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Karyotypical Identification of Some Important Alfalfa (*Medicago sativa* L.) Lines in Turkey

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ABSTRACT

Chromosome line and characterization of nine Turkish alfalfa (Medicago sativa L.) lines were investigated using karyological techniques in this study. Root tips were obtained from germinated alfalfa seeds in petri dishes at room temperature (25°C) for visualizing somatic chromosomes. The chromosome numbers of nine alfalfa lines were determined as 2n=4x=32. The karyotype formula of 2n=32= 30m+ 2sm (30 median + 2 submedian) were noted for Line 1, 2and 3. Whereas, Line 4, 6, 8, 9 showed the karyotype formula of 2n=32=32m (32 median). The karyotype formula of Line 5 and 7 were 2n=32=28m+4sm (28 median + 4 submedian). Satellite chromosome pairs were observed from Line 5. The results of the karyotype asymmetry index analysis showed that intrachromosomal asymmetry is higher than interchromosomal asymmetry in the karyotypes of alfalfa lines.

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Introduction

Cultivated alfalfa, *Medicago sativa* L., is an autotetraploid (Stanford, 1951) derived from the *Medicago sativa-falcata* complex, which includes a number of species and subspecies that share the same karyotype (Quiros and Bauchan, 1988). Also, cultivated tetraploid alfalfa is perennial autotetraploid (2n=4x=32), cross-pollinated forage legume and the most important cultivated forage plant in the World. *Medicago sativa* is composed of several perennial, outcrossing, and often interfertile taxa from section *Medicago* of the genus *Medicago*, which has agriculturally and economically important species (Small, 1989). In flora of Turkey, alfalfa has about 60 species in the World of which 40 annual and perennial taxa are reported. This shows that Turkey is a major gene hub for alfalfa. Three major alfalfa ecotypes namely Kayseri, Eastern Anatolian, and Bayindir are found in Turkey (Genckan, 1983). By virtue of alfalfa's wide adaptation ability, various subspecies that can grow in various climate and soil conditions, can contribute to increased crop yield and has facilitated inbreeding number of new varieties. All alfalfa varieties are divided into *Medicago sativa* L., Hybrid alfalfa (*M. sativa* × *M. falcata*), Turkistan and Peru group of varieties. Turkey has 7 different agro-climatic zones, and there is a dire need to develop alfalfa varieties specific for each region. Therefore, it is important to properly identify and breed alfalfa varieties that can be easily grown especially in the warm coastal and Southeastern Anatolia region with a good adaptation ability and suitability for these ecologies. Generally, alfalfas are synthetic populations with a wide gene variety (Sengul and Sagroz, 2003). The most important condition in synthetic populations is that it is made up of the number of lines with the same number of chromosomes and similar phenotypes but may have some variations in agronomic characteristics. Therefore, alfalfa varieties may carry similar features from their parental plants, especially in terms of cytology, and do not have problems in generating seeds. Therefore, there is a need to characterize these plants for morphological characteristics besides carrying cytological and molecular studies as an aid to selection in maternal plant selection. The affinity between the plants on this matter will resolve the problems in seed-set efficiency.

The materials used in this study belonged to the Peru variety and the plants included in this variety originated in the southern regions of the Americas. Alfalfa plants belonging to the Peru variety are sensitive to cold and they grow rapidly. These were selected for their features with have abundant leaves, low cellulose, and rapid growth after each cutting. Additionally, these plants are also utilized as synthetic materials with the intent of transferring their good features to other lines/varieties with desirable traits by pollination. It is presumed that these hybridization practices do not disturb the basic chromosome apparatus of the cells and are transferred to the next generations without any physical change. Therefore, it is very important to control if the hybrid or synthetic plants' have the same number of chromosomes, morphologies and karyotype. The fact that alfalfa has small chromosomes is a disadvantage carrying out cytological studies. This condition restricts to carry out of karyotype studies in alfalfa. The objective of this study was to identify the karyotypical features of alfalfa lines that have been eliminated according to certain features and touch on their assessment status in synthetic variety breeding.

Material and Method

The experimental material was selected from Peru alfalfas grown in Urfa and Akcakale and consisted of 49 superior alfalfa ecotypes in terms of fresh herbage yield and growth. These were multiplied in greenhouses of Ankara University in pots. Among these 9 plants (table 1) were re-selected in terms of rapid growth, the number of leaves, per plant after cutting, and seed yield. These were singular selection lines and named as 1, 2, 3, 4, 5, 6, 7, 8, 9.

All cytological observations were made from root tips. For visualizing somatic chromosomes, root tips were obtained from germinated alfalfa seeds germinated in petri dishes at room temperature (25°C). 2-3 days old root tips were pre-treated in 6% α-monobromonaphtalane in +4°C for 7.5 h and then fixed in glacial acetic acid for 30 minutes and transferred to 70% ethanol for long storage. When the root tips were analyzed, they hydrolyzed with 1 N HCl for 18 minutes at room temperature (25°C) after hydrolyzing, root tips stained with 2% aceto orcein in dark for 2.5 h. Subsequently, these were squashed in 45% acetic acid. The slides were observed with Olympus BX-51 microscope and photographed with an Olympus BX-51 and magnification was 8000×. Six chromosomal parameters were measured by Micro Measure 3.3 program (Reeves, 2001); i.e., chromosome length, relative length, the long arm and short arm lengths, arm ratio, centromeric index (Table 2). The ideograms were drawn based on long arm length/short arm length. Karyotype formulas of all *Medicago sativa* lines were determined using the method described by Levan et al. (1964). The ideograms were prepared with measurements taken on enlarged micrographs of ten well-spread metaphase plates.

Table 1. Localities of investigated of alfalfa lines used for karyological studies

Line Name	Localities	Latitude	Longitude
1	Koruklu Village-Akçakale-Sanliurfa	36.900160	38.924449
2	Tekyamac Village-Sanliurfa Merkez	37.050552	38.562597
3	Bakimli Village-Sanliurfa Merkez	37.190125	39.034939
4	Akziyaret Village- Sanliurfa Merkez	37.327905	38.819366
5	Bugluca Village-Bozova-Sanliurfa	37.247347	38.610326
6	Horzum Village-Karaköprü-Sanliurfa	37.306815	38.774623
7	Mustafacık St.-Karaköprü-Sanliurfa	37.329686	38.706869
8	Akpınar St-Sanliurfa	37.378006	38.757973
9	Gölgen St.-Sanliurfa	37.436183	38.908257

Table 2. Formula of chromosomal parameters and karyotype asymmetry

	Chromosomal parameter or asymmetry index	Formula	Reference
Chromosomal parameters	Arm ratio	AR=L/S	
	Chromosome Length	CL=L+S	
	Relative length of chromosome	RL(%)= (CL/ ∑ CL) ×100	
	Centromeric index	CI= S/CL	
	Chromosome type		Levan et al. 1964
Karyotypical parameters or asymmetry indices	The difference of relative length	DRL: MaxRL – MinRL	Zarco 1986
	Total form percentage	TF=100 × ΣS/CL	Huziwara 1962
	Intrachromosomal asymmetry index	A_1 = [1 – (Σ (short arm/long arm)/n)]	Zarco 1986
	Interchromosomal asymmetry index	A_2 = [standard deviation (S)/mean length (X)]	Zarco 1986
	Mean centromeric asymmetry (MCA)		Peruzzi and Eroglu 2013

Table 3. Average karyological values of nine Turkish alfalfa lines

Lines Number	2n	Karyotype Formula (2n)	S (μm)	L (μm)	C (μm)	R	CI	TCL (μm)	DRL	TF (%)	A_1	A_2	M_{CA}
1	32	30 m + 2 sm	0.37	0.47	0.84	1.31	0.44	26.76	2.24	43.72	0.22	0.11	12.44
2	32	30 m + 2 sm	0.33	0.44	0.76	1.33	0.45	24.40	3.36	43.36	0.24	0.15	13.74
3	32	30 m + 2 sm	0.42	0.54	0.96	1.31	0.44	30.62	2.53	43.83	0.22	0.11	12.30
4	32	32 m	0.28	0.34	0.62	1.25	0.45	19.74	1.82	44.68	0.19	0.08	10.64
5	32	28 m + 4 sm	0.39	0.52	0.93	1.37	0.45	29.74	6.32	42.35	0.24	0.18	14.13
6	32	32 m	0.22	0.28	0.50	1.32	0.43	16.04	2.50	43.64	0.23	0.11	13.03
7	32	28 m + 4sm	0.31	0.43	0.74	1.40	0.43	23.66	3.02	42.17	0.25	0.12	14.79
8	32	32 m	0.45	0.55	0.99	1.23	0.45	31.82	2.70	45.07	0.18	0.10	10.02
9	32	32 m	0.31	0.40	0.72	1.30	0.43	23.20	3.01	43.62	0.21	0.13	12.36

Abbreviations: CP: centromeric position; S: short arm length; L: long arm length; C: total chromosome length; R: arm ratio; RL: relative length; CI: centromeric index; DRL: difference of relative length; TF: total form percentage; A_1: intrachromosomal asymmetry index; A_2: interchromosomal asymmetry index; Mca: mean centromeric asymmetry

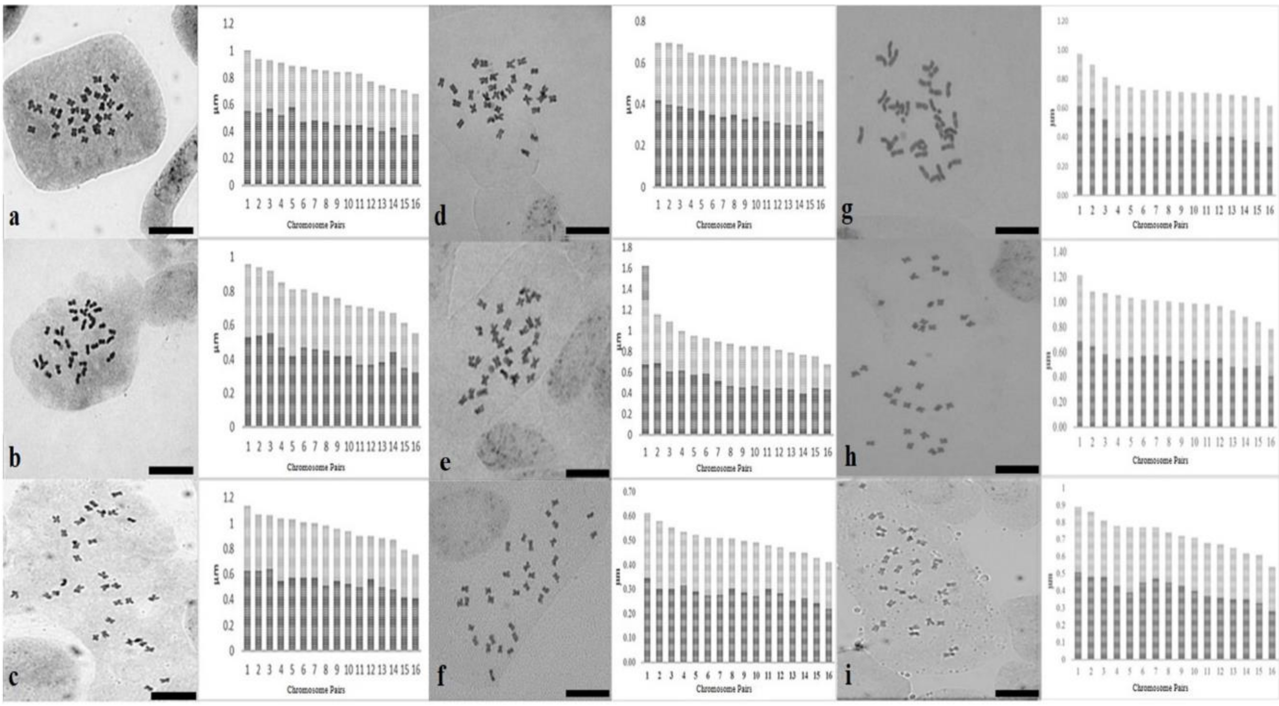

Figure 1. Somatic chromosomes and ideograms of Line; (a) 1, (b) 2, (c) 3, (d) 4, (e) 5, (f) 6, g) 7, h) 8, i) 9 of alfalfa (scale bar =10 µm)

Results

Somatic chromosome lines and cytological properties of nine alfalfa lines were determined. According to the results; chromosome lines of nine alfalfa were calculated as 2n=4x=32 (Falistocco, 1987; Mariani et al., 1996; Cluster et al., 1997; Zhang et al., 2008; Wang et al., 2009; Albayrak et al., 2015, Zarifi et al., 2018).

Average karyological characteristic values are presented in Table 3. The Line 1, 2 and 3 showed karyotype formula of (2n=32= 30m+ 2sm). Whereas, Line 4, 6, 8, 9 had metacentric chromosomes with (2n=32=32m). The Line 5, 7 showed (2n=32=28m+ 4sm) type of chromosomes, respectively. Satellite chromosome pairs were observed from Line 5. According to average karyologic characterization values; average short arm length varied 0.28 µm *(*Line 4) to 0.45 µm (Line 8*)*. Besides, average long arm length values ranged 0.28 µm (Line 6) to 0.55 (Line 8). Average chromosome length (C) ranged 0.50 (Line 6) to 0.99 (Line 8). Average arm ratio values varied 1.23 (Line 8) to 1.40 (Line 7). Average centromeric index values ranged 0.43-0.45 in nine alfalfa lines. Total chromosome lengths ranged from 16.04-31.82 µm.

Karyologic parameters are presented in Table 3. Somatic metaphases of nine alfalfa lines and their ideograms are illustrated in Figure 1. Comparing total chromosome length, Line 9 was the shortest (16.04 µm) and Line 8 was the longest (31.82 µm). The maximum long arm length was measured in *"*Line 8*"* (0.55 µm). Minimum short arm length was calculated with *"*Line 4*"* (0.28 µm). The maximum arm ratio of 1.40 was noted on "Line 7*"*. The smallest arm ratio of 1.23 was measured on "Line 8" with. *"*Line 2, 4, 5, 8*"* had the equal and the largest centromeric index (0.45). "Line 6, 7, 9" had the smallest centromeric index (0.42). The results of the karyotype asymmetry index analysis showed that intrachromosomal asymmetry was higher than interchromosomal asymmetry in the karyotypes of alfalfa lines (Table 3). Line 7 had the highest A_1 (0.25) and M_{ca} (14.79) value and the lowest TF (42.17%) value. Therefore, Line 7 was determined as the most asymmetric karyotype. On the contrary, Line 8 showed the most symmetric karyotype among the active genotypes with its A_1 (0.18) and M_{ca} (10.02) values and the highest TF (45.07%) value. When the interchromosomal asymmetry results were compared (Table 3), Line 5 showed the highest A_2 (0.18) and DRL (6.32) values and was determined as the most asymmetric karyotype among the lines. Line 4 had the lowest A_2 (0.08) and DRL (1.82) values. For the intrachromosomal asymmetry index; most asymmetric line was 7 (A_1: 0.25, TF: 42.17, Mca: 14.79), most symmetric line was 8 (A_1: 0.18, TF: 45.07, Mca: 10.02). For the interchromosomal asymmetry index; the most asymmetric line was 5 (A_2: 0.18, DRL: 6.32) and the most symmetric line was 4 (A_2: 0.08, DRL: 1.82).

Karyotype characteristics of Line 1 are shown in Table 3. Total chromosome length was determined as 26.76 µm. Maximum long arm length was calculated as 0.58 µm, minimum short arm length was measured as 0.29 µm. Arm ratio values ranged from 1.10-1.86. The relative lengths ranged 5.16-7.40 %. The results further showed that the centromeric index values ranged 0.35-0.48. Karyotype formula determined as 2n=4x=32= (30m + 2sm).

Karyotype characteristics of Line 2 are shown in Table 3. Total chromosome length was determined as 24.40 µm. The value of the minimum short arm length was 0.23 µm. Additionally; the maximum long arm length was measured as 0.55 µm. Arm ratios were calculated in range of 1.07-2.0. The relative lengths ranged from 4.51-7.87 %. The values of the centromeric index varied in a range of 0.34-0.74. Line 2's karyotype formula is 2n=4x=32= (30m + 2sm).

Karyotype characteristics of Line 3 are shown in Table 3. The value of total chromosome length and maximum long arm length was measured as 30.62 µm and 0.64 µm. Minimum short arm length was calculated as 0.34 µm. Arm ratio and relative length values were ranged 1.08-1.78 and 4.90-7.45 %, respectively. The centromeric index was calculated in a range of 0.38-0.48. Line 3 had a karyotype formula of 2n=4x=32= (30m + 2sm).

Karyotype characteristics of Line 4 are shown in Table 3. The value of the total chromosome length was calculated as 19.74 µm. Maximum long arm length was measured as 0.42 µm, while the minimum short arm length was calculated as 0.24 µm. Arm ratio and relative length values were among 1.05-1.49 and 5.27-7.09%, respectively. The centromeric index values ranged from 0.40-0.49 and the karyotype formula was 2n=4x=32=(32m).

Karyotype characteristics of Line 5 are shown in Table 3. Total chromosome length was determined as 29.74 µm. The maximum long arm and minimum short arm length were measured 0.69 µm and 0.24 µm, in the same order. Also, the satellite chromosome was observed in Line 5 and its length was calculated as 0.32 µm. Arm ratio values changed between 1.08-1.88. The relative lengths ranged from 4.57-10.89 %. Centromeric index of Line 5 was measured between 0.37-0.48. Line 5 has a karyotype formula 2n=4x=32= (28m + 4sm).

Karyotype characteristics of Line 6 are shown in Table 3. Line 6 has the smallest total chromosome length and it was determined as 16.04 µm. Maximum long arm length was calculated as 0.35 µm, minimum short arm length was measured as 0.18 µm. The arm length ratio has the values in a range of 1.07-1.66. The relative lengths ranged 5.14-7.64%. According to the results of the centromeric index for Line 6 was determined as 0.38-0.48. Line 6 has a formula 2n=4x=32=(32m).

Karyotype characteristics of Line 7 are shown in Table 3. Total chromosome length was determined as 23.66 µm. The maximum long arm length was calculated as with 0.62 µm, the minimum short arm length was measured as 0.27 µm. The arm ratio has the values in a range of 1.06-2.04. The relative lengths ranged from 5.20-8.22%. According to the results of the centromeric index, the values were determined among 0.34-0.49. Line 7 has a formula 2n=4x=32= (28m+ 4sm).

Karyotype characterization of Line 8 is shown in Table 3. Line 8 has the longest total chromosome length and it was determined 31.82 µm. Maximum long arm length was calculated as with 0.69 µm, minimum short arm length was measured 0.36 µm. The arm ratio has the values among 1.06-1.48. The relative lengths ranged from 4.93-7.64%. According to the results of the centromeric index, values were determined as 0.40-0.48. Line 8 had a karyotype formula of 2n=4x=32= (32m).

Karyotype characteristics of Line 9 are shown in Table 3. Total chromosome length was determined as 23.20 µm. The maximum long arm length was calculated as 0.51 µm, the minimum short arm length was measured as 0.26 µm. The arm ratio had the values in a range of 1.12-1.61. The relative lengths ranged from 4.66-7.67%. According to the results of centromeric index, the values were determined in between 0.39-0.47. Line 9 has a karyotype formula 2n=4x=32= (32m).

Discussion

The chromosome counting, measurements, and karyotype asymmetry are important data for the classification of plants in cytological way. The populations that have similar cytologic structure, are more successful in transferring good features from one to another in the process of synthetic variety breeding, such as alfalfa. Turkey is a very suitable area for genetic variability because of its location in a different climatological and geographical zone which are Mediterranean, Euro-Siberian, and Irano-Turanian (Martin et al., 2018). The genetic variability of alfalfa in Turkey is in the chromosome morphologies and chromosome numbers. The tetraploid alfalfa (*Medicago sativa* L.) (2n=4x=32) is the most commercially grown genus of *Medicago* (McCoy and Bingham, 1988). In this study, it is observed that alfalfa has 2n=4x=32 chromosomes, in the parallel results with Zhang et al. (2008), Lapina et al. (2011), Albayrak et al. (2015), and Fyad-Lameche et al. (2016), Zarifi et al. (2018). According to karyological results of Sadeghian and Hejazi (2014), Albayrak et al. (2015); arm ratios (R), total form percentage (TF) showed similar features, whereas total chromosome length and karyotype formula were not. Total chromosome length (TCL) of this study (exc. Line 4, 6) was bigger than Albayrak et al. (2015). Sadeghian and Hejazi (2014) karyotype formulas of their study were all metacentric. In this study, sub-metacentric chromosomes were specified in Line 1, 2, 3, 5, 7. Theoretically, differences observed in length and the structure of chromosome morphology could be explained away as gradual alterations, which occurred through the evolution of the karyotype during natural or manual selection (Benlioglu, 2020).

There is a lack of knowledges about karyotype asymmetry of *Medicago sativa* ssp. *sativa*. In the findings of this study, karyotype asymmetry of nine *Medicago* lines were determined. Sadeghian and Hejazi (2014), Farshadfar et al. (2018) which was studied karyotype asymmetry of the genus of *Medicago*, was stated that *Medicago* species had similar asymmetry properties (A_1, A_2, DRL). Zarifi et al. (2018) compared to Iranian tetraploid alfalfa cultivar to Turkey based tetraploid alfalfa populations, which was collected in Turkey's flora. Karyotype asymmetry of these alfalfas showed similar asymmetric features. In their study; intrachromosal asymmetry (A_1) ranged from 0.22-0.26, interchromosal asymmetry (A_2) between ranged from 0.10-0.13.

Conclusion

In the measurements done for the purpose of increasing seed-set efficiency and for their assessment as a maternal plant, lines numbered 1, 2 3 showed similar features in terms of the same karyotype formulas 2n=4x=32 (30 m +2 sm) and investigated features. Also, lines numbered as 4, 6, 8, 9 showed similar features in terms of karyotype formulas 2n=4x=32 (28 m +4 sm) and noted features. In terms of similar features, the line numbered as 4 showed the least similarities with other lines. Because Line 5 was the closest line to Line 1, 2, and 3 with a total chromosome length of 29.74 µm and in the maternal plant selection phase, it has a high potential to be included among these lines in hybridization programs for use as the maternal plant in synthetic variety breeding programs.

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