Caryologia

International Journal of Cytology,
Cytosystematics and Cytogenetics

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Comparative karyological analysis of some Turkish *Cuscuta* L. (Convolvulaceae)

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Abstract. This study investigated the somatic chromosome numbers and morphometric properties of 11 different taxa belonging to the genus *Cuscuta* L., which is one of the parasitic flowering plants and causes significant economic losses on agricultural products. For this purpose, the species were examined karyologically and compared statistically. Belonging to the genus *Cuscuta*, *C. campestris* Yunck., *C. hyalina* Roth, *C. kotschyana* Boiss., *C. babylonica* Aucher ex Choisy, *C. europaea* L., *C. kurdica* Engelm., *C. brevistyla* A.Braun ex A.Rich, *C. planiflora* Ten., *C. approximata* Bab., *C. lupuliformis* Krock. *C. palaestina* Boiss. the chromosome number and morphology of the species were investigated using karyological techniques. Chromosome numbers of the species; *C. kotschyana, C. babylonica, C. europaea, C. kurdica, C.planiflora* 2n=14; *C. campestris, C. hyalina, C. approximata, C. lupuliformis*, and *C. palaestina* 2n=28 and *C. brevistyla* 2n=42 is determined. Also, the species' chromosome number, total chromosome length, relative length, arm ratio, centromere index and centromere states, and karyotype asymmetry values were determined. Chromosome numbers of *C. kotschyana* and *C. kurdica* taxa were defined for the first time in this study. Thus, new data on the systematics of these species have been revealed.

Keywords: Convolvulaceae, *Cuscuta*, parasitic plant, Chromosome number, karyotype.

INTRODUCTION

Cuscuta (Dodder) is a member of the Convolvulaceae family, including about 200 different root parasite species. About 15-20 of these species cause severe problems in agricultural areas (Dawson et al., 1994). With this, most *Cuscuta* species are considered extinct in the wild, and some species even require conservation measures in nature (Costea and Stefanovic 2009a). *Cuscuta* species can be found in various habitats, including temperate, tropical, desert, riparian, coastal, high mountain, woodland, saltwater, and degraded environments (Costea et al., 2015). Like other parasitic plants, *Cuscuta* species play an essential role in ecosystems (Press et al. 1999).

Yunker (1932) divided the genus *Cuscuta* into three subgenera according to styles and stigma shapes. These; *Cuscuta* are Grammica (Lour.), Yunck, and Monogynella (Des Moul.). In the flora of Turkey, 15 species of these subgenera and two unknown species (*C. aratica* Butk. and *C. subuniflora* K. Koch), and one suspicious (*C. epilinum* Wiehe) species have been identified (Plitman, 1978). Since the vegetative parts of parasitic plants are generally reduced, flower characters are insufficient in taxonomy. This situation creates problems in diagnosis. For this reason, it is necessary to use some methods to identify species belonging to the genus *Cuscuta*. Studying chromosome numbers and structures can give valuable results in solving taxonomic problems (Taşar et al., 2018a; 2018b).

It has been determined that *Cuscuta* species generally have holocentric chromosomes and undergo inverted meiosis (Pazy and Putmann 1987; 1991; 1994).

Some morphological features are overlooked in plant determinations and classifications of classical taxonomy. Characteristics acquired according to environmental factors appear to be new features, confusing classification. For this reason, considering the characters in classical taxonomy, examining the chromosome numbers, structure and structures gives beneficial results in solving the problems (Taşar et al., 2018a; 2018b). In addition, statistical analyzes can be useful in morphological and anatomical studies recently (Genç et al. 2021; Arabacı et al. 2021; Dirmenci et al. 2019; Dirmenci et al. 2020; Açar and Satıl 2019; Açar and Taşar 2022). This study aims to reveal the karyological features of *Cuscuta* species distributed in Turkey, determine the relationships between them, and contribute to the genus's taxonomic classification.

MATERIAL AND METHODS

Material

Cuscuta samples, the study material, were obtained from the field. Localities of taxa are given in Table 1. Plant taxa were identified using the genus *Cuscuta* (Yuncker 1932) and Flora of Turkey. (Davis, 1978). The collected specimens have been turned into herbarium material and are kept in Van Yüzüncü Yıl University, Faculty of Agriculture, Plant Protection Department, and Bitlis Eren University, Biology Department.

Methods

Chromosome measurements

The seeds of the plant samples were sown in Petri dishes and germinated in an oven at 20-22 ºC. Roots reaching 1–2 cm in length from the germinated seeds were cut, kept in colchicine for 2 hours at room temperature, and subjected to pretreatment (Gedik et al., 2014). Then, the root tips were placed in Carnoy fixative (3:1) and fixed by keeping them in the refrigerator at +4 ºC for 24 hours. At the end of the period, root tips were hydrolyzed in 1N HCl in an oven at 60 ºC for 5-18 minutes. Root tips removed from hydrolysis were stained with Feulgen stain for 1 hour in a dark environment at room temperature. Then it was washed 2-3 times with tap water. For preparation, the growth meristem part was cut off with a sharp razor blade in a drop of 45% acetic acid on the slide, and the coverslip was closed. The best three somatic cells for each species were photographed using an Olympus BX53 microscope. The naming system of Levan (1964) was used to locate the centromere. The intra-chromosomal asymmetry index (A1) was calculated according to the formula proposed by Romero Zarco (1986). Interchromosomal asymmetry index (A2) and karyotype symmetry nomenclature were made according to Stebbins (1971).

Statistical analisyes

For analysis used, several formulas were established on chromosome characteristics. The measurements were built on haploid datasets. The calculations and abbreviations used in the analysis are as follows. TLC (total length of chromosomes), MTLC (mean of total length of chromosomes), MAX (maximum length of chromosome), MIN (minimum length of chromosomes), MLA (mean of long arms), MSA (mean of short arms), MrV (mean of r-value), MdV (mean of d value), MAR (mean of arm ratio), MCI (mean of chromosome index), MRLC (mean of the relative length of chromosomes), DRL (difference of range of relative length), TF% (total form percentage), S% (relative length of the shortest chromosome), A1 (intrachromosomal asymmetry index), A2 (interchromosomal asymmetry index), and A (Degree of asymmetry). Both arm ratios were assumed to be equally affected (Adhikary 1974). All karyotype formulas and asymmetry indexes were determined based on Huziwara (1962) (TF%), Levan et al. (1964) (r and d values), Zarco (1986) (A1 and A2), Watanabe (1999) (A), Peruzzi and Eroğlu (2013) (CI) as well. The abbreviations were taken from Rezeai et al. (2014) (RLC%, DRL, S%). The formulas are as follows.

Formulas

$$r\ value = \frac{Length\ of\ the\ long\ arm\ of\ chromosome}{Length\ of\ the\ short\ arm\ of\ chromosome}$$

d value=Length of the long arm of chromosome-Length of the short arm of chromosome

$$arm\ ratio = \frac{Length\ of\ the\ short\ arm\ of\ chromosome}{Length\ of\ the\ long\ arm\ of\ chromosome}$$

$$CI = \frac{Length\ of\ the\ short\ arm\ of\ chromosome}{Length\ of\ the\ long\ arm\ of\ chromosome + Length\ of\ the\ short\ arm\ of\ chromosome}$$

$$RLC\% = \frac{total\ length\ of\ each\ chromosome}{total\ length\ of\ chromosomes} \times 100$$

DRL=(maximum relative length)- (minimum relative length)

$$TF\% = \frac{total\ length\ of\ short\ arms}{total\ length\ of\ chromosomes} \times 100$$

$$S\% = \frac{length\ of\ shortest\ chromosome}{length\ of\ longest\ chromosome} \times 100$$

$$A = \left(\frac{1}{n}\right)\sum Ai, \quad Ai = \frac{li-si}{li+si}$$

(li = lengths of a long arm, si = lengths of a short arm, n = haploid chromosome number).

$$A1 = 1 - \frac{\sum_{i=1}^{n} \frac{b_i}{B_i}}{n}$$

(n = number of homologous chromosome pairs, b_i = the average length of short arms in every homologous chromosome pair, B_i = the average length of long arms in every homologous chromosome pair).

$$A2 = \frac{s}{\bar{x}}$$

(S = standard deviation of chromosome lengths, = mean of chromosome lengths).

A data matrix was constructed according to 17 chromosomal traits in Table 1. The Principal Component Analysis (PCA) was used based on the data matrix. Next, the cluster analysis was made using the Gower similarity index to determine the relationships between *Cuscuta* taxa's chromosome traits. Also, the Pearson correlation coefficient (r) analysis was performed to see strong and weak relationships between chromosome traits. At the same time, Shapiro - Wilk normality test was performed. Then, the one-way analysis of variance (ANOVA) was performed to determine whether the difference between the data was statistically significant. All the analyses were carried out with PAleontoSTatistics (PAST) (Hammer et al. 2001).

RESULTS

In this study, the karyological characteristics of 11 different *Cuscuta* taxa were investigated, and their details are given below.

Cuscuta campestris: The chromosome number of *C. campestris*, native to the United States of America and spread to many countries from there, and can be found almost everywhere in Turkey, was found to be 2n=2x=28. The haploid karyotype formula of this species is 10 median regions (m), 2 submedian regions (cm), and 2 dotted median (M) regions. Metaphase chromosome length varies between 2.48-1.48 μm. Chromosome arm ratios vary between 1.43-1 μm. Its centromere index ranges from 50.00 to 29.44 μm, and its relative length is between 10.93 and 18.32 μm. The intra-chromosomal asymmetric index (A1) is 0.32, and the inter-chromosomal asymmetric index (A2) is 0.04 (Table 2, Figure 1).

Cuscuta hyalina: The chromosome number of *C. hyalina* species, distributed in Turkey's local area (Bitlis

Table 1. The localities of studied taxa.

Taxa	Localities	Voucher specimen
Cuscuta campestris Yunck.	Adana, İmamoğlu, Alaybey village	1752
Cuscuta hyalina Roth.	Bitlis, Hizan, Karbastı village	2101
Cuscuta kotschyana Boiss.	Bitlis, Süphan mountain	2098
Cuscuta babylonica Aucher ex Choisy.	Van, Çatak, Sırmalı village	2100
Cuscuta europaea L.	Bitlis, Hizan	1993
Cuscuta kurdica Engelm.	Hakkâri, Ördekli village	14935
Cuscuta brevistyla A.Braun ex A.Rich	Bitlis, Hizan	1786
Cuscuta planiflora Ten.	Van, Tuşba	1766
Cuscuta approximata Bab.	Denizli, Honaz mountain	1801
Cuscuta lupuliformis.	Hakkâri Centre	2099
Cuscuta palaestina Boiss.	Van, Gürpınar	2095

Table 2. Chromosomes measurements of *Cuscuta* taxa (Ch. No: Chromosome No, C: Total length of the chromosome, L: Length of the long arm, S: Length of the short arm, CP: Centromeric position).

Ch. No	C	L	S	L/S	CI	RL	CP	Ch. No	C	L	S	L/S	CI	RL	CP
			Cuscuta campestris					17	2,03	1,10	0,93	1,18	45,81	16,42	m
1	2,48	1,46	1,02	1,43	41,13	0,00	m	18	1,94	1,16	0,78	1,49	40,21	17,18	m
2	2,4	1,4	1	1,40	41,67	0,00	m	19	1,92	1,00	0,92	1,09	47,92	17,36	m
3	2,29	1,39	0,9	1,54	39,30	0,00	m	20	1,89	1,05	0,84	1,25	44,44	17,63	m
4	2,1	1,31	0,79	1,66	37,62	0,00	m	21	1,78	0,92	0,86	1,07	48,31	18,72	m
5	2,06	1,03	1,03	1,00	50,00	0,00	M				*Cuscuta palaestina*				
6	2,06	1,16	0,9	1,29	43,69	0,00	m	1	4,80	2,40	2,40	1,00	50,00	10,78	M
7	1,98	1,21	0,77	1,57	38,89	0,00	m	2	4,74	2,44	2,30	1,06	48,52	10,92	m
8	1,9	1,08	0,82	1,32	43,16	0,00	m	3	4,69	2,53	2,16	1,17	46,06	11,04	m
9	1,84	1,12	0,72	1,56	39,13	0,00	m	4	4,36	2,97	1,39	2,14	31,88	11,87	sm
10	1,8	1,27	0,53	2,40	29,44	0,00	sm	5	4,28	2,93	1,35	2,17	31,54	12,09	sm
11	1,67	1,03	0,64	1,61	38,32	0,00	m	6	4,13	2,91	1,22	2,39	29,54	12,53	sm
12	1,54	0,84	0,7	1,20	45,45	0,00	m	7	3,93	2,37	1,56	1,52	39,69	13,17	m
13	1,51	1,03	0,48	2,15	31,79	0,00	sm	8	3,72	2,34	1,38	1,70	37,10	13,91	sm
14	1,48	0,74	0,74	1,00	50,00	0,00	M	9	3,42	1,71	1,71	1,00	50,00	15,13	M
			Cuscuta kotschyana					10	3,16	1,58	1,58	1,00	50,00	16,38	M
1	3,95	2,1	1,85	1,14	46,84	6,19	m	11	2,89	1,55	1,34	1,16	46,37	17,91	m
2	3,93	2,51	1,42	1,77	36,13	6,22	sm	12	2,79	1,56	1,23	1,27	44,09	18,55	m
3	3,51	1,89	1,62	1,17	46,15	6,96	m	13	2,62	1,44	1,18	1,22	45,04	19,76	m
4	3,47	1,93	1,54	1,25	44,38	7,04	m	14	2,23	1,23	1,00	1,23	44,84	23,21	m
5	3,36	1,68	1,68	1,00	50,00	7,27	M				*Cuscuta hyalina*				
6	3,18	1,61	1,57	1,03	49,37	7,69	m	1	5,18	2,63	2,55	1,03	49,23	11,08	m
7	3,04	1,52	1,52	1,00	50,00	8,04	M	2	5,05	2,58	2,47	1,04	48,91	11,36	m
			Cuscuta europaea					3	4,93	2,50	2,43	1,03	49,29	11,64	m
1	6,48	3,60	2,88	1,25	44,44	5,25	m	4	4,80	2,40	2,40	1,00	50,00	11,95	M
2	5,58	3,42	2,16	1,58	38,71	6,10	m	5	4,50	2,35	2,15	1,09	47,78	12,75	m
3	4,72	3,00	1,72	1,74	36,44	7,21	sm	6	4,40	2,20	2,20	1,00	50,00	13,04	M
4	4,53	2,63	1,90	1,38	41,94	7,51	m	7	4,25	2,15	2,10	1,02	49,41	13,50	m
5	4,37	2,45	1,92	1,28	43,94	7,79	m	8	4,18	2,30	1,88	1,22	44,98	13,72	m
6	4,35	2,52	1,83	1,38	42,07	7,82	m	9	4,00	2,00	2,00	1,00	50,00	14,34	M
7	4,00	2,70	1,30	2,08	32,50	8,51	sm	10	3,58	1,85	1,73	1,07	48,32	16,03	m
			Cuscuta brevistyla					11	3,42	1,71	1,71	1,00	50,00	16,77	M
1	3,95	2,10	1,85	1,14	46,84	6,19	m	12	3,19	1,61	1,58	1,02	49,53	17,98	m
2	3,93	2,51	1,42	1,77	36,13	6,22	sm	13	3,09	1,57	1,52	1,03	49,19	18,57	m
3	3,51	1,89	1,62	1,17	46,15	6,96	m	14	2,80	1,50	1,30	1,15	46,43	20,49	m
4	3,47	1,93	1,54	1,25	44,38	7,04	m				*Cuscuta babylonica*				
5	3,36	1,68	1,68	1,00	50,00	7,27	M	1	6,23	3,48	2,75	1,27	44,14	5,45	m
6	3,18	1,61	1,57	1,03	49,37	7,69	m	2	5,32	3,12	2,20	1,42	41,35	6,38	m
7	3,04	1,52	1,52	1,00	50,00	8,04	M	3	5,22	3,50	1,72	2,03	32,95	6,51	sm
8	3,01	1,85	1,16	1,59	38,54	11,07	m	4	4,69	3,24	1,45	2,23	30,92	7,24	sm
9	2,90	1,79	1,11	1,61	38,28	11,49	m	5	4,36	2,18	2,18	1,00	50,00	7,79	M
10	2,85	1,55	1,30	1,19	45,61	11,69	m	6	4,34	2,64	1,70	1,55	39,17	7,82	m
11	2,81	1,60	1,21	1,32	43,06	11,86	m	7	3,80	2,36	1,44	1,64	37,89	8,94	m
12	2,68	1,34	1,34	1,00	50,00	12,44	M				*Cuscuta kurdica*				
13	2,54	1,49	1,05	1,42	41,34	13,12	m	1	4,80	2,40	2,40	1,00	50,00	6,46	M
14	2,46	1,26	1,20	1,05	48,78	13,55	m	2	4,67	2,45	2,22	1,10	47,54	6,64	m
15	2,30	1,18	1,12	1,05	48,70	14,49	m	3	4,66	2,50	2,16	1,16	46,35	6,65	m
16	2,22	1,11	1,11	1,00	50,00	15,01	M	4	4,40	3,00	1,40	2,14	31,82	7,05	sm

Ch. No	C	L	S	L/S	CI	RL	CP
5	4,36	2,97	1,39	2,14	31,88	7,11	sm
6	4,25	2,95	1,30	2,27	30,59	7,30	sm
7	3,87	2,36	1,51	1,56	39,02	8,01	m
			Cuscuta approximata				
1	3,68	1,84	1,84	1,00	50,00	8,87	M
2	3,36	1,68	1,68	1,00	50,00	9,72	M
3	3,08	1,82	1,26	1,44	40,91	10,60	m
4	2,48	1,28	1,20	1,07	48,39	13,17	m
5	2,42	1,50	0,92	1,63	38,02	13,49	m
6	2,36	1,36	1,00	1,36	42,37	13,83	m
7	2,28	1,40	0,88	1,59	38,60	14,32	m
8	2,12	1,24	0,88	1,41	41,51	15,40	m
9	2,01	1,20	0,81	1,48	40,30	16,24	m
10	1,98	0,99	0,99	1,00	50,00	16,49	M
11	1,85	1,24	0,61	2,03	32,97	17,65	sm
12	1,84	1,06	0,78	1,36	42,39	17,74	m
13	1,62	1,00	0,62	1,61	38,27	20,15	m
14	1,57	0,84	0,73	1,15	46,50	20,80	m
			Cuscuta lupuliformis				
1	6,96	3,48	3,48	1,00	50,00	6,40	M
2	3,82	2,24	1,58	1,42	41,36	11,65	m
3	3,80	2,55	1,25	2,04	32,89	11,72	sm
4	3,20	1,60	1,60	1,00	50,00	13,91	M
5	3,14	1,82	1,32	1,38	42,04	14,18	m
6	3,07	1,83	1,24	1,48	40,39	14,50	m
7	2,98	1,76	1,22	1,44	40,94	14,94	m
8	2,94	1,80	1,14	1,58	38,78	15,14	m
9	2,76	1,70	1,06	1,60	38,41	16,13	m
10	2,52	1,56	0,96	1,63	38,10	17,67	m
11	2,43	1,33	1,10	1,21	45,27	18,32	m
12	2,40	1,20	1,20	1,00	50,00	18,55	M
13	2,30	1,26	1,04	1,21	45,22	19,36	m
14	2,20	1,10	1,10	1,00	50,00	20,24	M
			Cuscuta planiflora				
1	5,28	2,64	2,64	1,00	50,00	5,54	M
2	4,35	2,55	1,80	1,42	41,38	6,72	m
3	4,24	2,82	1,42	1,99	33,49	6,89	sm
4	4,08	2,04	2,04	1,00	50,00	7,16	M
5	3,96	2,26	1,70	1,33	42,93	7,38	m
6	3,78	2,15	1,63	1,32	43,12	7,73	m
7	3,54	2,18	1,36	1,60	38,42	8,26	m

province), was found as 2n=2x=28. The haploid karyotype formula of this species has 10 median regions (m) and 4 points median (M) regions. Metaphase chromosome length varies between 5.18-2.80 μm. Chromosome arm ratios vary between 1.03-1.15 μm. Its centromere index ranges from 50.00 to 44.98 μm and relative length from 11.08 to 20.49 μm. The intra-chromosomal asym-

metric index (A1) is 0.04, and the inter-chromosomal asymmetric index (A2) is 0.04 (Table 2, Figure 1).

Cuscuta kotshyana var. *caudata*: The chromosome number of this species was determined as 2n=2x=14. Haploid karyotype formula; It has 4 median regions (m), 2 points median (M) and 1 submedian region (cm) region. Metaphase chromosome length was measured in lengths ranging from 3.93-3.04 μm. Chromosome arm ratios vary between 1.77-1 μm. The centromere index is 50.00-36.13 μm. Its relative length was measured in the range of 6.22-8.04 μm. The intra-chromosomal asymmetric index (A1) is 0.15, and the inter-chromosomal asymmetric index (A2) is 0.07 (Table 2, Figure 1).

Cuscuta babylonica var. *babylonica*: The chromosome number of this species is mainly found in the Eastern Anatolia region of Turkey, at an altitude of 850-1200 m, whose stems are between thin filamentous and medium thickness, and which is yellowish-red is 2n=2x=14. The haploid karyotype formula of this species is 4 median regions (m), 2 submedian regions (cm), and 1 dotted median (M) region. Metaphase chromosome length varies between 6.23-3.80 μm. Chromosome arm ratios range from 1.64 to 1 μm. Its centromere index ranges from 50.00-30.92 μm, and its relative length ranges from 5.45 to 8.94 μm. The intra-chromosomal asymmetric index (A1) is 0.34, and the inter-chromosomal asymmetric index (A2) is 0.07 (Table 2, Figure 1).

Cuscuta europaea: C. europaea; has 2n=2x=14 chromosomes. The haploid karyotype formula has 5 median regions (m) and 2 submedian regions (cm). Metaphase chromosome length varies between 6.48-4 μm. Chromosome arm ratios vary between 2.08-1.25 μm. Its centromere index ranges from 44.44 to 32.50 μm, and its relative length ranges from 5.25 to 8.51 μm. The intra-chromosomal asymmetric index (A1) is 0.33, and the inter-chromosomal asymmetric index (A2) is 0.07 (Table 2, Figure 1).

Cuscuta kurdica: The chromosome number of this species was found to be 2n=2x=14. The haploid karyotype formula has 3 median regions (m), 3 submedian regions (cm), and 1 dotted median (M) region. Metaphase chromosome length varies between 4.80-3.87 μm. Chromosome arm ratios vary between 2.27-1 μm. Its centromere index ranges from 50.00-30.59 μm and relative length is between 6.46 and 8.01 μm. The intra-chromosomal asymmetric index (A1) is 0.34, and the inter-chromosomal asymmetric index (A2) is 0.07 (Table 2, Figure 1).

Cuscuta brevistyla: The chromosome number of C. brevistyla species, which is annual, parasitic, and generally distributed in the mountains, was determined as 2n=6x=42. The haploid karyotype formula has 15 medi-
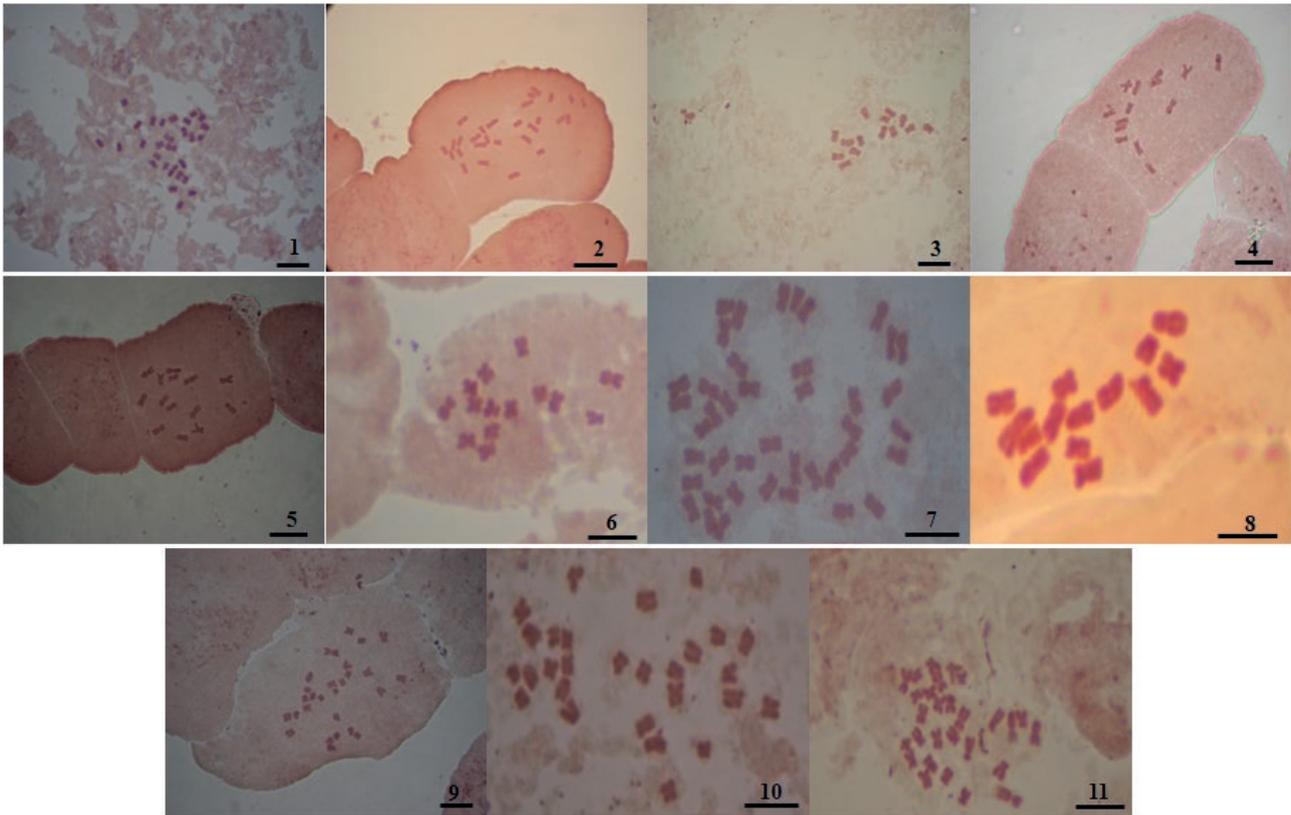
Figure 1. Mitotic metaphase chromosomes of *Cuscuta* taxa 1. *Cuscuta campestris*, 2. *Cuscuta hyalina*, 3. *Cuscuta kotschyana*, 4. *Cuscuta babylonica*, 5. *Cuscuta europaea* 6. *Cuscuta kurdica*, 7. *Cuscuta brevistyla*, 8. *Cuscuta planiflora*, 9. *Cuscuta approximata*, 10. *Cuscuta lupuliformis*, 11. *Cuscuta palaestina* (Scale:10 μm).

an regions (m), 3 submedian regions (cm), and 3 dotted median (M) regions. Metaphase chromosome length varies between 4.73-1.78 μm. Chromosome arm ratios vary between 1.97-1 μm. Its centromere index ranges from 50.00-33.63 μm, and its relative length varies between 12.63- 33.55 μm. The intra-chromosomal asymmetric index (A1) is 0.25, and the inter-chromosomal asymmetric index (A2) is 0.02 (Table 2, Figure 1).

Cuscuta planiflora: The chromosome number of this species was determined as 2n=2x=14. The haploid karyotype formula has 4 median regions (m), 1 submedian region (cm), and 2 dotted median (M) regions. Metaphase chromosome length varies between 5.28-3.54 μm. Chromosome arm ratios vary between 1.60-1 μm. Its centromere index ranges from 50.00 to 38.42 μm, and its relative length ranges from 5.54 to 8.26 μm. The intra-chromosomal asymmetric index (A1) is 0.24, and the inter-chromosomal asymmetric index (A2) is 0.07 (Table 2, Figure 1).

Cuscuta approximata: *C. approximata* has 2n=4x=28 chromosomes. The haploid karyotype formula has 10 median regions (m), 1 submedian region (cm), and 3 point median (M) regions. Metaphase chromosome length varies between 3.68-1.57 μm. Chromosome arm ratios vary between 1.60-1 μm. Its centromere index ranges from 50.00 to 32.97 μm and its relative length from 8.87 to 20.80 μm. The intra-chromosomal asymmetric index (A1) is 0.23, and the inter-chromosomal asymmetric index (A2) is 0.04 (Table 2, Figure 1).

Cuscuta lupuliformis: The chromosome number of this species was found to be 2n=2x=28. The haploid karyotype formula has 9 median regions (m), 1 submedian region (cm), and 4 point median (M) regions. Metaphase chromosome length varies between 6.96-2.20 μm. Chromosome arm ratios vary between 2.04-1 μm. Its centromere index ranges from 50.00-32.89 μm, and its relative length is 6.40-20.24 μm. The intra-chromosomal asymmetric index (A1) is 0.24, and the inter-chromosomal asymmetric index (A2) is 0.04 (Table 2, Figure 1).

Cuscuta palaestina: The chromosome number of this species was determined as 2n=4x=28. The haploid karyotype formula is 7 median regions (m), 4 submedian regions (cm), and 3 point median (M) regions. Metaphase chromosome length varies between 4.80-2.23 μm.

Table 3. Karyotype characteristics of *Cuscuta* taxa (TLC: Total Lenght of Chromosomes, MTLC (Mean of Total Length of Chromosomes, MAX: Maximum Length of Chromosome, MIN: Minimum Length of Chromosome, MLA: Mean of Long Arms, MSA: Mean of Short Arms, MrV: Mean of r Value, MdV: Mean of d Value, MAR: Mean of Arm Ratio, MCI: Mean of Chromosome Index, MRLC: Mean of Relative Length of Chromosomes, DRL: Difference of Range of Relative Length, TF%: Total Form Percentage, S%: Relative Length of Shortest Chromosome, A_1: Intrachromosomal Asymmetry Index, A_2: Interchromosomal Asymmetry Index).

Cuscuta Taxa	TLC	MTLC	MAX	MIN	MLA	MSA	MrV	MdV	MAR	MCI	MRLC	DRL	TF%	S%	A_1	A_2	A
C. campestris	27.11	0.97	1.46	0.48	1.14	0.78	1.92	0.36	1.51	40.69	14.37	7.39	0.41	0.33	0.32	0.04	0.19
C. hyalina	57.37	2.05	2.63	1.30	2.09	2.00	4.09	0.09	1.05	48.79	14.52	9.41	0.49	0.49	0.04	0.04	0.02
C.kotschyana	24.44	1.75	2.51	1.42	1.89	1.60	3.49	0.29	1.19	40.12	7.06	1.82	0.46	0.57	0.15	0.07	0.08
C. babylonica	33.96	2.43	3.5	1.45	2.93	1.92	4.85	1.01	1.59	39.49	7.16	3.49	0.40	0.41	0.34	0.07	0.21
C. europaea	34.03	2.43	3.6	1.30	2.90	1.95	4.85	0.95	1.53	40.01	7.17	3.26	0.40	0.36	0.33	0.07	0.20
C. kurdica	31.01	2.22	2.97	1.30	2.66	1.76	4.42	0.90	1.62	39.60	7.03	1.55	0.40	0.44	0.34	0.07	0.20
C. brevistyla	59.72	1.42	2.53	0.78	1.62	1.22	2.84	0.40	1.34	43.45	22.63	20.92	0.43	0.31	0.25	0.02	0.14
C. planiflora	29.23	2.09	2.82	1.36	2.37	1.79	4.16	0.58	1.38	42.76	7.01	2.72	0.43	0.48	0.24	0.07	0.14
C. approximata	32.65	1.17	1.84	0.61	1.31	1.01	2.32	0.30	1.37	43.87	14.89	11.92	0.43	0.33	0.23	0.04	0.13
C. lupuliformis	44.52	1.59	3.48	0.96	1.80	1.37	3.17	0.43	1.36	43.10	15.19	13.84	0.43	0.28	0.24	0.04	0.14
C. palaestina	51.76	1.85	2.97	1.01	2.14	1.55	3.69	0.59	1.43	42.48	14.80	12.43	0.42	0.34	0.28	0.04	0.16

Chromosome arm ratios vary between 2.39-1 μm. Its centromere index ranges from 50.00 to 44.84 μm and its relative length from 10.78 to 23.21 μm. The intra-chromosomal asymmetric index (A1) is 0.28, and the inter-chromosomal asymmetric index (A2) is 0.04 (Table 2, Figure 1).

Karyotypes in plants; According to the types of chromosomes, there are two types: symmetrical and asymmetrical. The symmetrical karyotype is characterized by the predominance of median and submedian chromosomes of approximately the same size. The increase in asymmetry caused by the centromere shift creates an asymmetric karyotype. Chromosomes change from the median and submedian type to subterminal and terminal (Babaarslan and Eroğlu, 2014). When the asymmetric indices of *Cuscuta* taxa were examined, it was seen that the TF% value changed between 0.41-0.49, the A index changed between 0.02 and 0.25, the A1 index between 0.21-0.38 and the A2 index between 0.09-0.31 (Table 3).

Statistical findings

Chromosome micromorphological features of 11 *Cuscuta* taxa were specified, and statistical analyses were performed using formulas created using various chromosome features. Mitotic metaphase chromosome images of *Cuscuta* taxa are given in Figure 1, and karyotype features are given in Table 2-3. One-way ANOVA test, which is one of the analyzes made according to the chromosome characteristics of the taxa, is given in Table According to the values obtained with the formulas using the micromorphological chromosome features

of taxa, the data show a normal distribution according to the Shapiro-Wilk test (p>0.05), and the residual plot graph is shown in Figure 2. Then, according to the one-way ANOVA test p-value, the difference between taxa was statistically significant (p<0.05) (Table 4).

Correlation analysis

According to the correlation analysis, there are relations between the r-values of chromosomal data according to the significance level less than p <0.05. Particularly a high relationship Although there was a strong positive relationship between MTLC and MIN, MAX, MLA, MSA, and MRV, it was observed that there was a strong negative relationship between MRLC and DRL. In addition, MAR and A1 and A characters are strongly positively correlated, while TF% is strongly negative; With MRLC, DRL is strongly positive while A2 is strongly negative; TF% was strongly negatively correlated with MAR, MDV, A1, A (Figure 3).

Principal Component Analysis (PCA)

According to PCA (Figure 4), the first two components explained most of the variation according to chromosome data between taxa. While the first two components explain 87.94 and 9.80% of the variance, these characters explained 97.75% of the total variation. The characters most affected by the variation were TLC, DRL%, MCl, and MRLC. The TLC value was the most influential one. The impact of other characters was very
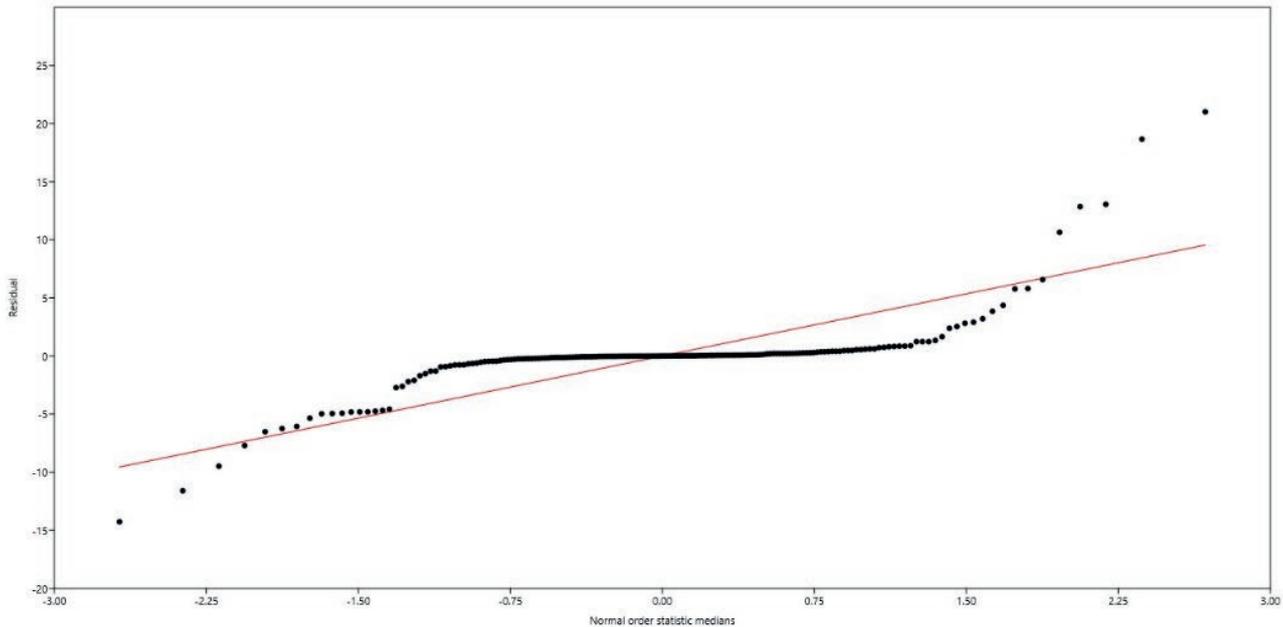
Figure 2. Shapiro - Wilk normality test(p=0.4809>0.05)-Residual plot.

Table 4. One way ANOVA test results.

Test for equal means					
	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	45343.3	16	2833.96	351.6	8.149E-179
					Permutation
Within groups:	2329.64	289	8.06104		p (n=99999)
Total:	47672.9	305			1E-05
omega2:	0.9483				

low. While this TLC value was positively correlated with MCL, MRLC, and DRL characters in the correlation analysis, it was negatively correlated with MAR, A, A1, A2, and S% characters.

Cluster analysis

According to the Cluster analysis results of the UPGMA algorithm and Gower similarity index, the taxa are divided into three main groups (Figure 5). *C. brevistyla*, *C. lupuliformis*, *C. palaestina*, *C. approximata*, and *C. campestris* were group, *C. kotschyana*, *C. planiflora*, *C. babylonica*, *C. europea*, *C. kurdica* had created a group. The *C. hyalina* species wholly separated from these groups were a group. As stated before, the fact that *C. hyalina* species spread in a local area directly correlates with the analysis result.

DISCUSSION

Cuscuta species show wide variation in chromosome numbers ranging from 2n = 8 to 2n = 60. Therefore, the genus is generally a polyploid complex resulting from two basic chromosome numbers x = 7 and x = 15 (Pazy & Plitmann, 1995; Hunziker, 1949-50).

The first step in combating parasitic plants is their correct diagnosis, as with other weeds. Due to the lack of true root and leaf structure of dodder, diagnosis is mainly made according to flower and fruit characteristics. These features are sometimes insufficient for diagnosis. Diagnosis of this genus is problematic in the World and Turkey. Therefore, determining the chromosome number and chromosomal morphology of the species belonging to this genus is of great importance in determining the systematic location of the species, identifying the species, and, when necessary, agriculturally struggling with these species. According to the karyotype analysis results of *Cuscuta* taxa, the primary chromosome number was determined as x=7. Among the study samples, *C. brevistyla* is polyploid, *C. campestris*, *C. hyalina*, *C. approximata*, *C. lupuliformis, C. palaestina* tetraploid, and other taxa are diploid.

According to the total length of chromosomes, The species with the longest chromosome length is *C. lupuliformis,* with 6.96 Mµ lengths. This species was morphological; *C. campestris,* with a total chromosome length of 2.48 Mµ was determined to be the shortest chromo-
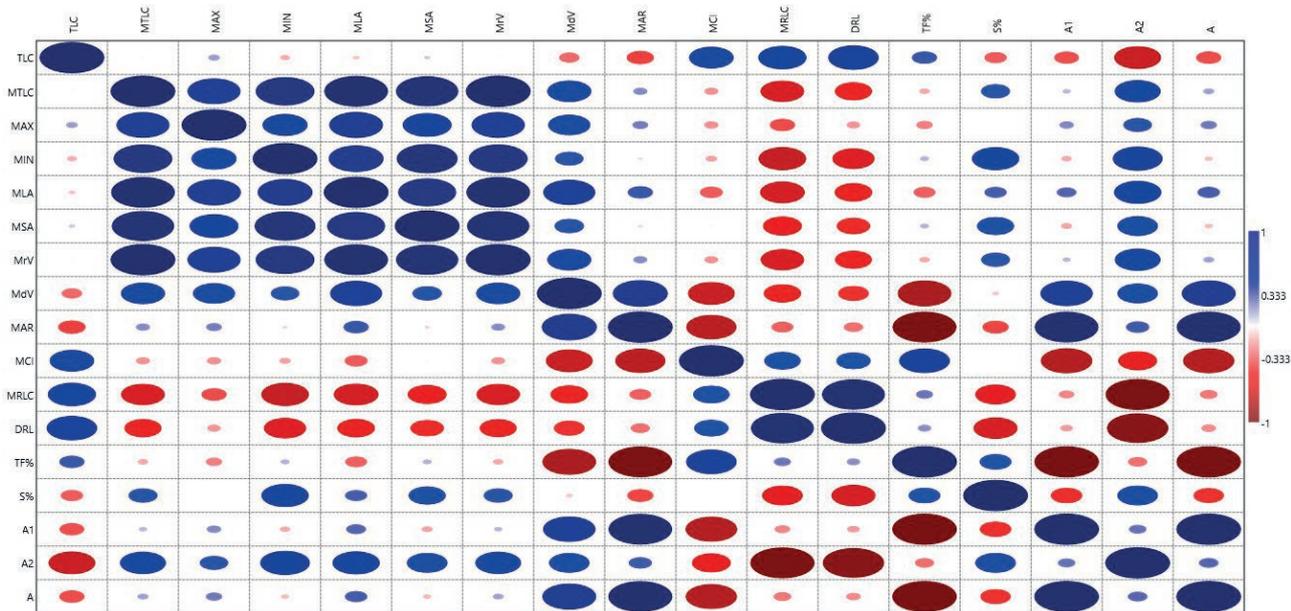
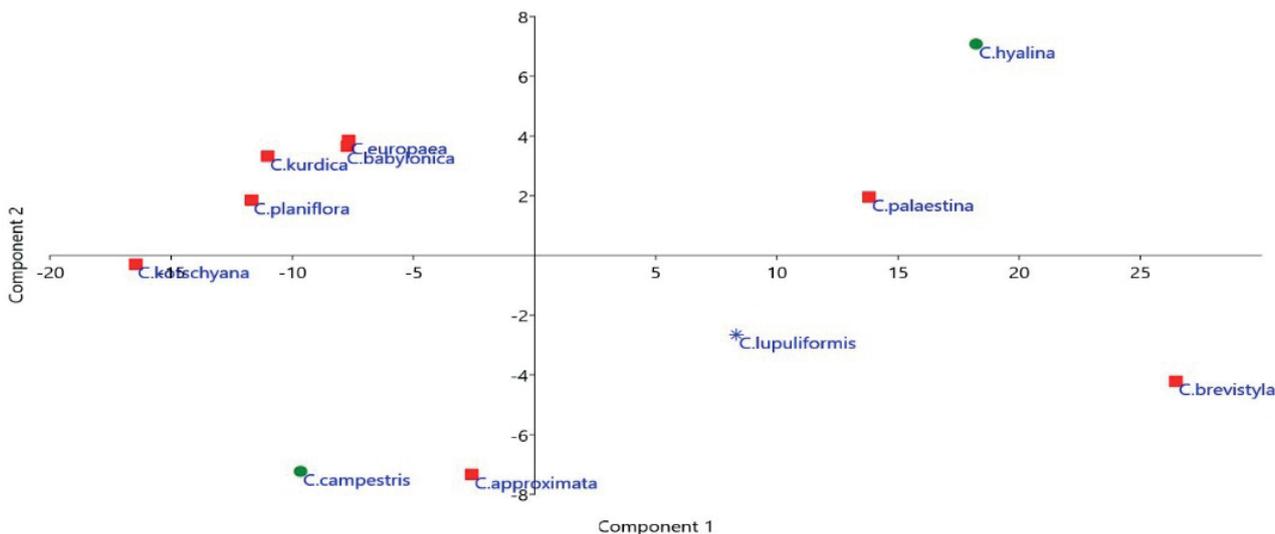**Figure 3.** Correlation analysis between karyotype characteristics.

Figure 4. PCA analysis scatter plot (same colors are the same subgenus).

some length. The chromosome number of *C. campestris* was first determined by Ward(1984) as 2n=28; later, Aryavand and García & Castroviejo (1987) 2n=56; Khatoon & Ali(1993) determined it as 2n=14, 28. According to our research results, the chromosome number of the species is 2n=28. It has been shown that the haploid karyotype formula is 10m+2sm +2M. The morphometric characteristics of the species were first revealed in this study. Singh and Roy(1970). collected *C. hyalina* from

India; The chromosome number of the species is 2n=30; Vu et al. determined as 2n=28. According to our study results, the chromosome number of the species is 2n=28. The haploid karyotype formula is 10m+4M. This study first revealed the chromosome number and morphometric characteristics of C. *kotschyana* species. Chromosome number 2n=14; Haploid karyotype formula; It has 4 median regions (m), 2 points median (M), and 1 submedian region (cm) region (Figure 6).
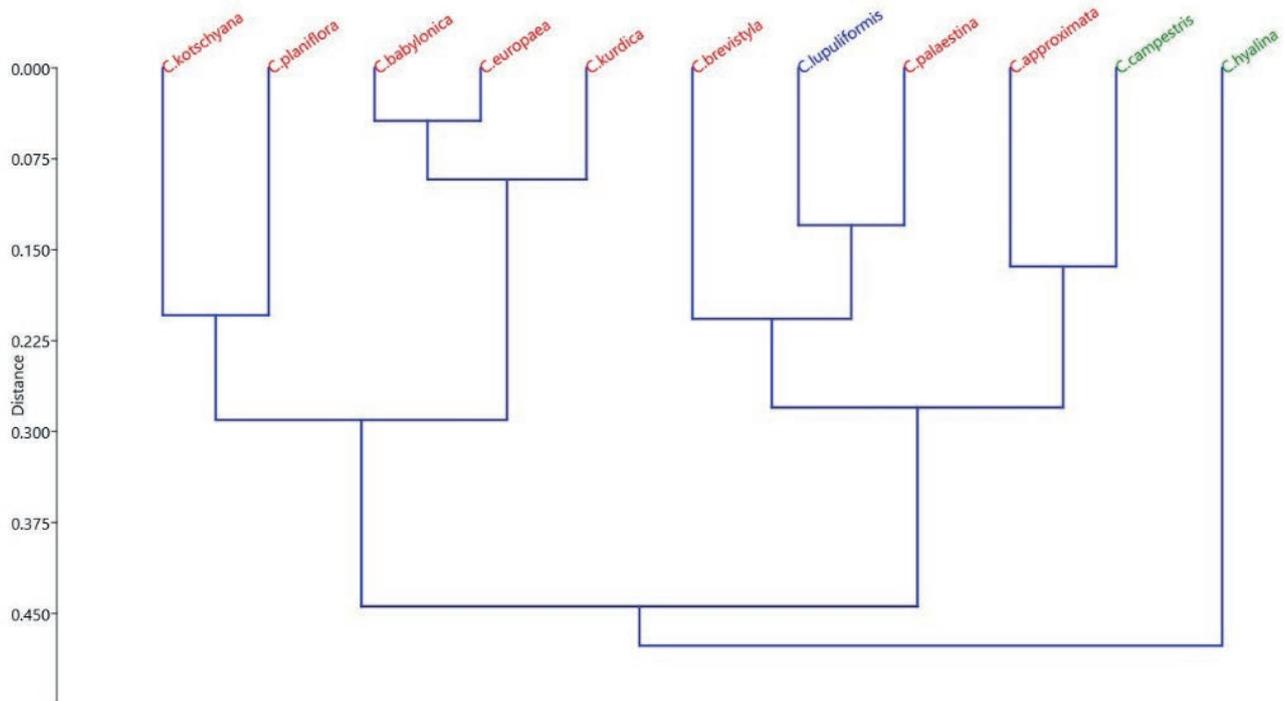
Figure 5. Cluster analysis according to karyotype characteristics Show that 3 main groups (Same colored taxa are located in the same section).

Pazy and Plitmann (2002) determined the chromosome number of *C. babylonica* as 2n=8, where they specified Israel as the locality. However, according to our research results, the chromosome number of the species is 2n=14, and the haploid karyotype formula is 4m + 2 cm + 1M.

The chromosome number of the *C. europaea* species was previously reported by Albers and Pröbsting(1998) and García and Castroviejo(2003) as 2n=14. Our research data also confirm this result. The haploid karyotype formula of the species, in which we found the chromosome number as 2n=14, is 5m + 2 cm.

Regarding chromosome number and morphology, the chromosome number of *C. kurdica* species, which was first discussed in this study, was determined as 2n=14. The haploid karyotype formula is 3m + 3 cm + 1 M.

Pazy and Plitman (1994) and Feinbrun and Taub(1978) found the chromosome number of *C. brevistyla* as 2n=42, where they specified Israel as a locality. According to our study results, the chromosome number of this species is 2n=42. The haploid karyotype formula is 15m + 3 cm + 3 M.

The chromosome number of *C. planiflora* has been determined by many researchers. Singh and Roy determined the chromosome number of this species as 2n=14; Pazy and Plitmann. (1991) 2n=14; García and Castroviejo.

(2003) 2n=26, 28; Aryavand(1987) reported 2n=28 and Vasudevan 2n=14. As a result of our research, the chromosome number of the species was determined as 2n=14. The haploid karyotype formula was 4m + 1 cm + 2 M.

C. approximata; García and Castroviejo (2003) and Guerra(2004). 2n=28 chromosomes have reported it. Our studies also confirm this result, and the chromosome number of this species is 2n=28. The haploid karyotype formula is 10m + 1 cm + 3M..

The chromosome number of *C. lupuliformis* was determined as 2n=28 by Vasudevan. According to our research results, the chromosome number of this species is 2n=28. The haploid karyotype formula is 9m + 1 cm + 4M.

Plazy and Plitmann (1991) showed the *C. palaestina* species as 2n=28 chromosomes. Our research confirms this result. We found the chromosome number of 2n = 28 of this species. The haploid karyotype formula is 7m + 4 cm + 3M.

Various karyological studies have been carried out on the chromosome number of species belonging to the *Cuscuta* genus. As a result of these studies, the Chromosome number of *Cuscuta japonica* Choisy. species is 2n= 32 (Leusova et al., 2005); the Chromosome number of *Cuscuta epithymum* L. species is 2n= 14 (Montgomery et al., 2003); the chromosome number of *Cuscuta australis* R. Br. species is 2n=56 (Yeh et al., 1995); Chromosome
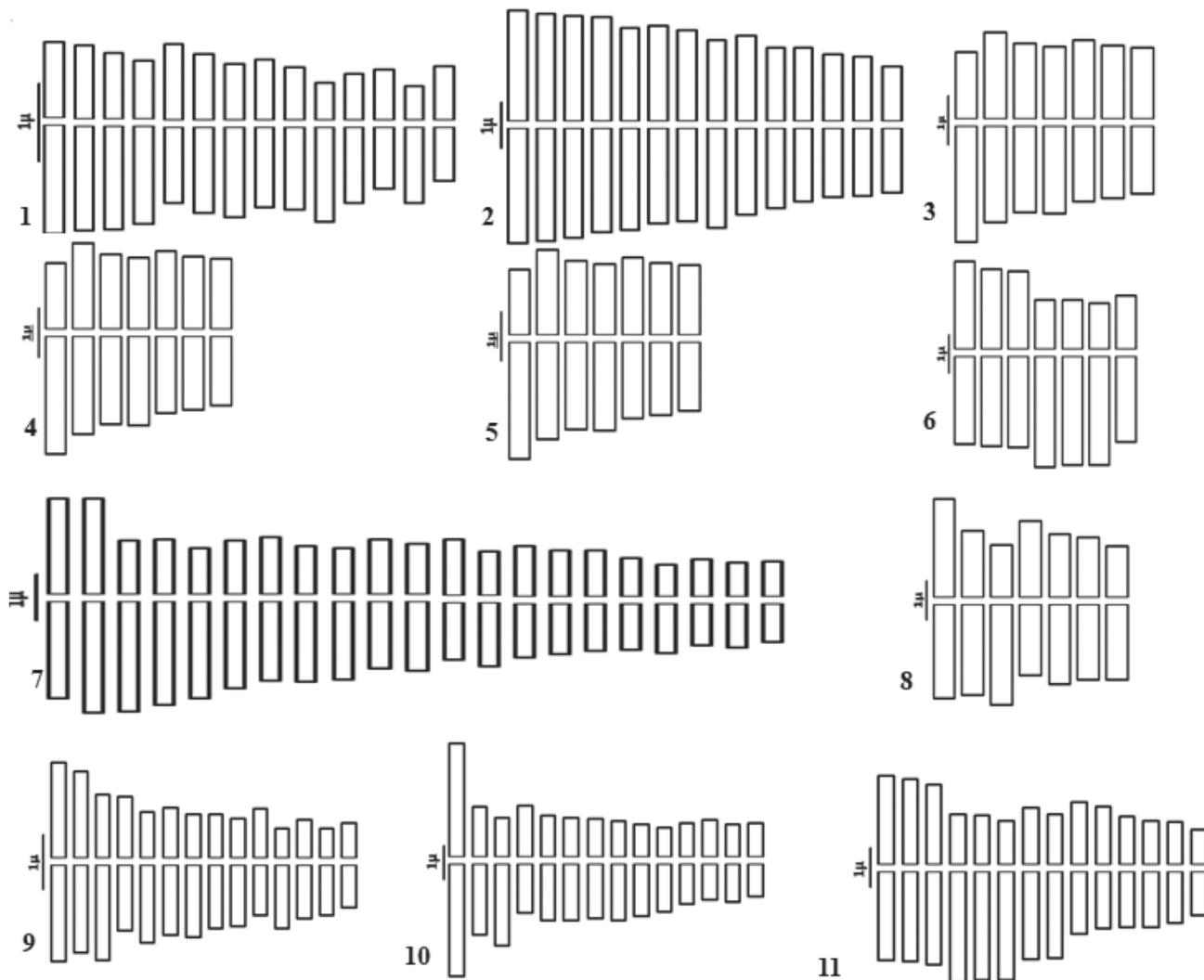
Figure 6. Haploid idiogram in *Cuscuta* taxa 1. *C. campestris,* 2. *C. hyalina*, 3. *C. kotschyana*, 4. *C. babylonica*, 5. *C. europaea* 6. *C. kurdica*, 7. *C. brevistyla*, 8. *C. planiflora*, 9. *C. approximata*, 10. *C. lupuliformis* 11. *C. palaestina..*

number of *Cuscuta triumvirati* Lange. Species 2n= 14 (García et al., 2003); Chromosome number of *Cuscuta pentagona* Engelm. is 2n= 44 (Pazy et al., 1995); The chromosome number of *Cuscuta pedicellata* Ledeb. species was determined as 2n= 10 (Pazy et al., 1991), and the chromosome number of *Cuscuta chinensis* Lam. species was determined as 2n= 60 (Mesĭcek et al., 1995).

According to cluster analysis, taxa were divided into 3 main groups. It is noteworthy that although *C. campestris* and *C. hyalina* are in the Grammica subgenus, they are in different groups according to chromosome micromorphological data. Here, it is estimated that some chromosomal features (According to PCA, such as TLC) may have differentiated over time, as the *C. hyalina* species was distributed in a local region in Turkey. It is seen

that *C. babylonica* and *C. europea* species in the *Cuscuta* subgenus and *C. kurdica* species are closely related. According to their morphological similarities, C. europea and *C. kurdica* species show very close similarities.

According to PCA, the most important character explaining the differentiation between taxa was seen as TLC (Total Lenght of Chromosomes) character. In addition, when the distribution of taxa in the diagram is examined, it is a compatible image with cluster analysis.

In this study, 11 species belonging to the genus *Cuscuta*, an essential part of Turkey's biological richness and consists of parasitic plants, were discussed in detail in terms of chromosome number and chromosome morphology and compared statistically. These karyological studies reveal the karyological differences and similari-

ties between the infrageneric and species. The results obtained increase our knowledge about these species. Thus, obtaining new data that can be used in the systematics of these species aims to reveal basic information about the systematics, karyology, and morphological features of taxa. In addition, it will form a fundamental step for future breeding and hybridization studies related to this genus and contribute to other biological research.

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