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## Statistical evaluation of chromosomes of some *Lathyrus* L. taxa from Turkey

HASAN GENÇ<sup>1</sup>, BEKİR YILDIRIM<sup>2,\*</sup>, MIKAIL AÇAR<sup>3</sup>, TOLGA ÇETİN<sup>4</sup>

<sup>1</sup>Department of Science Education, Faculty of Education, Burdur Mehmet Akif Ersoy University, Burdur, 15100, Turkey

<sup>2</sup>Department of Plant and Animal Production, Burdur Food, Agriculture and Livestock Vocational School of Higher Education, Burdur Mehmet Akif Ersoy University, Burdur, 15100, Turkey

<sup>3</sup>Department of Plant and Animal Production, Tunceli Vocational School of Higher Education, Munzur University, Tunceli, 62000, Turkey

<sup>4</sup>Science Teacher, Republic of Turkey Ministry of National Education, Seydikemer, Muğla, 48850, Turkey

\*Corresponding author. E-mail: bytr33@yahoo.com

**Abstract.** In this study, a statistical analysis was performed on mitotic metaphase chromosomes of 26 *Lathyrus* taxa, four of which are endemic. ANOVA, correlation analysis, PCA and cluster analysis were performed to determine the relationships between taxa based on chromosomal criteria. The morphological similarities of plant taxa and chromosomal statistics results may not be always parallel to each other. According to the findings obtained as a result of analysis, the following taxa, which are close to each other were determined: *L. hirsutus* - *L. odoratus*, *L. brachypterus* var. *haussknechtii* - *L. phaselitanus*, *L. stenophyllus* - *L. chloranthus*, *L. gorgoni* var. *gorgoni* - *L. nissolia* - *L. pratensis*, *L. tuberosus* - *L. annuus*.

**Keywords:** *Lathyrus*, chromosome, endemic, statistical analysis, Turkey.

### INTRODUCTION

*Lathyrus* L., a genus belonging to Fabaceae family consisting of more than 200 taxa is distributed almost all over the World (Allkin et al. 1986). The main diversity centers of *Lathyrus* are the Mediterranean region, Asia Minor and North America, as well as temperate South America and East Africa (Klamt & Schifino-Wittmann 2000). In Flora Europaea, 54 species of *Lathyrus* were reported from different areas of Europe (Tutin et al. 1968). *Lathyrus* is represented by 79 taxa in Turkey, 25 of which are endemic to Turkey (Davis 1970; Davis et al. 1988; Güner et al. 2000; Genç & Şahin 2008; Genç 2009; Genç & Şahin 2011).

Some agriculturally important species of the genus *Lathyrus* are grown for use as forage or human food (Yamamoto et al. 1984; Genç & Şahin 2001). Seeds of some *Lathyrus* species are used in the preparation of regional human food in different countries in the world (Kumar 1997; Uncuer et al. 2016).

A lot of studies such as taxonomical, cytotaxonomical, morphological, anatomical, etc. have been carried out on *Lathyrus* taxa which are so important in terms of agriculture. Caryological studies were conducted on *L. saxatilis* (Vent.) Vis., *L. vinealis* Boiss. & Noe, *L. inconspicuus* L., *L. setifolius* L. (Şahin & Altan 1990) and *L. rotundifolius* Willd. subsp. *miniatus* (Bieb. ex Stev.) Davis, *L. cassius* Boiss., *L. cicera* L., *L. aphaca* L. var. *modestus* P.H. Davis (Şahin 1993). Anatomical, morphological and palynological features of *L. inconspicuus*, *L. vinealis* from *Orobastrum* (Taub.) Boiss. section and *L. sativus* L., *L. hirsutus* L. from *Cicerula* (Medic.) Gren. & Godr. section were investigated qualitatively and quantitatively (Mantar et al. 2002; 2003). There are also studies conducted on the cytotaxonomical properties of *L. brachypterus* Čel. var. *haussknechtii* (Şirj.) Davis, *L. spathulatus* Čel., *L. ochrus* (L.) DC., *L. odoratus* L., *L. belinensis* N. Maxted & D. J. Goyder, *L. clymenum* L., *L. phaselitanus* Hub-Mor. & Davis and the morphological characteristics of grass pea (*L. sativus*) (Genç et al. 2009; Grela et al. 2010). A numerical taxonomic study on 54 of 58 *Lathyrus* taxa in Flora of Turkey was conducted (Doğan et al. 1992).

This study was carried out using the statistical evaluation of the findings of previous cytotaxonomical studies conducted by us (Şahin et al. 1998; Şahin et al. 2000; Genç & Şahin 2001; Genç et al. 2009).

In this study, by applying statistical analysis to the metaphase chromosome organization, it is aimed to see whether the taxa with the same chromosome morphology are similar taxonomically or not.

## MATERIAL AND METHODS

### Material

Plant specimens and seeds belonging to *Lathyrus* taxa were collected from natural habitats in Turkey during 1995-2007. Some plant specimens were stored at personal herbarium of Genç, while other herbarium specimens were stored at the FUH (Fırat University, Elazığ, Turkey) and GUL (Süleyman Demirel University, Isparta, Turkey) herbariums.

The eight sections of 28 investigated taxa according to morphological classification in Davis 1970 and Güner et al. 2000 are given in Table 1.

### Methods

#### Chromosome measurements

Determination of chromosome number and karyotype analyses of taxa were performed at mitotic metaphases.

**Table 1.** The sections of the investigated *Lathyrus* taxa.

Section	Taxa
<i>Platystylis</i>	<i>L. brachypterus</i> var. <i>haussknechtii</i> , <i>L. digitatus</i> (Bieb.) Fiori, <i>L. spathulatus</i>
<i>Pratensis</i>	<i>L. pratensis</i> L., <i>L. laxiflorus</i> (Desf.) O. Kuntze subsp. <i>laxiflorus</i>
<i>Lathyrus</i>	<i>L. tuberosus</i> L., <i>L. belinensis</i> , <i>L. odoratus</i> [ <i>L. odoratus</i> is cultivated as an ornamental plant in Turkey (Davis 1970)]
<i>Orobastrum</i>	<i>L. sphaericus</i> Retz., <i>L. inconspicuus</i> , <i>L. tauricola</i> P. H. Davis, <i>L. setifolius</i>
<i>Cicerula</i>	<i>L. annuus</i> L., <i>L. gorgoni</i> Parl. var. <i>gorgoni</i> , <i>L. cicera</i> , <i>L. sativus</i> , <i>L. stenophyllus</i> Boiss. & Heldr., <i>L. phaselitanus</i> , <i>L. hirsutus</i> , <i>L. chloranthus</i> Boiss.
<i>Clymenum</i>	<i>L. clymenum</i> , <i>L. ochrus</i>
<i>Nissolia</i>	<i>L. nissolia</i> L.
<i>Aphaca</i>	<i>L. aphaca</i> var. <i>affinis</i> (Guss.) Arc, <i>L. aphaca</i> var. <i>pseudoaphaca</i> (Boiss.) Davis, <i>L. aphaca</i> var. <i>modestus</i>

The seeds were germinated at room temperature in petri dishes covered with cotton. When the root tips reached 1 cm in length, they were cut off and pretreated with saturated paradichlorobenzene solution for 4 hours. At the end of the pretreatment process, root tips were washed and fixed with acetic acid-ethyl alcohol (1/3 v/v) for 24 hours. Then, the root tips were washed again and stored in 70% ethyl alcohol at 2-4 °C (Sharma & Gupta 1982).

After washed root tips had been hydrolyzed in 1 N HCl for 10-15 min at 60 °C. Feulgen method was used in the dyeing process (Elçi 1982; Sharma & Gupta 1982). Squashed preparats were prepared using root tips. The karyotypes were discussed according to Levan et al. (1964). Chromosomes measurements of *Lathyrus* taxa are given in Table 2.

#### Data analyses

For the analysis of karyotype characteristics, the following methods and formulas were used. The measurements were performed on haploid data sets. The following traits in each karyotype were measured: 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 relative length of chromosomes), DRL (difference of range of relative length), TF% (total form percentage), S% (relative length of shortest chromosome), A1 (intrachromosomal asymmetry index), A2 (inter-



chromosomal asymmetry index), and A (Degree of asymmetry). Both arm ratios were assumed to be equally affected (Adhikary 1974). All karyotype formulas 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 the Rezeai et al. (2014) (RLC%, DRL, S%). The formulas are as follows.

#### Formulas

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

$d \text{ value} = \text{Length of the long arm of chromosome} - \text{Length of the short arm of chromosome}$

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

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

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

$DRL = (\text{maximum relative length}) - (\text{minimum relative length})$

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

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

$A = \left(\frac{1}{n}\right) \sum A_i$ ,  $A_i = \frac{l_i - s_i}{l_i + s_i}$  ( $l_i$  = lengths of a long arm,  $s_i$  = 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,  $\bar{x}$  = mean of chromosome lengths).

A data matrix was constructed according to 17 karyotype characteristics mentioned in Table 3. The principal component analysis (PCA) was used based on the data matrix (Jolliffe 2002). The cluster analysis was made using Gower (dis)similarity index for determining the relationships between chromosome properties of *Lathyrus* taxa (Romesburg 2004). In addition, the Pearson correlation coefficient ( $r$ ) analysis was performed to see strong and weak relationships between chromosome properties. 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

Statistical studies on the chromosome morphologies of 26 *Lathyrus* taxa were conducted. Images of the mitotic metaphase chromosomes of *Lathyrus* taxa are given in Figure 1. Karyotype characteristics of *Lathyrus* taxa are given in Table 3.

The chromosome properties of taxa are summarized in the Stacked bar (Figure 2). Shapiro - Wilk normality test and One way ANOVA test results are given in Figure 3 and Table 4. According to the values obtained with the formulas using chromosome morphological properties of taxa, the data show a normal distribution (Figure 3), and then the one-way ANOVA test is statistically significant according to the p-value ( $p < 0.05$ ) (Table 4).

#### Correlation analysis

According to the correlation analysis, there are relations between the  $r$ -values of chromosome data according to the significance level less than  $p < 0.05$ . Especially a strong positive relationship between TLC, MTLC, MAX, MIN, MLA, MSA, and a strong negative relations between MRV, MDV and MAR, MCI and A1 and A values (Figure 4).

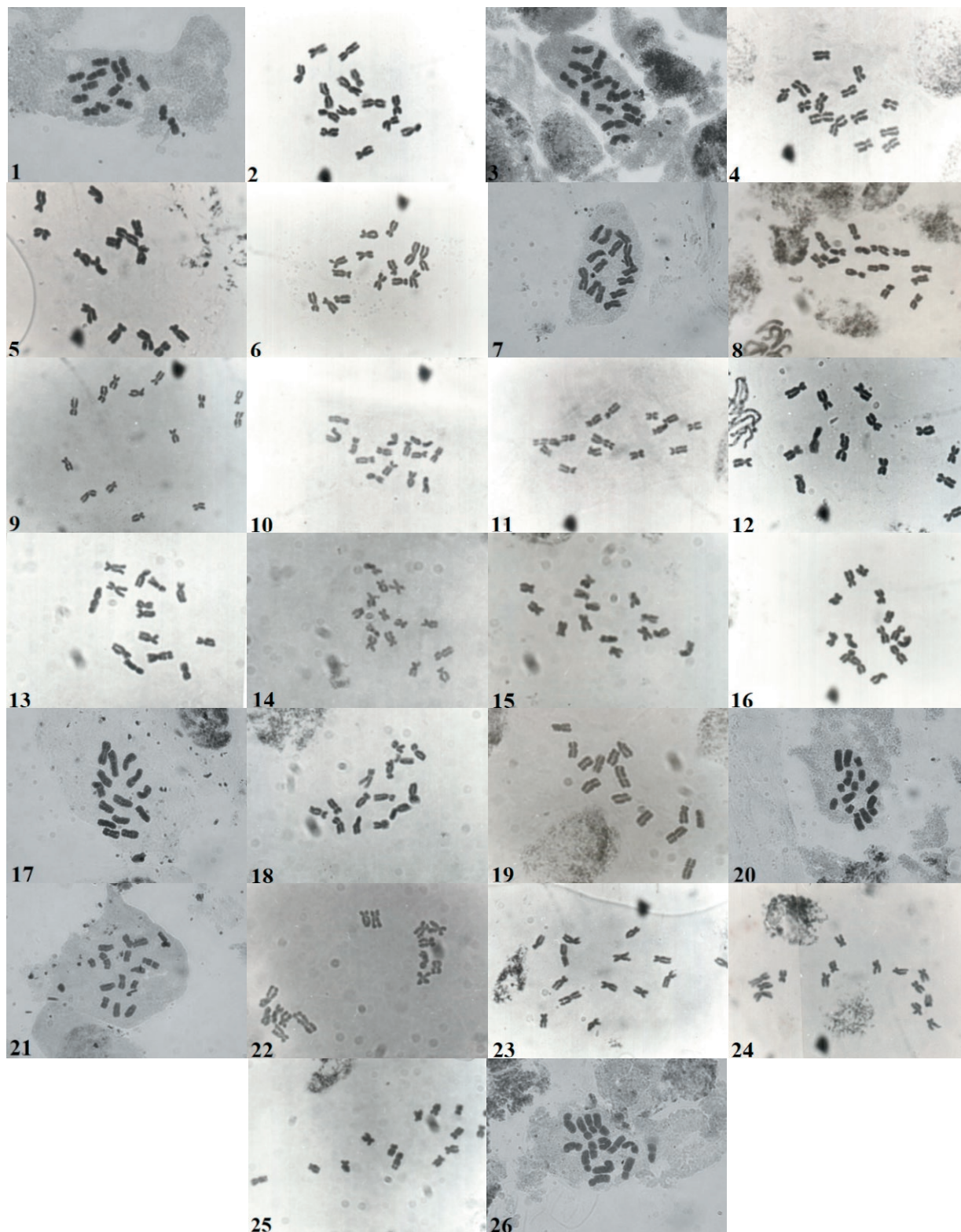
#### Principal component analysis (PCA)

According to PCA (Table 5, Figure 5), the first two components explained the majority of the variation according to chromosome data between the taxa. While the first two components explain 57.98 and 38% of the variance, respectively, these characters explained 96% of the total variation. The characters that affected the variation most were S%, TLC, DRL and TF%. Similarly, since some variables (such as A, A1) have lower values than calculations, the effects on variation in PCA have been low.

#### Cluster analysis

According to the UPGMA algorithm Gower index Cluster analysis results, the taxa are divided into 4 groups (Figure 6). These groups are also divided into subgroups among themselves. Especially *L. hirsutus* - *L. odoratus*, *L. brachypterus* var. *haussknechtii* - *L. phaselitanus*, *L. stenophyllus*, - *L. chloranthus*, *L. gorgoni* var. *gorgoni* - *L. nissolia* - *L. pratensis*, *L. tuberosus* - *L. annuus* taxa are closely related.





**Figure 1.** Mitotic metaphase chromosomes of *Lathyrus* taxa (1. *L. brachypterus* var. *haussknechtii*, 2. *L. digitatus*, 3. *L. spathulatus*, 4. *L. pratensis*, 5. *L. laxiflorus* subsp. *laxiflorus*, 6. *L. tuberosus*, 7. *L. belinensis*, 8. *L. sphaericus*, 9. *L. inconspicuus*, 10. *L. tauricola*, 11. *L. setifolius*, 12. *L. annuus*, 13. *L. gorgoni* var. *gorgoni*, 14. *L. cicera*, 15. *L. sativus*, 16. *L. stenophyllus*, 17. *L. phaselitanus*, 18. *L. hirsutus*, 19. *L. chloranthus*, 20. *L. clymenum*, 21. *L. ochrus*, 22. *L. nissolia*, 23. *L. aphaca* var. *affinis*, 24. *L. aphaca* var. *pseudoaphaca*, 25. *L. aphaca* var. *modestus*, 26. *L. odoratus*).

**Table 3.** Karyotype characteristics of *Lathyrus* taxa (TLC: Total Length 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<sub>1</sub>: Intrachromosomal Asymmetry Index, A<sub>2</sub>: Interchromosomal Asymmetry Index).

<i>Lathyrus</i> Taxa	TLC	MTLC	MAX	MIN	MLA	MSA	MrV	MdV	MAR	MCI	MRLC	DRL	TF%	S%	A <sub>1</sub>	A <sub>2</sub>	A
<i>L. brachypterus</i> var. <i>haussknechtii</i>	36.93	5.28	6.48	4.32	3.00	2.08	1.47	0.92	0.69	0.41	14.28	5.85	39.37	66.67	0.90	0.14	0.184
<i>L. digitatus</i>	50.13	7.16	9.16	6.03	4.15	3.01	1.41	1.15	0.72	0.42	14.28	6.24	41.99	65.83	0.90	0.13	0.166
<i>L. spathulatus</i>	44.10	6.30	8.00	5.09	3.60	2.53	1.43	1.07	0.70	0.41	14.28	6.60	40.16	63.62	0.90	0.15	0.176
<i>L. pratensis</i>	40.41	5.77	7.62	4.65	3.49	2.09	1.68	1.39	0.60	0.37	14.28	7.35	36.30	61.02	0.91	0.16	0.249
<i>L. laxiflorus</i> subsp. <i>laxiflorus</i>	54.52	7.79	9.27	6.32	4.86	2.78	1.79	2.08	0.58	0.36	14.28	3.96	35.67	68.18	0.92	0.11	0.272
<i>L. tuberosus</i>	43.14	6.16	8.10	5.01	3.74	2.21	1.71	1.53	0.59	0.37	14.28	7.16	35.84	61.85	0.91	0.15	0.258
<i>L. belinensis</i>	36.52	5.22	6.56	4.48	3.15	2.07	1.54	1.08	0.65	0.39	14.28	5.70	39.68	68.29	0.91	0.13	0.210
<i>L. sphaericus</i>	39.61	5.66	6.92	4.68	3.37	2.28	1.50	1.08	0.67	0.40	14.28	5.65	40.37	67.63	0.90	0.12	0.195
<i>L. inconspicuus</i>	28.06	4.01	4.66	3.28	2.41	1.48	1.66	0.93	0.62	0.38	14.28	4.92	37.03	70.39	0.91	0.12	0.241
<i>L. tauricola</i>	33.19	4.74	6.06	3.98	2.85	1.72	1.68	1.08	0.61	0.38	14.28	6.27	36.34	65.68	0.91	0.14	0.355
<i>L. setifolius</i>	34.07	4.87	7.16	3.46	3.04	1.60	1.94	1.44	0.54	0.35	14.28	10.86	32.81	48.32	0.92	0.22	0.304
<i>L. annuus</i>	43.17	6.17	8.28	4.97	3.67	2.24	1.67	1.43	0.62	0.38	14.28	7.67	36.27	60.02	0.91	0.16	0.240
<i>L. gorgoni</i> var. <i>gorgoni</i>	41.62	5.95	7.50	4.77	3.56	2.15	1.68	1.40	0.62	0.38	14.28	6.56	36.26	63.60	0.91	0.13	0.241
<i>L. cicera</i>	30.35	4.34	5.29	3.30	2.67	1.61	1.65	1.06	0.61	0.37	14.28	6.55	37.07	62.38	0.91	0.14	0.242
<i>L. sativus</i>	37.40	5.34	6.21	4.47	3.19	1.95	1.65	1.23	0.62	0.38	14.28	4.65	36.58	71.98	0.91	0.10	0.238
<i>L. stenophyllus</i>	39.55	5.65	7.16	4.65	3.47	2.20	1.58	1.27	0.64	0.39	14.28	6.35	39.04	64.94	0.91	0.14	0.219
<i>L. phaselitanus</i>	36.27	5.18	6.41	4.36	2.97	2.03	1.47	0.93	0.69	0.41	14.28	5.65	39.20	68.02	0.90	0.12	0.185
<i>L. hirsutus</i>	44.75	6.39	8.36	5.07	4.12	2.27	1.86	1.84	0.56	0.35	14.28	7.35	35.57	60.64	0.92	0.16	0.289
<i>L. chloranthus</i>	40.53	5.79	7.35	4.70	3.50	2.28	1.57	1.22	0.65	0.39	14.28	6.54	39.48	63.94	0.91	0.15	0.217
<i>L. clymenum</i>	35.32	5.05	7.22	2.83	3.20	1.64	1.96	1.55	0.53	0.34	14.28	12.43	32.59	39.20	0.93	0.30	0.310
<i>L. ochrus</i>	33.84	4.83	6.00	3.33	2.91	1.73	1.69	1.17	0.63	0.38	14.28	7.89	35.81	55.50	0.91	0.18	0.238
<i>L. nissolia</i>	39.42	5.63	6.86	4.36	3.35	2.04	1.65	1.30	0.62	0.38	14.28	6.34	36.28	63.55	0.91	0.14	0.237
<i>L. aphaca</i> var. <i>affinis</i>	35.17	5.02	6.77	4.12	3.17	1.68	1.95	1.48	0.53	0.36	14.28	7.53	33.44	60.86	0.92	0.16	0.311
<i>L. aphaca</i> var. <i>pseudoaphaca</i>	29.35	4.19	5.53	3.45	2.62	1.41	1.90	1.21	0.54	0.35	14.28	7.09	33.63	62.39	0.92	0.15	0.300
<i>L. aphaca</i> var. <i>modestus</i>	33.21	4.74	5.89	3.95	2.77	1.77	1.61	1.00	0.65	0.39	14.28	5.84	37.40	67.06	0.91	0.13	0.219
<i>L. odoratus</i>	42.92	6.13	7.49	5.13	3.99	2.14	1.90	1.84	0.55	0.35	14.28	5.50	34.97	68.49	0.92	0.12	0.297

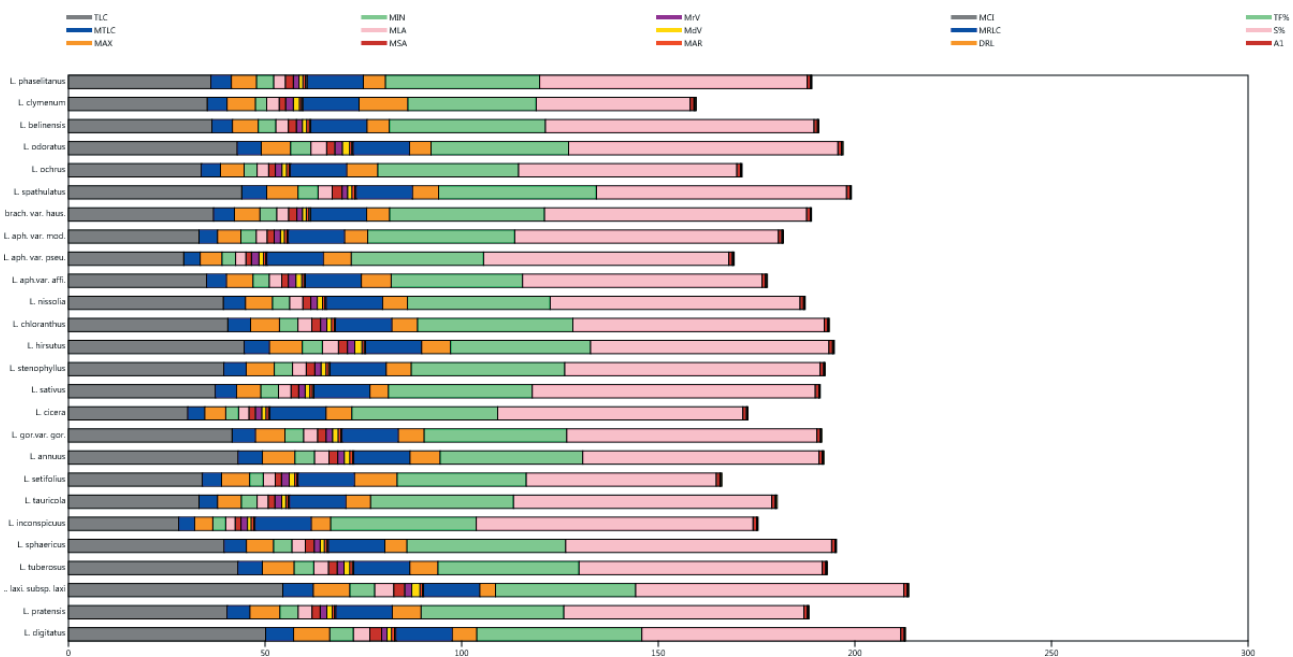


Figure 2. *Lathyrus* taxa karyotype characteristics of Stacked bar.

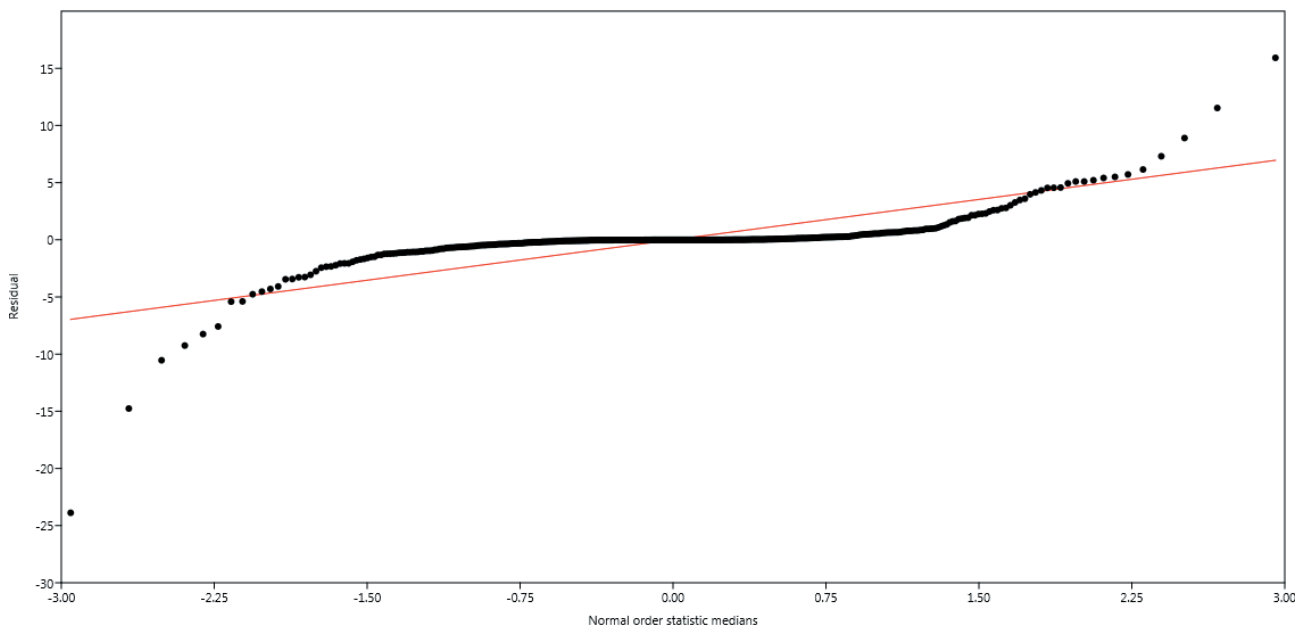


Figure 3. Shapiro - Wilk normality test.

### DISCUSSION

To best of our knowledge no statistical analysis of chromosomes belonging to such a number of taxa in the genus *Lathyrus* is available in literature. In this study, 26 taxa belonging to 8 sections of genus *Lathyrus* were

investigated. Among the investigated taxa, *Lathyrus brachypterus* var. *hausknechtii*, *L. belinensis*, *L. tauricola*, *L. phaselitanus* are endemic to Turkey.

In some studies, the cluster analysis data can yield similar trees with the morphological classification of the taxa (Açar & Satıl 2019; Dirmenci et al. 2019).

**Table 4.** One way ANOVA test results.

Test for equal means	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	133605	16	8350.28	1464	0
Within groups:	2423.77	425	5.70299		Permutation p (n=99999)
Total:	136028	441			1E-05
$\omega^2$ :	0.9815				

**Table 5.** Principal component analysis of *Lathyrus* taxa showing the eigen values of total variance.

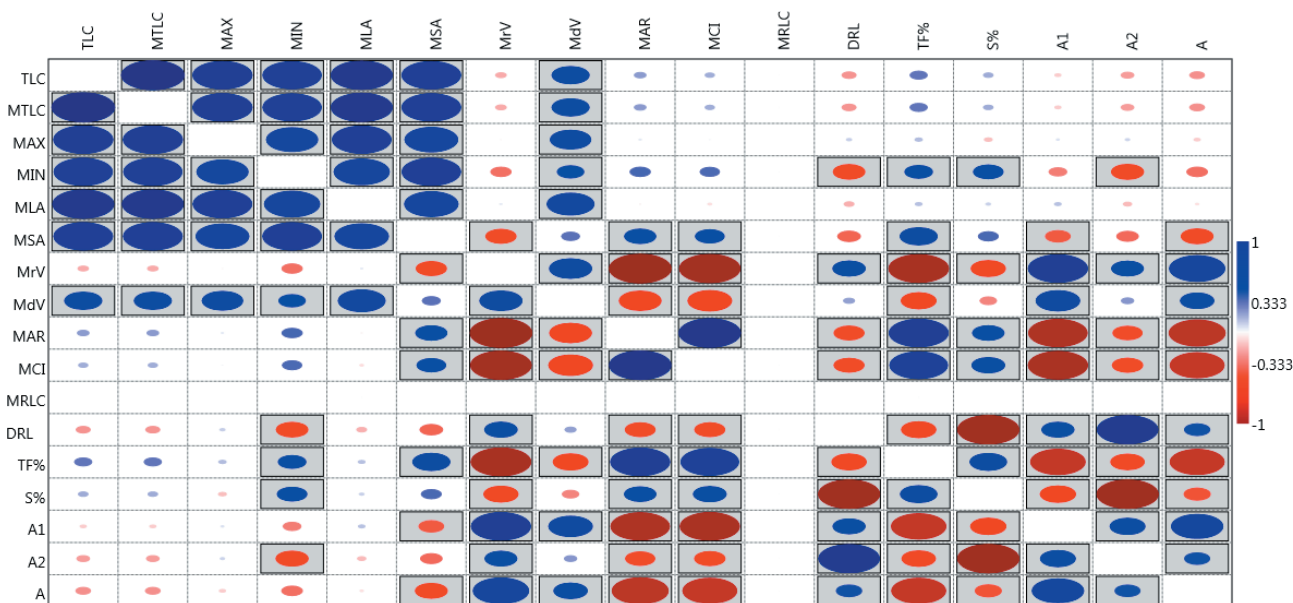
PC	Eigen value	% variance
1	56.2106	57.978
2	36.8804	38.04
3	3.66766	3.783

According to the data of Doğan et al (1992), obtained in the study using forty morphological characters, the *Lathyrus* genus was divided into two subgenus and nine sections. However, the results obtained do not show compatibility with Davis (1970).

The statistical results obtained in our study are also not consistent with Davis (1970). This situation suggests that the statistical results obtained from taxa may not always be completely compatible with morphological features.

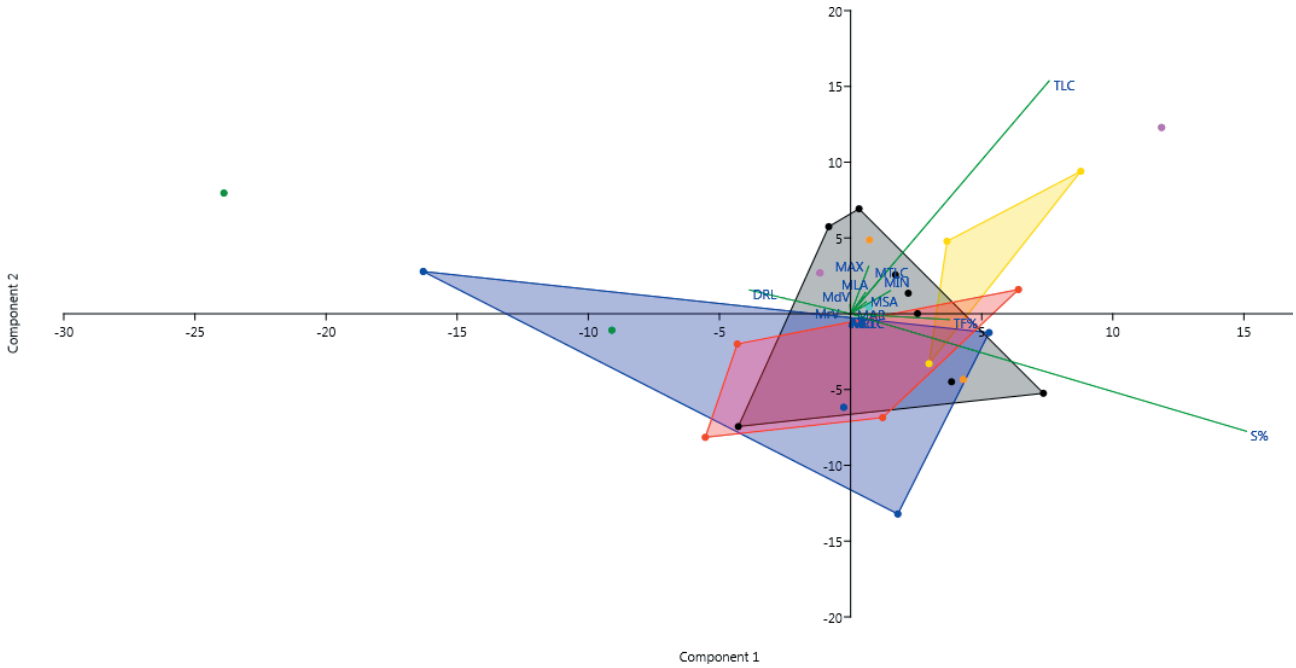
However, in our study, the caryological data were not generally similar to the morphological classification of the taxa, but similarities and close relationships among some taxa were also similar to morphological data (Figure 6). According to the PCA scatter diagram, like the cluster analysis results, the sections were observed to be intertwined in the distribution formations of taxa (Figure 5). Cluster analysis made according to karyotype features successfully distinguished the taxa from each other. However, it was also found to be an inconsistency with morphological classification.

According to the caryological examination, *L. hirsutus* - *L. odoratus*, *L. brachypterus* var. *haussknechtii* - *L. phaselitanus*, *L. stenophyllus* - *L. chloranthus*, *L. gorgoni* var. *gorgoni* - *L. nissolia* - *L. pratensis*, *L. tuberosus* - *L. annuus* taxa are closely related (Figure 6). *L. hirsutus* and *L. odoratus* are morphologically similar, and have been observed to be close to each other as a result of caryological analysis. *L. brachypterus* var. *haussknechtii* and *L. phaselitanus* differ morphologically and are located in different sections; however, these taxa are similar according to caryological data we obtained. *L. stenophyllus* and *L. chloranthus* belonging to the same section are similar to each other according to caryological analysis. And conversely, *L. gorgoni* var. *gorgoni*, *L. nissolia* and *L. pratensis* belonging to different sections are similar to each other according to the analysis of its metaphase chromosome morphology. Similarly, the two species, *L. tuberosus* and *L. annuus* from different sections are similar to each other according to cluster analysis.

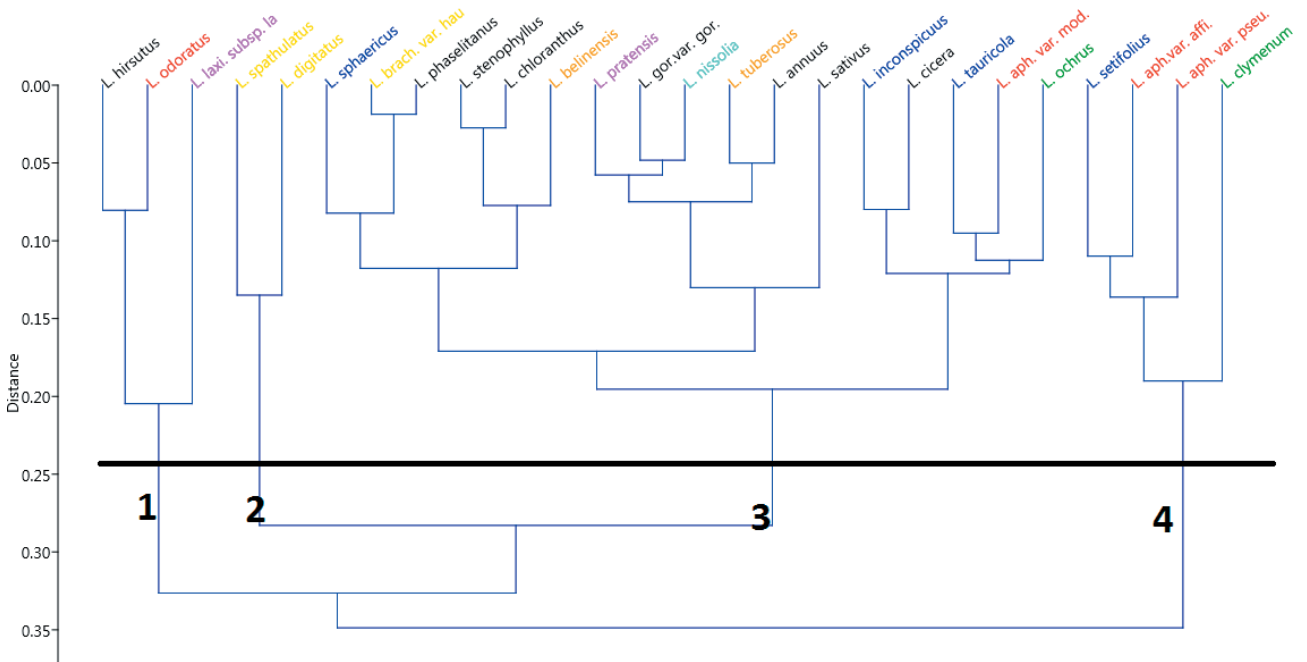


**Figure 4.** Correlation analysis between karyotype characteristics.





**Figure 5.** PCA scatter plot diagram (Different colors refer to different sections and the lines with variables indicate the effect and direction of variation).



**Figure 6.** Cluster analysis according to karyotype characteristics (Same coloured taxa are located in the same section except *L. odoratus*. It is an ornamental plant).

In terms of similarities of the taxa, the presence of satellite and distribution was not found to be significant. This study revealed that the morphological similari-

ties of plant taxa and chromosomal statistics results may not be always parallel to each other.

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