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Karyotype and C-banding Patterns of Mitotic Chromosomes in *Heteranthelium piliferum*

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Abstract: The C-banded karyotype of *Heteranthelium piliferum* species was studied in a natural population from northwest of Iran using aceto-iron-hematoxilin staining and C-banding technique. Chromosome measurements including long arm, short arm and chromosome lengths, arm ratio index, relative chromosome length, heterochromatin percent per chromosome and per chromosome set were made. It was revealed that the karyotype of this species is symmetric and consists of 7 pairs of metacentric chromosomes. Arm ratio index values ranged from 1.01 in chromosome G to 1.44 in chromosome D. One of the chromosomes had a satellite located on the end of its long arm (chromosome G). The Q genome of this species like A, B, D, S, M and M_t genomes in diploid species of *Aegilops-Trticum* group, H genome in *Hordeum*, E genome in *Agropyron* and R genome in *Secale* has metacentric or sub-metacentric chromosomes.

Key words: Genetic resources, grass species, Iran, Triticeae

INTRODUCTION

The tribe Triticeae includes world strategically crops such as wheat, barley and rye. It also includes important, mostly perennial, fodder grasses such as *Agropyron*, *Elytrigia*, *Elymus*, *Leymus*, *Psathyrostachys* and others. Many wild annual grasses of the Triticeae tribe 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.

Heteranthelium piliferum is an annual, diploid (2n = 2x = 14, QQ) grass species native to middle and west Asia (Watson and Dallwitz, 1994). In the manner of disarticulation of spikelets, *Heteranthelium* remotely resembles the genus *Eremopyrum*, in the structure of awns it resembles *Aegilops* and in the structure of glumes, the genus *Hordeum* (Chennaveeraiah and Sarkar, 1965). It belongs to Triticeae and can be used as a genetic resource for desirable genes in cereal breeding.

The development and application of differential staining techniques on plant chromosomes, including Giemsa C-banding method (Vosa and Marchi, 1972; Vosa 1975), which stains constitutive heterochromatin (Arrighi and Hsu, 1971), have resulted in many detectable markers for karyotype analysis. This method is the most applicable one which has been widely used for chromosome identification in many species of Triticeae species such as *Aegilops* (Friebe *et al*., 1992a, b, 1993, 1995, 1996; Badaeva *et al*., 2002, 2004), *Agropyron* and *Elymus* (Endo and Gill, 1984), *Henrardia persica* (Asghari-Zakaria *et al*., 2002), *Hordeum* (Linde-Laursen *et al*., 1980, Kakeda *et al*., 1991), *Secale* (Weimark 1975; Giraldez *et al*., 1979) and *Triticum* (Gill *et al*., 1991; Badaeva *et al*., 1994).

According to Chennaveeraiah and Sarkar (1965) *H. piliferum* is a diploid species with the base chromosome number of x = 7. We could not find any published report on detailed karyotype and C-banding patterns of chromosomes in this species. The objective of this study is constructing a detailed karyotype of this species using aceto-iron-hematoxilin staining and C-banding techniques.

MATERIALS AND METHODS

This study was conducted at cytogenetic laboratory of Mohaghegh Ardabili University, Iran, in 2005. Seeds of a natural population of *H. piliferum*, collected from East Azarbayjan province, northwest 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 and C-banding technique has been used as described earlier by Asghari-Zakaria *et al*. (2002).

Chromosome measurements including long arm, short arm and chromosome lengths, total length of chromosome set, arm ratio index, relative chromosome length, heterochromatin percent per chromosome and per chromosome set were made from 15 enlarged well-spread metaphase cells using Micromeasure software developed by the Biology Department of Colorado State University, available on Internet at http://www.colostate.edu/

Depts/Biology/Micromeasure. Homologous chromosomes were identified based on position of centromere and similarities of C-banding patterns. The nomenclature followed Levan *et al*. (1964) and chromosomes were named as A, B, C, D, E, F and G in descending order of length.

RESULTS AND DISCUSSION

Mitotic chromosomes and their C-banding patterns are shown in Fig. 1a and b, respectively. Karyotypic characters of the seven mitotic chromosomes are shown in Table 1.

The distinct morphological characters and C-banding patterns for each chromosome are described as follows:

Chromosome A was the largest chromosome among the chromosomes of *H. piliferum*. There were one proximal, two interstitial and one telomeric band on its long arm and one proximal, one distal and one telomeric band on its short arm. Another faint interstitial band was also observed in some of metaphase cells in both arms.

Chromosome B the banding pattern of this chromosome consisted of one proximal, one interstitial and one telomeric band on its long and short arms. Another faint interstitial band on its long arm was also observed in some metaphase cells.

Chromosome C it showed one proximal, one interstitial and one telomeric band on its long arm and one proximal and one telomeric band on its short arm.

Chromosome D three sharp bands were observed at proximal, interstitial and telomeric regions on its long arm and two others at proximal and telomeric regions on its short arm.

Chromosome E banding pattern of this chromosome consisted of one proximal and one interstitial band on its long arm and one proximal band on its short arm and a faint band in telomeric region of its long arm (in some metaphase cells).

Chromosome F had the two sharp bands at proximal and telomeric regions of its both long and short arms.

Chromosome G (SAT chromosome), the smallest one in the chromosome set of *H. piliferum* was distinguishable from other chromosomes through a satellite located on the end of its long arm. It had one proximal and one telomeric band on its short arm and one proximal, one interstitial, one telomeric band near NOR region of its long arm.

The analysis of karyotype showed that *H. piliferum* has 2n = 2x = 14 chromosomes (Fig. 2). This is in agreement with Chennaveeraiah and Sarkar (1965), where they concluded that it was a diploid species with the base chromosome number of x = 7. Chromosome length in *H. piliferum* ranged from 7.53 μm in chromosome G to 9.96 μm in chromosome A. On the other hand, arm ratio index values ranged from 1.01 in chromosome G (with considering satellite in the long arm length of this chromosome) to 1.44 in chromosome D (Table 1). The ratio between the largest and the smallest chromosome was 1.3: 1. Since, *H. piliferum* had chromosomes with

Table 1: Karyotypic characters of seven mitotic chromosomes of *H. piliferum*

Chr	Type	Long arm (μm)*	Short arm (μm)	Chromosome (μm)	Relative length (%)	Arm ratio	Satellite (μm)	(%) Constitutive heterochromatin
A	m	5.57±0.16	4.39±0.16	9.96±0.30	16.41±0.16	1.29±0.03	-	37.85±2.12
B	m	5.14±0.15	4.15±0.14	9.29±0.29	15.30±0.13	1.25±0.02	-	32.41±2.21
C	m	4.92±0.16	4.24±0.16	9.15±0.31	15.08±0.15	1.17±0.02	-	36.98±2.86
D	m	5.17±0.21	3.63±0.16	8.80±0.35	14.50±0.14	1.44±0.03	-	27.81±2.62
E	m	4.61±0.15	3.63±0.13	8.24±0.27	13.56±0.15	1.28±0.03	-	21.53±1.32
F	m	4.13±0.16	3.61±0.14	7.74±0.28	12.75±0.16	1.15±0.01	-	32.40±1.78
G	m	3.78±0.12	3.75±0.13	7.53±0.25	12.40±0.12	1.01±0.02	0.98±0.04	33.65±1.62

m: metacentric, *: mean±standard error

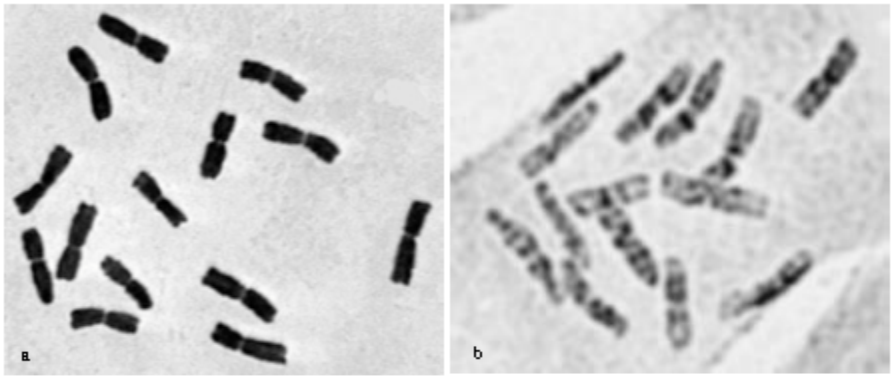

Fig. 1: Metaphase chromosomes of *H. piliferum* stained with aceto-iron-hematoxilin (a) and Giemsa C-banding technique

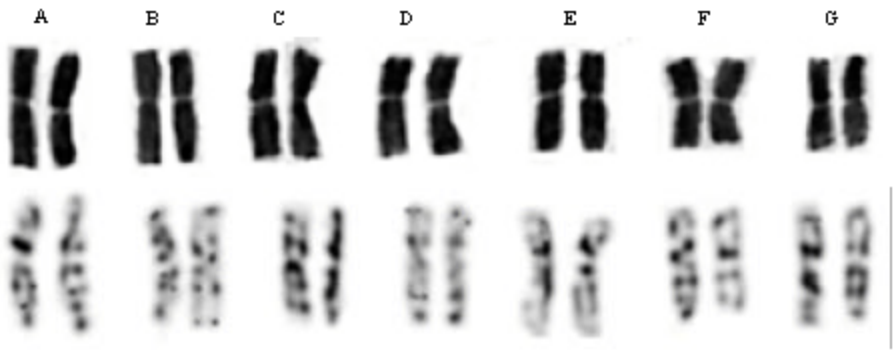

Fig. 2: Karyogram of somatic metaphase chromosomes of *H. piliferum*

median centromere and mostly equal arms, it showed a symmetric karyotype. Each chromosome had a distinct C-banding pattern and this technique provided adequate information to identify all of *H. piliferum* chromosomes. *H. piliferum* has only one SAT chromosome located on the end of long arm of chromosome G. Total length of chromosomes in this species was 121.14±1.06 μm and the amount of constitutive heterochromatin in each chromosome was approximately equal. The mean value of heterochromatin in *H. piliferum* was nearly 31%.

In the tribe *Triticeae* the Q genome of this species like A, B, D, S, M and M_s genomes in diploid species of *Aegilops-Trticum* group, H genome in *Hordeum*, E genome in *Agropyron* and R genome in *Secale* has metacentric or sub-metacentric chromosomes and symmetric karyotype. Cultivated species of this tribe including *T. aestivum*, *T. turgidum*, *Hordeum vulgare* and *Secale cereale* have also chromosomes with median and sub-median centromere. The C, U and U_s genomes in *Ae. caudata*, *Ae. umbellulata* and *Ae. uniaristata* species have also as many as four chromosomes with sub-terminal centromere (Badaeva *et al*., 1994, 2002, 2004; Friebe *et al*., 1992a, 1992b, 1993, 1995, 1995, 1996; Gill *et al*., 1991; Linde-Laursen *et al*., 1980; Endo and Gill, 1984; Weimark, 1975; Giraldez *et al*., 1979). Where as, the O genome of *Henrardia persica* in the tribe *Triticeae* has seven acrocentic chromosomes (Asghari-Zakaria *et al*., 2002).

CONCLUSION

The results showed that the karyotype of Q genome in this species is symmetric and consists of 7 pairs of metacentric chromosomes. One of the chromosomes had a satellite located on the end of its long arm. Study of karyological characteristics of *H. piliferum* is valuable in using this species as a genetic resource for cereal breeding.

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