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KARYOTYPE ANALYSIS OF EIGHT TURKISH VETCH (*VICIA SATIVA* L.) CULTIVARS

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Abstract

Mitotic chromosomes of 8 Turkish cultivars of *Vicia sativa* L. were subjected to karyotype analysis using squash method. They were analysed cytologically by conventional feulgen staining to identify variation in the morphology of indvidual chromosomes and relationship between them. The results showed that the chromosome numbers of *Vicia sativa* L with 2n=12 were metacentric with submedian orientation. Karyotypes of the studied cultivars had small variations and divergence in positions of short and long arms, which showed that they have considerable potential for use in breeding programmes.

Introduction

The genus *Vicia* includes over 160 species with a primarily Euro Asiatic distribution (Kupicha 1976, Allkin *et al*., 1986, Maxted, 1995). The speciation in the genus is accompanied by variation in chromosome size (Creomonini *et al*., 1993) and hybridization is common in the genus (Yamamoto, 1986). This along with anthropogenic disturbance of primary habitats (Zohary & Plitmann, 1979) could explain the formation of *Vicia sativa* aggregate, a group considered to be in active evolution (Weber & Schifino-Wittmann, 1999). The aggregate is morphologically, karyologically and ecologically variable, which makes taxonomical limitation difficult (Hanelt & Mettin, 1989, Weber & Schifino-Wittmann, 1999) and makes them an interesting model for the study of karyotype.

The karyotypes of Turkish common vetch cultivars except for cultivars Sarıelçi and Karaelçi (Elçi, 1965) based on chromosome size and arm ratio has not been established previously. The objective of the study was to determine and compare karyotype variability among 8 Turkish vetch cultivars to find the hybridization compatibility. The results would provide information for production of hybrids responsible for increased yield, insect pest resistance and cold tolerance, which would serve as an important data base for future research and use of these cultivars in hybridization programmes.

Materials and Methods

Seeds of 8 cultivars of vetch viz., Karaelçi, Sarıelçi, Kubilay 82, Ürem 79, Emir, Uludağ, Nilüfer and Çubuk used in the study were obtained from the germplasm collection of the Department of Field Crops, Faculty of Agriculture, University of Ankara, Turkey. 10-15 mm long roots of two-three day old seedlings germinated from seeds in Petri dishes were used for accumulation of cells in metaphase as described by Elçi (1965). These roots were cut and treated with α monobromonaphthlene for 2 hours,

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followed by 30 minutes fixation with glacial acetic acid. All operations were performed at room temperature. Thereafter, roots were hydrolysed with 1 N HCl for 12 minutes and stained with feulgen. Synchronised root tips were then squashed in 1% lactopropionic orcein for chromosome preparations as described by Busch *et al*., (1996) and the lengths of chromosome and arm ratio were measured. At least 8 complete metaphase figures with clearly distinguishable chromosomes were measured using LUCIA software and average values of relative sizes of the chromosomes and their arms were used to construct idiograms. The total chromosome length were measured with the computer system. The rules for numbering chromosomes within idiograms generally followed those used by Hollings & Stace (1974).

The photomicrographs were taken with Zeiss research microscope fitted with a microphotographic attachment, using an oil immersion objective (100x). The karotypes were determined by examining about a well spread metaphase for each of the eight varieties. Five cells which were flat and the chromosomes well spread were photographed and arm lengths measured on prints enlarged to a total magnification of 4700. Each chromosome was identified on the basis of its total chromosome length. Analysis and identification of each chromosome was made using following parmeters.

Arm ratio = length of short arm / length of long arm (Heneen, 1962; Elçi, 1965)
Chromosome length = length of short arm + length of long arm (Elçi, 1965)
Relative chromosome length = length of indvidual chromosome/Total length of all chromosomes in the genome X 50 (Elçi, 1965)

Results and Discussion

The stained chromosomes of each of the respective cultivars and their idiograms are presented in Fig. 1 and 2. Relative chromosome length and arm ratio of the karyotyupes of cultivars is given in Table 1. The table shows that all cultivars presented 2n=12 karyotyotype. Relative chromosome length of cultivar Karaelçi and Sarielçi were compatible with the previous findings by Elçi (1965). No sattellite was observed on karyotype of cultivars, all having normal structure. All chromosomes were metacentric with centromeres in submedian region in confirmation with the results of Elçi (1965) and contradicted findings of Navratilova *et al*., (2003) who found one metacentric, four subcrocentric and one acrocentric chromosome. The results obtained by Hannelt & Metin (1966), Ladizinsky (1978) Weber & Shifino-Wittmann (1999), are also contradictory. They found five acrocentric chromosome pairs, one with a secondary constriction in the long arm and one marker metacentric chromosome.

All chromosomes were clearly discriminated and their relative sizes could be determined based on measurements of mitotic chromosome lengths; however, these differences were not big. Despite co existence of different taxa of *Vicia sativa,* the cultivars were not of mixed origin and had no or different cytotypes. This suggests that *Vicia sativa* is probably more stable. A comparison of chromosome length reveals that cultivar Ürem 79 and Uludağ were more variable compared to other six (Table 1). On the average, the longest chromosome length was found on chromosomes of Ürem 79 (1.94 ± 0.18 to 4.52 ± 0.60 μ) and the smallest on cultivar Uludağ (1.80 ± 0.06 to 3.77 ± 0.04 μ). The highest variability in standard deviation of chromosome length was found on chromosomes of Nilüfer followed by that of cv. Emir and Karaelçi. All other cultivars included in the study had lower standard deviation on each of the respective chromosomes.

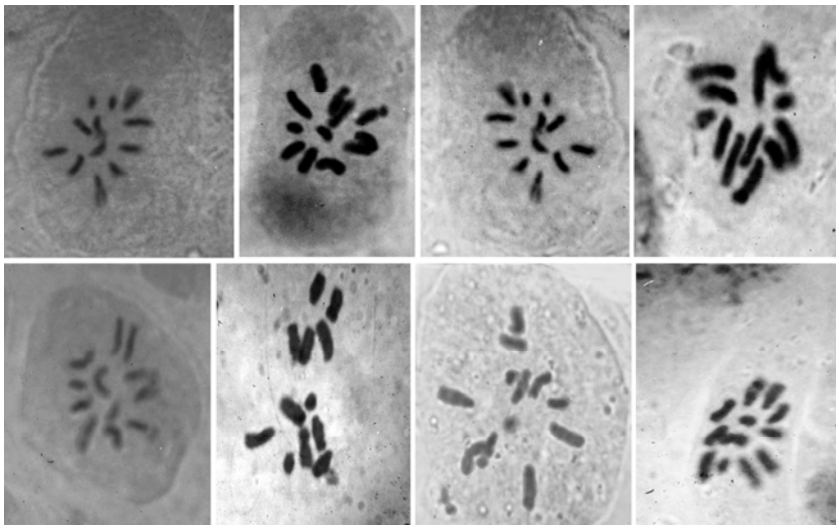

Fig. 1. Chromosomes of cultivars Kubilay, Karaelçi, Çubuk, Nilüfer, Ürem, Emir, Sarıelçi, and Uludağ of *Vicia sativa* L. stained with feulgen.

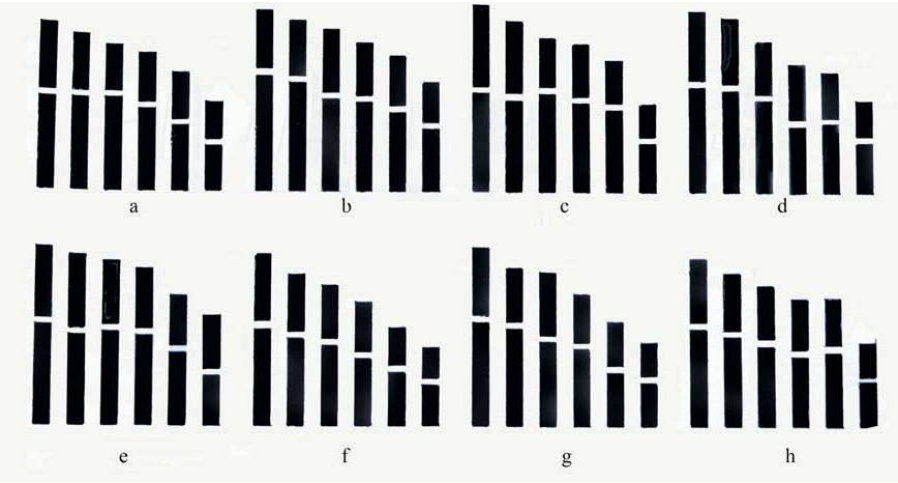

Fig. 2. Idiogram of cultivars Kubilay, Karaelçi, Çubuk, Nilüfer, Ürem, Emir, Sarıelçi, and Uludağ

A great homology of chromosomes could be identified, in the karyotypes of the studied cultivars, with small variations and divergence in chromosome length and positions of short and long arms. Although, chromosome length in itself has limited value in identification;. the variations in the arm ratio of the same chromosome among varieties is attributed to inversions, translocations as well as deletions during evolution. It was supposed that the differences in the length of chromosomes was due to chromatin elimination in evolution. These small variations in the morphology of chromosomes further indicates that all cultivars belonged to *V. sativa* and did not belong to different cytotypes or mixed genotypes.

Table 1. Chromosome length, arm ratios (length of short arm/length of long arm) and relative chromosome lengths of eight Turkish vetch cultivars supplied with standard deviations.

Chr. No.	Chromosome length	Arm ratio ± sd	Relative chromosome length ± sd	Chromosome length	Arm ratio ± sd	Relative chromosome length ± sd
	Kubilay 82			**Karaelçi**		
1	3.78 ± 0.41	0.69 ±0.16	5.15 ± 0.17	4.21 ± 0.73	0.64 ± 0.27	5.26 ± 0.30
2	3.62 ± 0.37	0.60 ±0.12	4.76 ± 0.28	3.99 ± 0.84	0.55 ± 0.20	4.82 ± 0.50
3	3.23 ± 0.48	0.60 ±0.28	4.41 ± 023	3.60 ± 0.52	0.68 ± 0.24	4.36 ± 0.22
4	3.09 ± 0.46	0.62 ±0.18	4.23 ± 0.21	3.42 ± 0.50	0.62 ± 0.13	4.04 ± 0.24
5	2.66 ± 0.44	0.66 ±0.13	3.54 ± 0.28	3.53 ± 0.32	0.63 ± 0.14	3.69 ± 0.39
6	2.05 ± 0.34	0.73 ±0.21	2.68 ± 0.31	2.38 ± 0.53	0.69 ± 0.22	2.86 ± 0.24
	Çubuk			**Nilüfer**		
1	4.40 + 0.61	0.80 ± 0.09	5.31 ± 0.25	4.44 ± 1.57	0.69 ± 0.23	5.36 ± 0.20
2	4.15 + 0.49	0.75 ± 0.09	4.92 ± 0.18	4.16 ± 1.57	0.65 ± 0.19	4.94 ± 0.30
3	3.81 + 0.70	0.60 ± 0.18	4.46 ± 0.14	3.74 ± 1.32	0.65 ± 0.16	4.42 ± 0.13
4	3.48 + 0.59	0.56 ± 0.18	4.17 ± 0.20	3.52 ± 1.45	0.71 ± 0.11	4.08 ± 0.33
5	3.17 + 0.70	0.57 ± 0.15	3.68 ± 0.29	2.84 ± 0.91	0.77 ± 0.18	3.54 ± 0.43
6	2.07 + 0.30	0.70 ± 0.12	2.49 ± 0.38	2.18 + 0.72	0.71 ± 0.12	2.56 ± 0.23
	Ürem 79			**Emir**		
1	4.52 ± 0.60	0.62 ± 0.18	5.11 ± 0.25	3.89 ± 0.87	0.71 ± 0.16	5.80 ± 0.80
2	4.36 ± 0.67	0.70 ± 0.08	5.09 ± 0.34	3.31 ± 0.55	0.70 ± 0.13	5.04 ± 0.29
3	3.84 ± 0.47	0.72 ± 0.16	4.50 ± 0.16	2.86 ± 0.58	0.78 ± 0.19	4.40 ± 0.33
4	3.61 ± 0.35	0.69 ± 0.18	4.22 ± 0.06	2.69 ± 0.44	0.74 ± 0.13	3.77 ± 0.51
5	3.03 ± 0.09	0.67 ± 0.02	3.58 ± 0.40	2.22 ± 0.55	0.69 ± 0.25	3.12 ± 0.69
6	1.94 ± 0.18	0.69 ± 0.01	2.29 ± 0.39	1.81 ± 0.59	0.74 ± 0.15	2.54 ± 0.74
	Sarıelçi			**Uludağ**		
1	3.94 ± 0.58	0.66 ± 0.20	5.57 ± 0.60	3.77 ± 0.04	0.65 ± 0.01	5.21 ± 0.10
2	3.62 ± 0.37	0.56 ± 0.08	5.05 ± 0.39	3.52 ± 0.01	0.64 ± 0.16	4.86 ± 0.06
3	3.20 ± 0.18	0.66 ± 0.18	4.45 ± 0.33	3.20 ± 0.09	0.68 ± 0.05	4.41 ± 0.08
4	2.92 ± 0.17	0.60 ± 0.11	4.15 ± 0.22	2.94 ± 0.07	0.72 ± 0.09	4.07 ± 0.06
5	2.33 ± 0.27	0.78 ± 0.14	3.27 ± 0.45	2.85 ± 0.00	0.64 ± 0.13	3.94 ± 0.04
6	1.77 ± 0.50	0.76 ± 0.14	2.44 ± 0.61	1.80 ± 0.06	0.73 ± 0.05	2.48 ± 0.06

The karyotype study of the vetch cultivars further indicate that they are cytologically stable and have considerable potential for use in breeding programmes. Morphologic differences in the size and morphology of the chromosomes could be attributed to progressive change in evolution of karyotype during natural or manual selection. Establishment of karyotype also allowed us to address the question of identification of homologous chromosomes among the cultivars. Included cultivars except Sarielçi have almost same growing pattern and are suitable for cultivation in warm Aegean region of Turkey both during summer and winter. The cultivar Sarielçi is better adapted to the severe colds of Central Anatolia, where temperature drops down to -10^{o}C during winter. Homology of chromosomes suggests easy crossability of cultivars to breed new winter hardy cultivars suitable for cold of Central and Eastern Anatolia Turkey.

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