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Karyological analysis on wheat Tir (*Triticum aestivum* var. Aestivum L. spp. Leucospermum Körn.) ecotypes in Lake Van Basin, Turkey

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Abstract Karyotypic is studied within ecotypes species which are importantly found with different ecotypes, and it may possible showing specific genomic adaptation with their environmental growing conditions. Chromosome karyotype and morphology in wheat Tir by analyzing chromosomes in five ecotypes (Ahlat, Ercis, Muradiye, Patnos and central part of Van) in order to present the best instruction for cytogenetic studies in chromosome analysis was investigatred. Pre-treatment with %1 alpha-bromo naphthalene, fixation in karnoy 1, hydrolysis in NaOH and staining by %2 Aceto-Orcein were proved using root meristem segments followed by studying the microscopic preparations. Karyotype analysis of each ecotype. There was separately performed and showing several indices (TL: Total Length, LA: Long Arm, SA: Short Arm, Sat: Satellite and AR: Arm Ratio). The somatic chromosome numbers of all studied ecotypes are hexaploid with 2n=6x=42 and the averages of chromosomes length ranged from 4.60 ± 0.02 µm to 16.05 ± 0.02 µm. The longest chromosome was observed in chromosome number 1 from ecotype 3 which belongs to Muradiye and the shortest one was related to the chromosome number 21 from ecotype 4 from Patnos.

Keywords: Karyotype analysis, Wheat Tir, *Triticum aestivum*, Ecotype, Van basin

Introduction

Today, wheat (*Triticum aestivum*) is the staple food of people in many countries, so that it provides more than 20% of the calories needed by the world's population. Wheat is preferred over other cereals due to its starch, protein and good baking properties. Although bread can be made from other grains, to date no plant has been able to compete with wheat in preparing bread for human consumption (Farshadfar *et al*., 2020). Studies on cytogenetic diversity are used in different plant breeding programs, which have specific applications based on the chromosomal structure of the plant as well as

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breeding goals. Considering the most land areas in arid and semi-arid regions of Turkey devoted to wheat cultivation, plant breeders mainly focus on cultivars resistant to biotic (including pests and diseases, etc.) and abiotic (including salinity and drought) stresses. Using of wild wheat species such as Triticum urartu and Triticum booticum, with a wide cytogenetic diversity, can be very useful. It is also necessary to know how wheat originates and spreads in the collection, protection and optimal use of wild wheat in breeding programs (Ranjbar *et al*., 2007; Devos ad Gale, 1993).

The chromosomes in different species of the *Triticae* family are very diverse in number and size, but the number of base chromosomes in this family is similar and equal to 7 (Hilu, 2004). Autoploidy, alloploidy and aneuploidy caused high diversity within this family (Sliai and Amer, 2011; Kharazian, 2008). Wheat species are classified into three groups including diploids, tetraploids and hexaploids. Karyotypic studies are performed to compare differences among individuals in a group and to reveal the evolution of changes in the chromosomes that make up the genome (Safari and Mehrabi, 2021). Therefore, a breeder with cytogenetic information can easily decide which species could be used in a crossbreeding program to produce fertile offspring (Stebbins, 1971). Differences in the structure and size of chromosomes in mitosis can indicate genetic diversity, so, karyotypic studies can be useful to classify plants and solve classical taxonomic problems and determine the affinity of species, especially wild and native plants, as the first step in phylogenetic analysis and evolution of related species (Borem and Fritch-neto, 2014). Cluster analysis could not completely separate Triticum and Aegilops species. Variation among the species was mainly based on chromosomal asymmetry and chromatin content. *Ae. cylindrica* and *T. monococcum* karyological study showed that T. monococcum have symmetrical chromosomes and *Ae. cylindrica* has sub-metacentric and sub-telocentric chromosomes (Ghorbani-Sini and Arzani, 2015). Ehtemam *et al.* (2014) evaluated karyotypic wheat species with genome A. Species were classified into three clusters based on two indicators A1 and A2. The results showed that *T. durum* was more primitive than *T. turgidum* and *T. monoccocum* donates genome A to *T. durum* and *T. aestivum*. In other study, chromosomal homology among three Aegilops species with genome D (*Ae. cylindrica, Ae. crassa* and *Ae. tauschii*) with bread wheat was determined in order to show the possibility of gene transfer from Aegilops species to wheat (Jaffar-Aghaei *et al*., 2007). The results showed that it is possible to prepare hybrids between these species at least using the embryo rescue technique. In another study, the cytogenetic analysis of Aegilops species using C-banding techniques showed that the chromosomes of the genome D in *Ae. ventricosa* are more similar to *Ae. crassa* chromosomes (Bedava *et al*., 2012). Karyotypic analysis of wild wheat showed that karyotypic characteristics were not able to distinguish two species *Ae. tauschii* and *T. boeticum* (Karimiafshar *et al*., 2016). There is no study on the karyotype of hexaploid Tir wheat ecotypes in the Van Basin. The present study was performed on several ecotypes from this area.

Materials and methods

The chromosomes morphology in wheat Tir ecotypes was studied using cytogenetic indices. For this purpose, samples from five different ecotypes of Tir wheat (Ahlat, Ercis, Muradiye, Patnos and central part of Van) were collected in Van Lake Basin. Pre-treatment with %1 alpha-bromo naphthalene, fixation in karnoy 1, hydrolysis in NaOH and staining by %2 Aceto-Orcein were conducted using root meristem segments followed by studying the microscopic preparations. Finally, ten replications (suitable metaphase plate) were selected and analyzed using Micro Measure software. Karyotype analysis of each ecotype was conducted separately and several indices (TL: Total Length, LA: Long Arm, SA: Short Arm, Sat: Satellite, AR: Arm Ratio and Chr. Type: Chromosome Type according to Levan method) were measured (Levan, 1964). Ideogram of each ecotype was also determined. Data were analyzed using Micromeasure 3.3, SPSS and Excel.

Results

The chromosomes numbers, morphometric parameters and karyotype formula of all Tir wheat ecotypes were determined (Table 1-5) and ideogram were made on them (Figure 1-5).

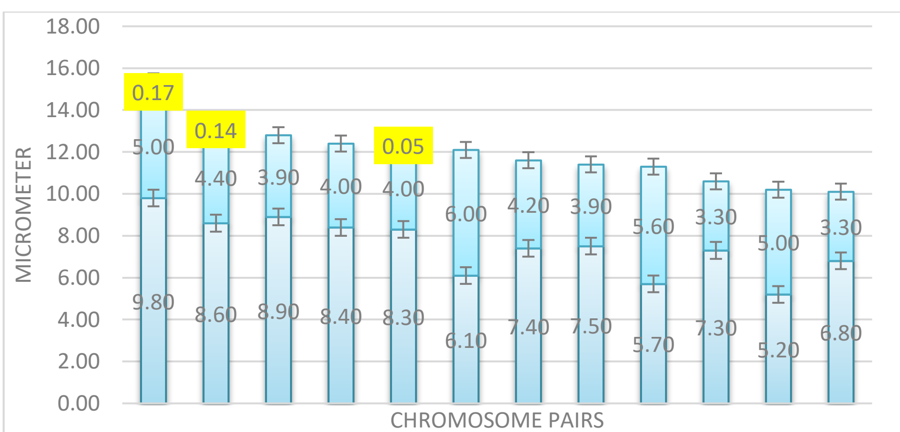

Figure 1. Somatic haploid ideogram in Ahlat ecotypes (Wheat Tir)

Table 1. Karyotype characteristics in Ahlat ecotypes (Wheat Tir)

Pair	L (µm)	S (µm)	Sat (µm)	TL (µm)	AR	Chromosome Type
1	9.80 ±0.03	5.00 ±0.04	0.17 ±0.02	14.97 ±0.06	1.96 ±0.05	sm
2	8.60 ±0.09	4.40 ±0.03	0.14 ±0.01	13.14 ±0.05	1.95 ±0.10	sm
3	8.90 ±0.01	3.90 ±0.094	-	12.80 ±0.03	2.28 ±0.06	sm
4	8.40 ±0.07	4.00 ±0.02	-	12.40 ±0.04	2.10 ±0.03	sm
5	8.30 ±0.11	4.00 ±0.09	0.05 ±0.01	12.35 ±0.19	2.08 ±0.09	sm
6	6.10 ±0.04	6.00 ±0.01	-	12.10 ±0.07	1.02 ±5.05	m
7	7.40 ±0.08	4.20 ±0.08	-	11.60 ±0.05	1.76 ±0.02	sm
8	7.50 ±0.09	3.90 ±0.09	-	11.40 ±0.03	1.92 ±0.05	sm
9	5.70 ±0.07	5.60 ±0.08	-	11.30 ±0.02	1.02 ±0.01	m
10	7.30 ±0.06	3.30 ±0.03	-	10.60 ±0.03	2.21 ±0.03	sm
11	5.20 ±0.03	5.00 ±0.09	-	10.20 ±0.04	1.04 ±0.08	m
12	6.80 ±0.11	3.30 ±0.02	-	10.10 ±0.15	2.06 ±0.04	sm
13	6.50 ±0.06	3.10 ±0.06	-	9.60 ±0.03	2.10 ±0.02	sm
14	6.20 ±0.01	2.80 ±0.08	-	9.00 ±0.05	2.21 ±0.09	sm
15	5.80 ±0.08	2.80 ±0.01	-	8.60 ±0.01	2.07 ±0.06	sm
16	5.70 ±0.02	2.40 ±0.07	-	8.10 ±0.01	2.38 ±0.04	sm
17	5.40 ±0.08	2.50 ±0.08	-	7.90 ±0.08	2.16 ±0.03	sm
18	5.20 ±0.06	2.30 ±0.05	-	7.50 ±0.07	2.26 ±0.04	sm
19	4.90 ±0.08	2.00 ±0.02	-	6.90 ±0.04	2.45 ±0.02	sm
20	4.30 ±0.05	2.10 ±0.04	-	6.40 ±0.08	2.05 ±0.06	sm
21	4.20 ±0.02	2.10 ±0.03	-	6.30 ±0.03	2.00 ±0.07	sm

Karyotype formula: 15sm+3m+3smsat µm: Micrometer, L: Long arm, S: Short arm, Sat: Satellite, TL: Total chromosome length, AR: Arm Ratio, m: Metacentric, sm: Submetacentric.

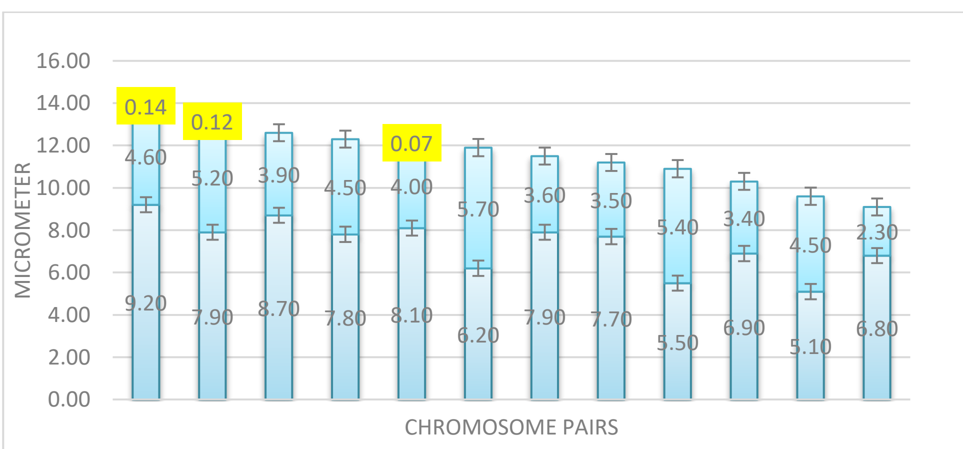

Figure 2. Somatic haploid ideogram in Ercis ecotypes (Wheat Tir)

Table 2. Karyotype characteristics in Ercis ecotypes (Wheat Tir)

Pair	L (µm)	S (µm)	Sat (µm)	TL (µm)	AR	Chromosome Type
1	9.20 ± 0.04	4.60 ± 0.09	0.14 ± 0.04	13.94 ± 0.01	2.06 ± 0.04	sm
2	7.90 ± 0.06	5.20 ± 0.03	0.12 ± 0.07	13.22 ± 0.07	1.52 ± 0.08	m
3	8.70 ± 0.08	3.90 ± 0.03	-	12.60 ± 0.04	2.23 ± 0.03	sm
4	7.80 ± 0.01	4.50 ± 0.12	-	12.30 ± 0.07	1.73 ± 0.01	sm
5	8.10 ± 0.09	4.00 ± 0.02	0.07 ± 0.01	12.17 ± 0.03	2.03 ± 0.02	sm
6	6.20 ± 0.06	5.70 ± 0.08	-	11.90 ± 0.02	1.09 ± 0.09	m
7	7.90 ± 0.07	3.60 ± 0.03	-	11.50 ± 0.09	2.19 ± 0.04	sm
8	7.70 ± 0.02	3.50 ± 0.06	-	11.20 ± 0.05	2.20 ± 0.03	sm
9	5.50 ± 0.04	5.40 ± 0.03	-	10.90 ± 0.02	1.02 ± 0.09	m
10	6.90 ± 0.04	3.40 ± 0.07	-	10.30 ± 0.09	2.03 ± 0.08	sm
11	5.10 ± 0.02	4.50 ± 0.02	-	9.60 ± 0.04	1.13 ± 0.02	m
12	6.80 ± 0.03	2.30 ± 0.08	-	9.10 ± 0.07	2.96 ± 0.04	sm
13	5.60 ± 0.09	2.70 ± 0.06	-	8.30 ± 0.02	2.07 ± 0.02	m
14	5.70 ± 0.05	2.40 ± 0.02	-	8.10 ± 0.01	2.38 ± 0.08	sm
15	5.30 ± 0.02	2.50 ± 0.04	-	7.80 ± 0.04	2.12 ± 0.09	sm
16	5.10 ± 0.08	2.40 ± 0.08	-	7.50 ± 0.02	2.13 ± 0.01	sm
17	4.90 ± 0.09	2.20 ± 0.03	-	7.10 ± 0.08	2.23 ± 0.03	sm
18	4.80 ± 0.03	2.10 ± 0.04	-	6.90 ± 0.02	2.29 ± 0.07	sm
19	4.40 ± 0.05	1.90 ± 0.11	-	6.30 ± 0.09	2.32 ± 0.03	sm
20	4.20 ± 0.07	1.70 ± 0.02	-	5.90 ± 0.05	2.47 ± 0.05	sm
21	4.20 ± 0.01	1.50 ± 0.04	-	5.70 ± 0.03	2.80 ± 0.03	sm
Karyotype formula: $14sm+2sm^{sat}+1m^{sat}$						

µm: Micrometer, L: Long arm, S: Short arm, Sat: Satellite, TL: Total chromosome length, AR: Arm Ratio, m: Metacentric, sm: Submetacentric.

Table 3. Karyotype characteristics in Muradiye ecotypes (Wheat Tir)

Pair	L(µm)	S(µm)	Sat(µm)	TL (µm)	AR	Chromosome Type
1	10.20 ± 0.01	5.70 ± 0.05	0.15 ± 0.03	16.05 ± 0.03	1.79 ± 0.03	sm
2	10.10 ± 0.08	5.00 ± 0.09	0.21 ± 0.01	15.31 ± 0.07	2.02 ± 0.08	sm
3	9.90 ± 0.09	4.70 ± 0.05	-	14.60 ± 0.03	2.11 ± 0.09	sm
4	9.60 ± 0.05	4.70 ± 0.04	-	14.30 ± 0.02	2.04 ± 0.04	sm
5	9.20 ± 0.03	4.70 ± 0.03	0.03 ± 0.02	13.93 ± 0.09	1.96 ± 0.04	sm
6	6.80 ± 0.08	6.70 ± 0.02	-	13.50 ± 0.03	1.01 ± 0.05	m
7	8.30 ± 0.02	4.50 ± 0.08	-	12.80 ± 0.04	1.84 ± 0.02	sm
8	8.10 ± 0.03	4.30 ± 0.01	-	12.40 ± 0.04	1.88 ± 0.03	sm
9	6.30 ± 0.06	5.80 ± 0.01	-	12.10 ± 0.08	1.09 ± 0.04	m
10	7.70 ± 0.04	3.80 ± 0.06	-	11.50 ± 0.03	2.03 ± 0.01	sm
11	5.80 ± 0.02	5.50 ± 0.07	-	11.30 ± 0.02	1.05 ± 0.02	m
12	7.70 ± 0.07	3.20 ± 0.03	-	10.90 ± 0.05	2.41 ± 0.03	sm
13	6.80 ± 0.09	3.30 ± 0.09	-	10.10 ± 0.03	2.06 ± 0.02	sm
14	6.70 ± 0.01	3.20 ± 0.05	-	9.90 ± 0.01	2.09 ± 0.01	sm
15	6.50 ± 0.07	3.20 ± 0.07	-	9.70 ± 0.03	2.03 ± 0.02	sm
16	6.30 ± 0.11	2.90 ± 0.01	-	9.20 ± 0.02	2.17 ± 0.05	sm
17	5.70 ± 0.06	3.20 ± 0.05	-	8.90 ± 0.01	1.78 ± 0.02	sm
18	5.60 ± 0.05	3.10 ± 0.04	-	8.70 ± 0.05	1.81 ± 0.07	sm
19	5.50 ± 0.04	2.70 ± 0.06	-	8.20 ± 0.04	2.04 ± 0.05	sm
20	5.10 ± 0.08	2.50 ± 0.05	-	7.60 ± 0.06	2.04 ± 0.04	sm
21	4.90 ± 0.02	2.40 ± 0.02	-	7.30 ± 0.04	2.04 ± 0.03	sm

Karyotype formula: $15sm+3m+3sm^{sat}$

µm: Micrometer, L: Long arm, S: Short arm, Sat: Satellite, TL: Total chromosome length, AR: Arm Ratio, m: Metacentric, sm: Submetacentric.

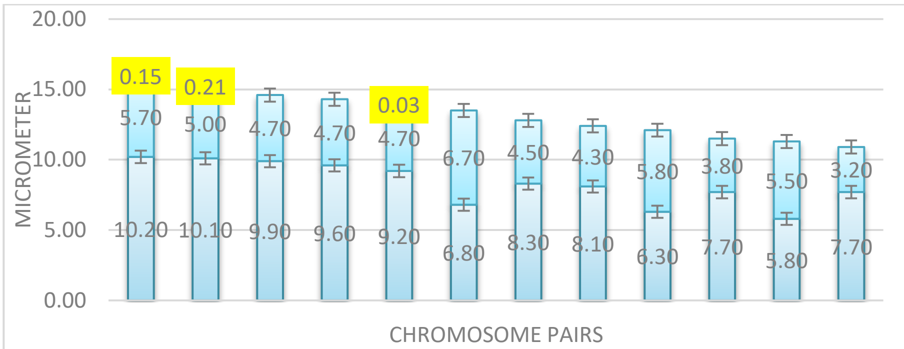

Figure 3. Somatic haploid ideogram in Muradiye ecotypes (Wheat Tir)

Table 4. Karyotype characteristics in Patnos ecotypes (Wheat Tir)

Pair	L (µm)	S (µm)	Sat (µm)	TL (µm)	AR	Chromosome Type
1	8.10 ±0.05	4.60 ±0.04	0.18 ± 0.01	12.88 ±0.08	1.76 ±0.03	sm
2	7.90 ±0.07	4.20 ±0.02	0.11 ± 0.04	12.21 ±0.07	1.88 ±0.04	sm
3	7.90 ±0.03	3.80 ±0.03	-	11.70 ±0.02	2.08 ±0.01	sm
4	7.60 ±0.02	3.70 ±0.07	-	11.30 ±0.15	2.05 ±0.07	sm
5	7.50 ±0.09	3.60 ±0.05	0.03 ± 0.03	11.13 ±0.05	2.08 ±0.02	sm
6	5.50 ±0.01	5.30 ±0.02	-	10.80 ±0.03	1.04 ±0.08	m
7	7.20 ±0.03	3.30 ±0.02	-	10.50 ±0.01	2.18 ±0.02	sm
8	6.90 ±0.02	3.20 ±0.05	-	10.10 ±0.07	2.16 ±0.03	sm
9	5.10 ±0.03	4.70 ±0.05	-	9.80 ±0.08	1.09 ±0.02	m
10	6.70 ±0.04	2.90 ±0.03	-	9.60 ±0.05	2.31 ±0.09	sm
11	4.40 ±0.02	4.30 ±0.06	-	8.70 ±0.05	1.02 ±0.03	m
12	5.60 ±0.13	2.50 ±0.06	-	8.10 ±0.04	2.24 ±0.02	sm
13	4.90 ±0.03	2.50 ±0.03	-	7.40 ±0.06	1.96 ±0.06	sm
14	4.80 ±0.07	2.30 ±0.02	-	7.10 ±0.02	2.09 ±0.03	sm
15	4.60 ±0.07	2.00 ±0.08	-	6.60 ±0.01	2.30 ±0.05	sm
16	4.20 ±0.02	2.10 ±0.03	-	6.30 ±0.04	2.00 ±0.06	sm
17	3.90 ±0.06	2.00 ±0.03	-	5.90 ±0.02	1.95 ±0.07	sm
18	3.70 ±0.01	1.80 ±0.09	-	5.50 ±0.04	2.06 ±0.01	sm
19	3.50 ±0.03	1.60 ±0.02	-	5.10 ±0.03	2.19 ±0.03	sm
20	3.30 ±0.03	1.60 ±0.03	-	4.90 ±0.03	2.06 ±0.04	sm
21	3.10 ±0.02	1.50 ±0.04	-	4.60 ±0.02	2.07 ±0.08	sm

Karyotype formula: 15sm+3m+3sm^{sat}

µm: Micrometer, L: Long arm, S: Short arm, Sat: Satellite, TL: Total chromosome length, AR: Arm Ratio, m: Metacentric, sm: Submetacentric.

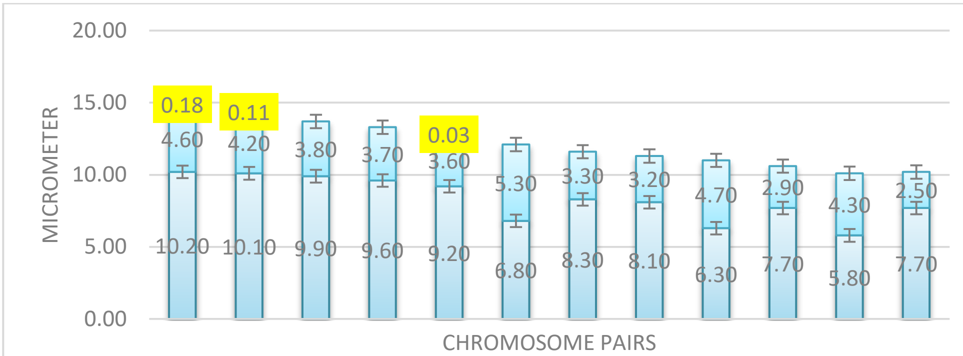

Figure 4. Somatic haploid ideogram in Patnos ecotypes (Wheat Tir)

Table 5. Karyotype characteristics in Van ecotypes (Wheat Tir)

Pair	L (µm)	S (µm)	Sat (µm)	TL (µm)	AR	Chromosome Type
1	8.10 ±0.02	4.60 ±0.07	0.16 ± 0.07	12.86 ±0.08	1.76 ±0.11	sm
2	8.30 ±0.08	4.10 ±0.08	0.11 ± 0.04	12.51 ±0.06	2.02 ±0.07	sm
3	8.50 ±0.05	3.60 ±0.04	-	12.10 ±0.05	2.36 ±0.08	sm
4	8.10 ±0.07	3.80 ±0.06	-	11.90 ±0.03	2.13 ±0.03	sm
5	7.90 ±0.03	3.80 ±0.03	0.03 ± 0.03	11.73 ±0.07	2.08 ±0.05	sm
6	7.60 ±0.08	3.60 ±0.06	-	11.20 ±0.06	2.11 ±0.02	sm
7	7.30 ±0.03	3.50 ±0.06	-	10.80 ±0.03	2.09 ±0.03	sm
8	7.10 ±0.02	3.50 ±0.04	-	10.60 ±0.09	2.03 ±0.02	sm
9	6.80 ±0.05	3.30 ±0.04	-	10.10 ±0.03	2.06 ±0.07	sm
10	6.60 ±0.07	3.20 ±0.03	-	9.80 ±0.03	2.06 ±0.01	sm
11	6.40 ±0.05	3.10 ±0.01	-	9.50 ±0.02	2.06 ±0.04	sm
12	6.20 ±0.01	2.90 ±0.06	-	9.10 ±0.02	2.14 ±0.04	sm
13	6.10 ±0.05	2.80 ±0.02	-	8.90 ±0.07	2.18 ±0.06	sm
14	5.70 ±0.03	2.80 ±0.07	-	8.50 ±0.04	2.04 ±0.03	sm
15	5.40 ±0.03	2.70 ±0.03	-	8.10 ±0.04	2.00 ±0.08	sm
16	4.90 ±0.02	2.40 ±0.01	-	7.30 ±0.06	2.04 ±0.02	sm
17	4.70 ±0.04	2.30 ±0.06	-	7.00 ±0.09	2.04 ±0.06	sm
18	4.60 ±0.05	2.20 ±0.03	-	6.80 ±0.02	2.09 ±0.05	sm
19	4.40 ±0.02	2.10 ±0.01	-	6.50 ±0.03	2.10 ±0.02	sm
20	3.70 ±0.02	1.60 ±0.16	-	5.30 ±0.06	2.31 ±0.08	sm
21	3.50 ±0.017	1.60 ±0.05	-	5.10 ±0.04	2.19 ±0.02	sm

Karyotype formula: $18sm+3sm^{sat}$

µm: Micrometer, L: Long arm, S: Short arm, Sat: Satellite, TL: Total chromosome length, AR: Arm Ratio, m: Metacentric, sm: Submetacentric.

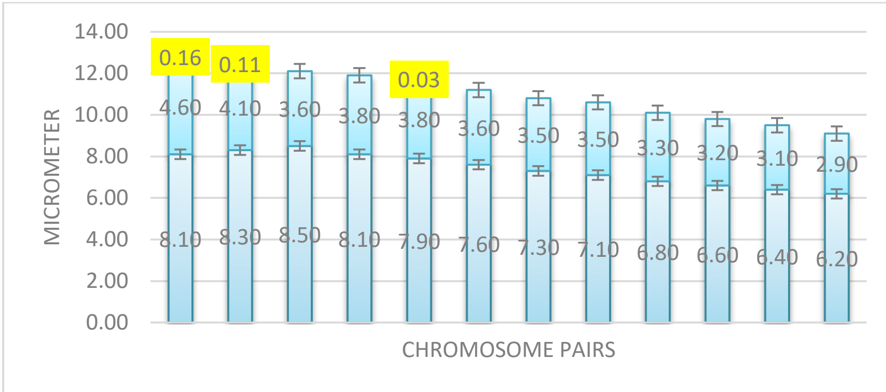

Figure 5. Somatic haploid ideogram in Van ecotypes (Wheat Tir)

Discussion

Microscopic examination and comparison of the results obtained by applying different types and compounds (two types of pretreatment solution with two time treatments, two types of fixative solution and two types of stain solution) on root tip meristem cells showed that using of pretreatment α-bromonaphthalene 1% for 3 hours, fixation of samples with Karnoy 1 I for 24 hours and staining of chromosomes with %2 aceto-orcein solution have the best results in terms of observational comparison of chromosome resolution and the ability to measure their properties compared to other compounds presented. The somatic chromosome numbers of all investigated ecotypes are hexaploid with 2n=6x=42 and the averages of chromosomes length ranged from 4.60 ± 0.02 µm to 16.05 ± 0.02 µm. The longest chromosome was observed in chromosome number 1 from ecotype 3 belongs to Muradiye and the shortest one was related to the chromosome number 21 from ecotype 4 in Patnos. The comparison of morphometric parameters of studied ecotypes are given in Table 1-5. Patnos has the smallest value, according to parameter of haploid complement. The karyotypic characteristics of wheat and Agilops species was previously studied (Feridooni *et al.*, 2017). Cytogenetic analysis on thirty populations from six species of wheat and Agileops showed that the studied populations had an almost symmetrical karyotype. Since 1970, there are several cytogenetic studies on chromosomes in hexaploid wheat Triticum aestivum L. (2n=6x=42) using different banding and molecular methods (Gerlach, 1977; Gill *et al.*, 1977; Gill, 1987). However, the banding pattern reported by various researchers showed the inconsistencies due to intra- and inter band polymorphism, which may be due to the non-standard chromosome analysis and banding techniques used in different laboratories. Therefore, in the absence of appropriate molecular facilities, using of normal and accurate karyotype analysis could be useful to observe the structural differences in different ecotypes. In fact, standard karyotype analysis based on chromosome length and arm ratio is still valid.

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