromosomes in archesporial division or at an early stage of meiotic cycle. Mc Coy and Law (1968) and Assadi (1995) also reported that the various forms of Aneuploids, chromosomes with satellites and B chromosomes were found in *Agropyron* species. Kenneth *et al.* (1999) reported that the small differences in DNA content within *Agropyron* species may be due to the presence or absence of B chromosomes and satellites. B chromosomes are additional chromosomes that are

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found in a wide variety of plants and animals (Perfectti and Werren, 2001). Among flowering plants, Bs are more likely to occur in out crossing than in inbred species (Palestis *et al*., 2004).

This study was performed to identifying of polyploidy levels, differences of studded species in cytogenetically characters such as present or absence of satellites and B chromosomes in four *Agropyron* species in Iran using Aghayev method.

MATERIALS AND METHODS

In this study four Agropyron species-*A. desertorum*, *A. cristatum*, *A. pectinoforum* and *A. imbricatum* Karyologicaly were assayed in Ardebil Unversity in 2006. Seeds from each species were germinated in 25°C to preparing root tips. The germinated seeds incubated in 4°C for four days and then transferred to growth chamber (25°C) to continuing the germination. The roots with 2-3 cm length were used for aceto-iron-hematoxilin staining method as fallow (Aghayev, 1998): Stored germinated seeds whit 2-3 cm roots in 8-hydroxyl-quinalin (0.002 Mol) 5 h as pretreatment, fixing the roots in Lewitscky solution (acid cromic 1% and formalin 10% with ratio of 1:1 W/V) 24 h in 4°C, washing with flowing water for 3 h, storing in ethanol 70% in 4°C during long time, hydrolyzing the roots with 1 cm length in 1 N NaOH for 10 min and 60°C, washing with distilled water for 30 min, staining with aceto- iron-hematoxilin (4%) during 4 h in 30°C, washing with distilled water for 30 min, cutting 1 mL of root tips and treating them in Cytase enzyme 1 h in 25°C, squashing the root tips in one drop of acetic acid (5%) on a lame using squash method (Aghayev, 1998). Chromosomes in metaphase cells were seen with Olympus microscope (BH2) and photographs were prepared. For each species, total length of each chromosome, length of short and long arms, arm ratio, presence or absence of B chromosomes and satellites was measured and mean and variance of each trait was calculated and studied species was compared based on these statistics.

RESULTS

In all of the mitotic metaphase cells of *A. desertorum* that were studied, 28 chromosomes without supernumerary chromosomes were observed and some of the cells had one B chromosome (Fig. 1). The chromosomes 2 and 4 of this species had satellites (Fig 1 and 5). In studied metaphase cells in this species, arm ratio of the chromosomes ranged between 1.24±0.02 and 1.66±0.05 (Table 1) and all of the chromosomes with arm ratio lower than 1.7 were metacentric (Levan *et al*., 1964).

The mitotic metaphase cells of *A. cristatum* that were studied had 28 to 31 chromosomes without B chromosomes (Fig. 2). The chromosomes 3 and 5 of this species had satellites (Fig. 6). In studied metaphase cells in this species, arm ratio of the chromosomes ranged between 1.2±0.04 and 1.78±0.04 (Table 1) and chromosomes 2 and 5 were submetacentric and other chromosomes of this species were metacentric (Table 1).

The studied metaphase cells in *A. imbricatum* had 28 to 33 chromosomes inclined to 1 to 3 B chromosomes and chromosome 7 of this species had satellite (Fig. 3 and 7). Arm ratio index of this species ranged between 1.18±0.02 and 1.83±0.09 and chromosomes 2 and 5 of this species were submetasentric and others were metasentric (Table 2).

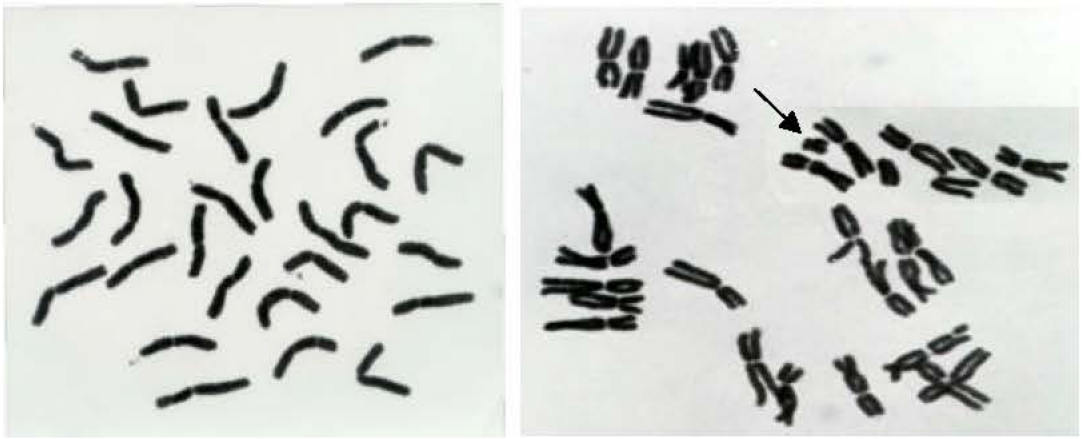

Fig. 1: Two metaphase cells of the A. desertorum with 28 chromosomes and one B chromosome (right) and 6 chromosomes with satellites (left)

Table 1: Length and relative length of chromosomes and arm ratio index in *A. imbricatum* and *A. pectinoforum* based on 10 metaphase cells observations

	A. desertorum			*A. cristatum*		
Chr. No.	Ch. Length (μm)	Relative length (%)	Arm ratio	Ch. length (μm)	Relative length (%)	Arm ratio
1	12.31±0.36	16.6±0.3	1.38±0.04	12.61±0.53	16.69±0.4	1.47±0.04
2	11.17±0.35	15.1±0.3	1.24±0.02	11.38±0.46	15.07±0.4	1.77±0.08
3	11.07±0.28	14.9±0.25	1.66±0.05	11.41±0.47	15.12±0.4	1.2±0.04
4	10.65±0.33	14.3±0.3	1.50±0.05	10.54±0.42	13.95±0.4	1.67±0.06
5	10.00±0.31	13.5±0.3	1.61±0.04	10.23±0.39	13.54±0.38	1.78±0.1
6	9.70±0.26	13.1±0.27	1.47±0.07	10.14±0.40	13.42±0.39	1.29±0.03
7	903.00±0.27	12.5±0.29	1.46±0.05	9.20±0.37	12.18±0.4	1.53±0.06
Mean	10.60±0.31	14.3±0.23	1.47±0.05	10.37±0.43	12.18±0.4	1.53±0.06

Table 2: Length and relative length of chromosomes and arm ratio index in *A. imbricatum* and *A. pectinoforum* based on 10 metaphase cells observations

	A. Imbrication			*A. pectinoforam*		
Chr. No.	Ch. Length (μm)	Relative length (%)	Arm ratio	Ch. length(μm)	Relative length (%)	Arm ratio
1	13.12±0.29	16.54±0.2	1.76±0.09	12.20±0.29	16.81±0.24	1.55±0.07
2	12.62±0.21	15.91±0.17	1.24±0.05	11.20±0.24	15.43±0.21	1.25±0.05
3	11.39±0.20	14.36±0.18	1.40±0.04	10.84±0.28	14.93±0.26	1.58±0.07
4	11.26±0.29	14.20±0.26	1.68±0.07	10.25±0.21	14.12±0.20	1.27±0.05
5	10.74±0.27	13.54±0.25	1.18±0.02	9.80±0.24	13.50±0.24	1.66±0.07
6	10.38±0.22	13.09±0.21	1.83±0.09	9.52±0.20	13.12±0.21	1.23±0.04
7	9.79±0.32	12.34±0.33	1.65±0.11	8.77±0.23	12.08±0.26	1.61±0.06
Mean	11.33±0.26	14.29±0.23	1.53±0.07	10.37±0.24	14.29±0.23	1.45±0.06

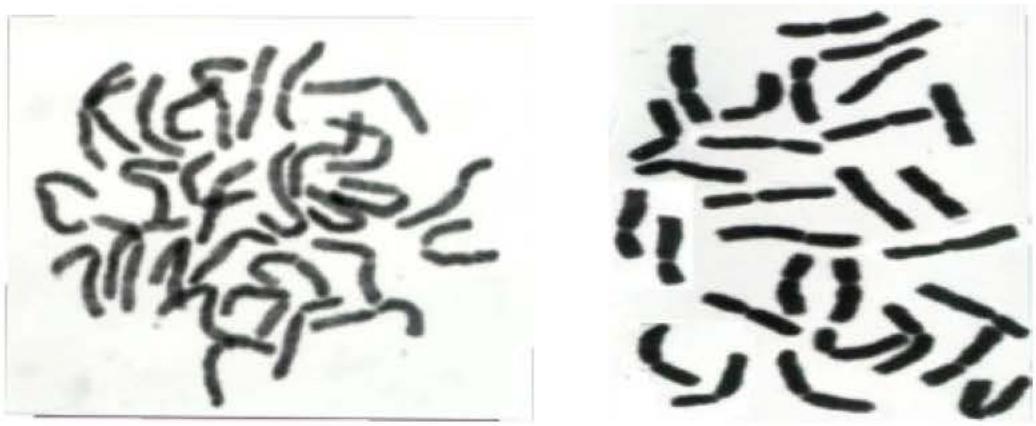

Fig. 2: Two metaphase cells of the *A. cristatum* with 29 (right) and 31 chromosomes (left)

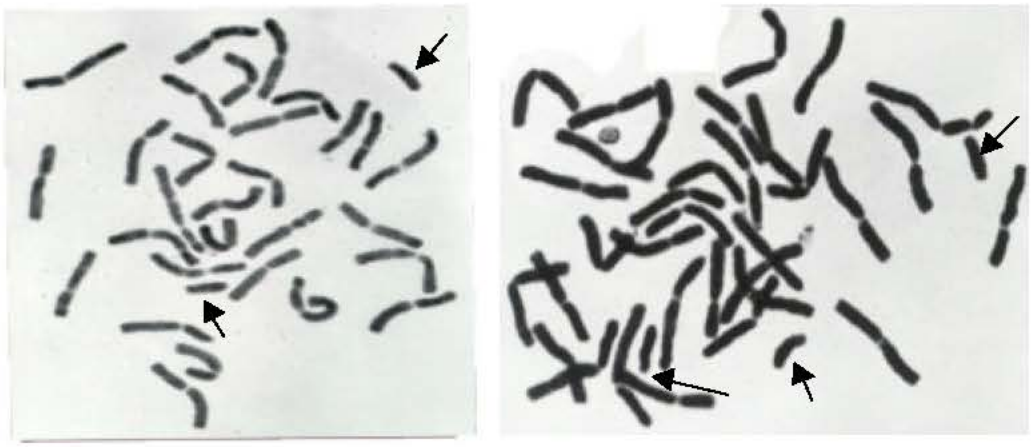

Fig. 3: Two metaphase cells of the *A. imbricatum* with 28 ordinary chromosomes and two B chromosomes (left) and 33 ordinary chromosomes with three B chromosomes (right)

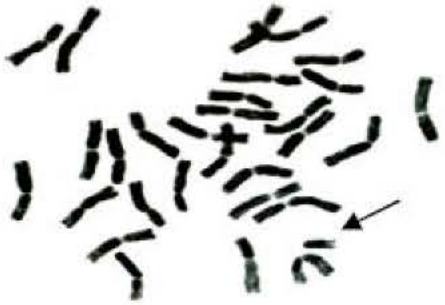

Fig. 4: One metaphase cell of the *A. pectinoforum* with 29 ordinary chromosomes and one B chromosome

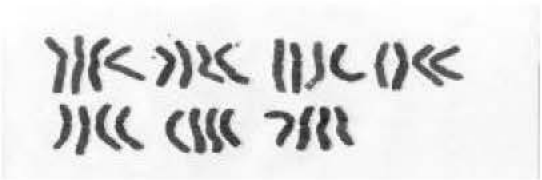

Fig. 5: Karyotype of metaphase chromosomes in *A. desertrum*

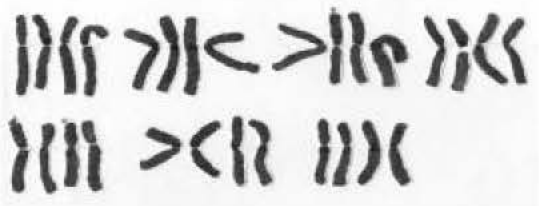

Fig. 6: Karyotype of metaphase chromosomes in *A. cristatum*

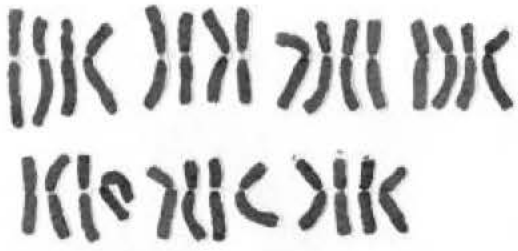

Fig. 7: Karyotype of metaphase chromosomes in *A. imbricatum*

Table 3: ANOVA table for chromosomal traits in studied species

	MS			
SOV	df	Ch. length	Relative length	Arm ratio
Species	3	1.19^{ns}	0.003^{ns}	0.013^{ns}
Error	24	1.23	2.003	0.044
CV	-	13.12	10.81	10.3

ns: Non significant

Fig. 8: Karyotype of metaphase chromosomes in *A. pectinoforum*

Twentyeight to twentynine chromosomes with one B chromosome were exist in studied cells of *A. pectinoforum* (Fig. 4). The chromosome 3 of this species had satellite (Fig. 8). In studied metaphase cells in this species, arm ratio of the chromosomes ranged between 1.23±0.04 and 1.66±0. 07 (Table 2) and all of the chromosomes with arm ratio lower than 1.7 were metasentric (Levan *et al*., 1964).

Four species that were studied, don't have significant differences with each other in chromosomal characteristics (Table 3) but these species have differences in absence or presence of B chromosomes, position of satellites on karyogram and number of excess chromosomes (aneuploidy forms).

DISCUSSION

Counting of the chromosomes in four *Agropyron* species showed that these species with basic set of chromosomes, x = 7 are tetraploides. Dewey and Asay (1975). Reported that tetraploid species and subspecies of crested wheat grass complex accounting for about 90% of the natural populations. The species that were studied don't have significant differences in chromosome length, relative length of chromosomes and arm ratio index and this make complex the cytotaxonomy of *Agropyron* species. Therefore, the proper classification of species and subspecies in this genus needs to advance cytotaxonomical methods such as chromosome banding methods. Schulz *et al*. (1963) and Kenneth *et al*. (1999) reported that the species of *Agropyron* genus don't have differences in chromosomal indexes and the differences of species come from chromosomal rearrangement after interspecific hybridization. Genomic similarity caused that the species and subspecies hybridized with each other and new fertile hybrids are produced (Knowles, 1955; Asay and Dewey, 1979). Interspecific and intergeneric hybrids are common among the perennial grasses of the *Triticeae* tribe. They may also become new species through induced amphploidy, or may be used as a means of gene transfer between parent species (Napier and Walton, 1982; Watson and Dallwitz, 1994).

In this study the number of chromosomes varied from 28 to 33 and this show the chromosomal variations between and within *Agropyron* species. In

A. pectinoforum, tetraploid Irannian materials, different meiotic cells within the same anther had chromosome numbers which ranged from 28 to 36 and the present of cells with 33 chromosomes were 72% (Asay and Dewey, 1975). Dewey (1974) reported that plants whit chromosome numbers 2n = 32 and 33, were probably a derivative of interspecific hybrids.

In three species that were studied, various numbers of B chromosomes were exist and in other investigations, B chromosomes were found in *Agropyron* species (Mc Coy and Law, 1968; Assadi, 1995). For this, the species of this genus are suitable for assaying the structure and role of the B chromosomes.

REFERENCES

Aghayev, Y.M., 1998. Advanced squash methods for investigation of plant chromospmes. Presented Papers in the 4th Iranian Congress on Crop Production and Breeding Sciences, Isfahan University of Technology, Isfahan, Iran, pp: 1-20.

Assadi, M., 1995. Meiotic configuration and chromosome number in some Iranian species of *Elymus* L. and *Agropyron* Gartner. Bot. J. Linnean Soc. London, 117: 159-168.

Asay, K.H. and D.R. Dewey, 1979. Bridging ploidy differences in crested wheatgrass with hexaploid diploid hybrids. Crop Sci., 19: 519-523.

Dewey, D.R., 1969. Synthetic hybrids of *Agropyron albicas*, *A. dasystachyum*, *Sitanion hystrix* and *Elymus Canadensis*. Am. J. Bot., 56: 664-670.

Dewey, D.R., 1974. Reproduction in crested wheat grass pentaploids. Crop Sci., 8: 607-611.

Dewey, R.D. and K.H. Asay, 1975. The crested wheatgrasses of Iran. Crop Sci., 15: 844-849.

Dewey, D.R., 1983. Historical and current taxonomic perspectives of *Agropyron*, *Elymus* and related genera. Crop Sci., 23: 637-642.

Kenneth, P.V., K. Arumuganathan and K.B. Jensen, 1999. Nuclear DNA content of perennial grasses of the triticeae. Crop Sci., 39: 661-667.

Knowles, R.P., 1955. A study of variability in crested whetgrass. Can. J. Bot., 33: 534-546.

Levan, A., K. Fredga and A. Sandberg, 1964. Nomenclature for centromeric position on chromosomes. Hereditas, 52: 201-220.

McCoy, G.A. and A.G. Law, 1968. Satellite chromosomes in two diploid species of crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) or *Agropyron cristatiforme* Sarkar and *Agropyron imbricatum* (M.B.) Roem. Et Schult). Agron. Abstr., 13: 221-223.

Napier, K.V. and P.D. Walton, 1982. A hybrid between *Agropyron trachycaulum* and *A. desertrum*. Crop Sci., 22: 657-660.

Nevski, S.A., 1933. Agrostologische stuien. IV. Uber das system der tribe Hordeae Beenth. Acta Inst. Bot. Acad. Sci., URSS Ser. I.

Palestis, B.G., R. Trivers, A. Burt and R.N. Jones, 2004. The distribution of B chromosomes across species. Cytogenet. Genom Res., 106: 151-158.

Perfectti, F. and J. Weeren, 2001. The interspecific origin of B chromosomes: Experimental evidence. Evolution, 55: 1069-1073.

Sakamoto, S., 1964. Cytogenetical problems in *Agropyron* hybrids. Seiken Jiho., 16: 38-47.

Schulz-Schaeffer, J.R., P.W. Allderice and G.C. Creel, 1963. Segmental allopolyplidy in tetraploid and hexaploid *Agropyron* species of the crested wheagrass complex (section *Agropyron*). Crop Sci., 3: 525-530.

Watson, L. and M.J. Dallwitz, 1994. The Grass Genera of the World. Cabi Publ., pp: 81-82.