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The Detailed Karyotype Analysis, Karyotype Asymmetry and Polyploidy in Hemp (*Cannabis sativa* L.)

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Abstract: Hemp (*Cannabis sativa* L.) is an economical plant with a diploid chromosome number of $2n = 20$ and used in many areas, especially in industry. The aim of this study is to perform detailed karyotype analysis and detailed chromosomal measurements using five different genotypes of the plant known as diploid chromosome number $2n = 20$, to determine the karyotype asymmetry for the first time, and to investigate the polyploidy variations. The diploid chromosome number and karyotype formula were $2n = 2x = 20 = 18m + 2sm$. The karyotype had small metacentric and submetacentric chromosomes. The smallest chromosome length, largest chromosome length, total haploid chromosome length, and average haploid chromosome length were 2.41, 3.55, 29.87, and 2.99 µm, respectively. Intrachromosomal and interchromosomal karyotype asymmetries were calculated using many different parameters, mainly M_{CA} (mean centromeric asymmetry) and CV_{CL} (variation coefficient of chromosome length). According to the asymmetry values, the species had quite symmetrical karyotype. Although the diploid chromosome number was known as $2n = 20$, detailed karyotype analysis and asymmetry data were presented for the first time by this study. In addition, in the study carried out on five different genotypes, polyploidy variation was detected in one genotype.

Kenevirde (*Cannabis sativa* L.) Detaylı Karyotip Analizi, Karyotip Asimetrisi ve Poliploidi

Anahtar Kelimeler
Kenevir,
Kromozom,
Karyoloji

Öz: Kenevir (*Cannabis sativa* L.), diploid kromozom sayısı $2n = 20$ olan ve sanayi başta olmak üzere birçok alanda kullanılan ekonomik bir bitkidir. Bu çalışmanın amacı, diploid kromozom sayısı $2n = 20$ olarak bilinen bitkinin beş farklı genotipini kullanarak detaylı karyotip analizini ve detaylı kromozomal ölçümlerini yapmak, karyotip asimetrisini ilk kez belirlemek ve poliploidi varyasyonlarını araştırmaktır. Diploid kromozom sayısı ve karyotip formülü, $2n = 2x = 20 = 18m + 2sm$'dir. Karyotip, küçük metasentrik ve submetasentrik kromozomlara sahiptir. En küçük kromozom uzunluğu, en büyük kromozom uzunluğu, toplam haploid kromozom uzunluğu ve ortalama haploid kromozom uzunluğu sırasıyla 2.41, 3.55, 29.87 ve 2.99 µm'dir. İntrakromozomal ve interkromozomal karyotip asimetrileri, M_{CA} (ortalama sentromerik asimetri) ve CV_{CL} (kromozom uzunluğunun varyasyon katsayısı) parametreleri kullanılarak hesaplandı. Asimetri değerlerine göre tür oldukça simetrik karyotipe sahiptir. Diploid kromozom sayısı $2n = 20$ olarak bilinmesine rağmen detaylı karyotip analizi ve asimetri verileri ilk kez bu çalışma ile ortaya konmuştur. Ayrıca beş farklı genotip üzerinde yapılan çalışmada bir genotipte poliploidi varyasyonu tespit edilmiştir.

1. INTRODUCTION

Hemp (*Cannabis sativa* L.) is an economical plant with a diploid chromosome number of 2*n* = 20 and used in many areas, especially in industry. Its fibers, stems and seeds are used in many different areas such as energy, construction, medicine, and food. In archaeological studies, the remains of fabric made from hemp were found in 8000 BC and the hemp was the first plant used to make rope. It is a plant originating from a very wide distribution area extending from the Caspian Sea and the Himalayan mountains to China and Siberia. It is known that it was found in Anatolia in 700-800 BC [1, 2]. Today, it is classified as drug type (medicinal cannabis or marijuana) and fibre type (industrial hemp), depending on its cannabinoid content and use [3].

Modern phylogenetic research mainly relies on morphological and molecular characters, but chromosomal characters and karyotype evolution play an important role to evaluate origin of the plants and interspecific relationships [4, 5]. The fundamental characters of chromosomal data are basic number (*x*), diploid number (2*n*), and chromosome lengths, which are total length, long arm length, short arm length, and relative length. These basic characters can be changed numerically by aneuploidy and polyploidy mechanisms, or structurally by rearrangements such as deletions, inversions, duplications, and translocations. All these mechanisms produce the intrachromosomal and interchromosomal variations in karyotypes by changing centromere position and chromosome morphology [6, 7].

In *Cannabis sativa*, the diploid chromosome number is 2*n* = 20 consisting of eighteen autosomal chromosomes and one pair sex chromosomes [8-12]. The aim of this study is to perform detailed karyotype analysis and detailed chromosomal measurements using five different genotypes of the plant known as diploid chromosome number 2*n* = 20, to determine the karyotype asymmetry (intrachromosomal and interchromosomal asymmetry) for the first time, and to investigate the polyploidy variations.

2. MATERIAL AND METHOD

2.1. Plant Material

Within the scope of the study, five different hemp genotypes were used as material. They were Eminönü, Kavacık, Maltepe, Spice bazaar, and Van genotypes. The hemp seeds were obtained from the Institute of Hemp Research.

2.2. Cytogenetic Procedure

(i) Germination; the hemp seeds were germinated between moist filter papers in petri dishes at room temperature. (ii) Pretreatment; the germinated seeds were pretreated in alpha-monobromonaphthalene solution at 4°C for 16 h. (iii) Fixation; then the materials were fixed in fixative solution containing ethanol:glacial acetic acid (3:1, v:v) at 4°C for 24 h. (iv) Hydrolysis; the materials were hydrolyzed in 1 N HCl at 60°C for 12 min. (v) Staining; the materials were stained in acetoorcein (2%) for 2 h. (vi) Preparation; the preparations were made by the squash method [13].

2.3. Karyotype Analysis

At least 10 well-spread metaphase plates were used for chromosomal measurements. The chromosomal measurements were performed by the KaryoType 2.0 software using well-spread metaphase plates. The following parameters were used to karyotype analysis: short arm length (p), long arm length (q), total chromosome length (CL = p + q), total haploid length (THL = CL_1 + CL_2 + CL_3 + ……. CL_n), relative length (RL) = [(p + q)/THL] × 100, mean chromosome length (MCL = THL/*n*), and centromeric index (CI) = [(p)/(p + q)] × 100. The karyotype formulae were detected based on arm rates (r = q/p) as described by Levan et al. [14]. The monoploid ideograms were drawn by the ChemDraw Professional software depending on short arm and long arm lengths.

Karyotype asymmetries were estimated by intrachromosomal asymmetry (M_{CA} = mean [(Σq − Σp) / (Σq + Σp)] × 100) and interchromosomal asymmetry (CV_{CL} = [SD (standard deviation) / MCL] × 100). M_{CA} and CV_{CL} refer to the mean centromeric asymmetry and variation coefficient of chromosome length, respectively [15, 16].

3. RESULTS AND DISCUSSION

The somatic metaphase chromosomes and monoploid ideogram of *Cannabis sativa* were represented in Figure 1 and Figure 2. In *Cannabis sativa*, the diploid chromosome number was determined as 2*n* = 20 in all genotypes and 2*n* = 20, 40 in Eminönü genotype. No satellites and seconder construction were observed in *Cannabis sativa* karyotype measurements. Srivastava et al. [8] reported the secondary constriction in chromosome 3.

The chromosomes were type of metacentric and submetacentric and the karyotype formulae were 2*n* = 2*x* = 20 = 18m + 2sm in all genotypes and 2*n* = 4*x* = 36m + 4sm in Eminönü genotype (Table 1). It was reported that the diploid chromosome number of *Cannabis sativa* was 2*n* = 20 [10-12] and the karyotype formula was 18m + 2sm [8, 9]. These data were compatible with our study results.

Eminönü genotype showed polyploidy variation with ploidy level of 4*x*. There was only one report in the literature regarding the polyploidy variation of *Cannabis sativa*. Moteg [17] reported that the chromosome number and ploidy level were 2*n* = 20, 40, 80 and 2*x*, 4*x*, 8*x*, respectively. Since our country has very different climate, soil, and geographical characteristics, it has a suitable environment for plant karyological variations. Plant communities can also show morphological and genetic variations at various levels by adapting themselves to their environment.

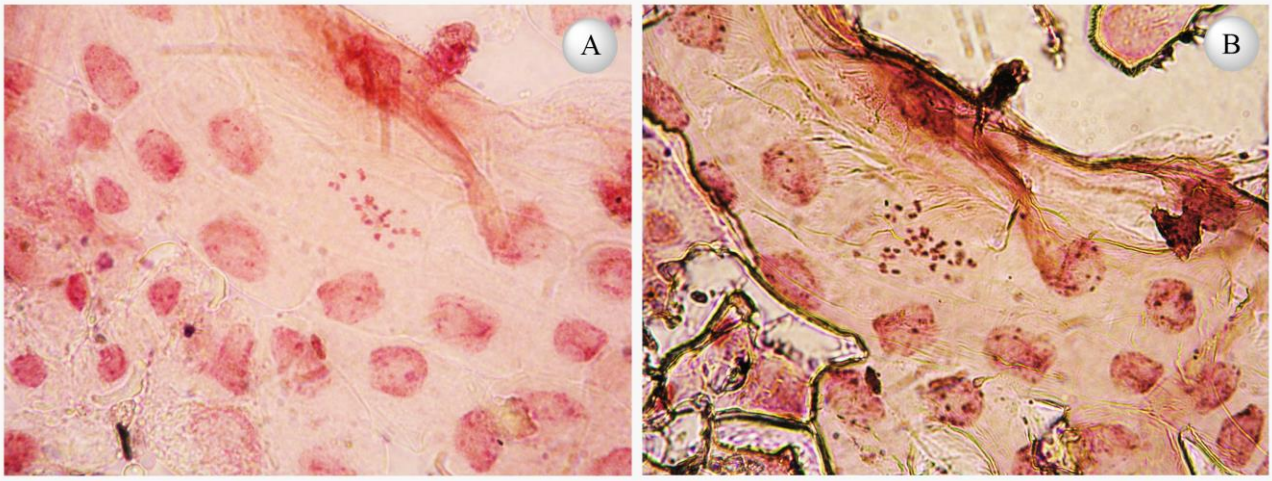

Figure 1. Metaphase chromosomes (2*n* = 20) of the spice bazaar genotype (A). Polyploidy variation observed in Eminönü genotype (2*n* = 40) (B).

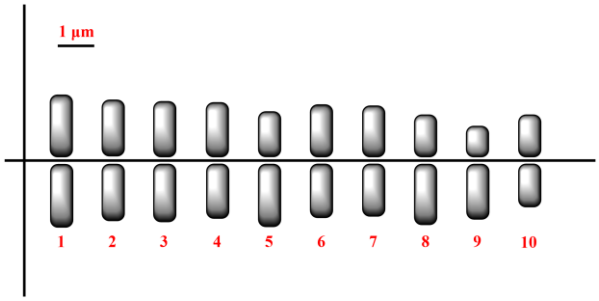

Figure 2. The monoploid ideogram of *Cannabis sativa*.

Table 1. The diploid chromosome numbers and karyotype formulae of hemp genotypes.

Genotype	2*n*	Karyotype formula
Eminönü	20 40	18m + 2sm 36m + 4sm
Kavacık	20	18m + 2sm
Maltepe	20	18m + 2sm
Spice bazaar	20	18m + 2sm
Van	20	18m + 2sm

The smallest chromosome length, the largest chromosome length, total haploid chromosome length was average chromosome length were 2.41 µm, 3.55 µm, 29.87 µm, and 2.98 µm, respectively. The values of relative length and centromeric index ranged from 8.06-11.88% and 35.77-50.00%, respectively (Table 2). The low centromeric index indicated movement away from the median region. The chromosome 9 was an asymmetric chromosome with the lowest centromeric index and the highest arm ratio.

Karyotype asymmetries were calculated with CV_{CL} and M_{CA} values, which were considered the most reliable interchromosomal and intrachromosomal parameters. CV_{CL} and M_{CA} values were 11.50 and 7.07, respectively. According to these values, the karyotype of *Cannabis sativa* was quite symmetrical.

Table 2. Detailed chromosome measurements of *Cannabis sativa*.

Chromosome pair	Length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio	Relative length (%)	Centromeric index (%)	Chromosome type
1	3.55	1.80	1.75	1.03	11.88	49.30	metacentric
2	3.25	1.65	1.60	1.03	10.87	49.23	metacentric
3	3.20	1.63	1.57	1.04	10.71	49.06	metacentric
4	3.08	1.54	1.54	1.00	10.30	50.00	metacentric
5	3.06	1.78	1.28	1.39	10.24	41.83	metacentric
6	3.02	1.52	1.50	1.01	10.10	49.67	metacentric
7	2.94	1.49	1.45	1.03	9.84	49.32	metacentric
8	2.92	1.72	1.20	1.43	9.77	41.10	metacentric
9	2.46	1.58	0.88	1.80	8.23	35.77	submetacentric
10	2.41	1.22	1.19	1.03	8.06	49.38	metacentric

4. CONCLUSION

In the present study, it was recorded only one chromosome number (2*n* = 20) in all genotypes excluding polyploidy (2*n* = 40) in Eminönü genotype. The polyploidy was not very common in *Cannabis sativa* and in this respect, it was an important variation. In addition, the karyotype asymmetry was reported for the first time. The results made a significant contribution to *Cannabis* cytotaxonomy.

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