COMPARATIVE BIOSYSTEMATICS STUDIES ON *Lathyrus* L. (FABACEAE) IN BOLU

BERK PÜRAL

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COMPARATIVE BIOSYSTEMATICS STUDIES ON *Lathyrus* L. (FABACEAE) IN BOLU

by

BERK PÜRAL

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Approval of the Graduate School of Natural and Applied Sciences.

Prof. Dr. Yaşar DÜRÜST

Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master.

Prof. Dr. Ekrem GÜREL

Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of master.

Assist. Prof. Dr. Emel USLU

Supervisor

Examining Committee Members

1. Prof. Dr. Mecit VURAL ……

2. Prof. Dr. M. Tekin BABAÇ ……

3. Assist. Prof. Dr. Emel USLU ……

ABSTRACT

COMPARATIVE BIOSYSTEMATICS STUDIES ON *Lathyrus* L. (FABACEAE) IN BOLU

PÜRAL, Berk

M.Sc., Department of Biology

Supervisor: Assist. Prof. Dr. Emel USLU

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Morphometric characters, quantitative and qualitative, and two molecular markers, RAPD and ISSR, were used to determine the phenetic classification of 14 taxa of *Lathyrus* L. (Fabaceae) collected from Bolu Province. In morphometric part, 12 quantitative and 13 qualitative morphological characters were scored. In molecular part, a total of 320 and 92 bands were produced by 12 RAPD and 4 ISSR markers, respectively. Data obtained from morphometric and molecular analyses were used to construct phenograms according to the UPGMA method based upon Euclidean distance matrices in package program of Statistica 8.0. Congruences were found between the groups based on morphological and molecular data in some extent. Especially, at different distances levels, groups of sections showed some similarities to the sectional classifications of *Lathyrus* carried out previously by some researchers. According to the results obtained from the numerical analyses indicated that the RAPD and ISSR molecular techniques may reliably be used for the infrageneric classifications of *Lathyrus*. Additionaly, because of different molecular and especially morphological characteristics of *L. hirsutus* samples collected from Gerede population might be taken into account as being a new subspecies.

Keywords: Bolu, Fabaceae, ISSR, *Lathyrus* L., RAPD, UPGMA

ÖZET

BOLU *Lathyrus* L.'LARI (FABACEAE) ÜZERİNDE KARŞILAŞTIRMALI BİYOSİSTEMATİK ÇALIŞMALAR

PÜRAL, Berk
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Tez Danışmanı: Yrd. Doç. Dr. Emel USLU

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Bolu ilinden toplanan 14 *Lathyrus* L. (Fabaceae) taksonunun fenetik sınıflamasını belirlemek için nicel ve nitel morfolojik karakterler, RAPD ve ISSR moleküler markörleri kullanılmıştır. Morfometrik kısımda, 12 nicel ve 13 nitel morfolojik karakter ölçülmüştür. Moleküler kısımda, 12 RAPD ve 4 ISSR pirimerinden sırasıyla 320 ve 92 bant elde edilmiştir. Morfometrik ve moleküler analizler sonucunda elde edilen veriler Statistica 8,0 paket programında, UPGMA yöntemine göre Öklid uzaklığına dayalı fenogramların oluşturulmasında kullanılmıştır. Gruplar arasında morfolojik ve moleküler verilere dayalı kısmi uyumlar bulunmuştur. Özellikle farklı seksiyon seviyelerindeki gruplamalar daha önce *Lathyrus* taksonu üzerinde bazı araştırmacılar tarafından yapılan seksiyon sınıflamalarına benzer olarak bulunmuştur. Sayısal analizlerden elde edilen sonuçlara göre, RAPD ve ISSR moleküler tekniklerinin, *Lathyrus*' un infrageneric sınıflandırmasında güvenilir bir şekilde kullanılabileceği gösterilmiştir. Ek olarak, *L. hirsutus*'un Gerede populasyonundan toplanan örneklerin farklı moleküler ve özellikle morfolojik karakteristiklerinden dolayı yeni bir alttür olarak dikkate alınabileceği gösterilmiştir.

Anahtar Kelimeler: Bolu, Fabaceae, ISSR, *Lathyrus* L., RAPD, UPGMA

To my dear aunt, Vildan Yalçın.

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ABBREVIATIONS

CA	Cluster Analysis
DNA	Deoxyribonucleicacid
ISSR	Inter-Simple Sequence Repeat
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RPM	Revolutions Per Minute
Taq	*Thermus aquaticus*
UPGMA	Unweighted Pair-Group Method of Analysis

1. INTRODUCTION

1.1 The Genus *Lathyrus* L. in the World

The genus *Lathyrus* L. (The sweet peas) was first recognized, by Linnaeus in his "Species Plantarum" (Linnaeus, 1753). The genus *Lathyrus* is a member of tribe *Fabeae* Rchb. in family Leguminosae Juss. The modern composition of the tribe *Fabeae* which is placed in the "temperate herbaceous" papilionoid group of Leguminosae, includes five genera; *Lathyrus* L., *Lens* Mill., *Pisum* L., *Vavilovia* Fed. and *Vicia* L.. The characters, which define the tribe, are leaves with terminal tendrils, unusual stem vascular system supplying the stipules, adaxially pubescent styles, and several floral characters. (Gunn, 1969; Davis, 1970; Polhill, 1981; Wojciechowski et al., 2000; Kenicer et al., 2005; Lock & Maxted 2005; Belaid et al., 2006).

The genus *Lathyrus* consists of about 160 annual and perennial species in the world. Approximately 140 of them are found throughout the temperate northern hemisphere (Kupicha, 1983; Tsui, 1984; Allkin et al., 1985; Goyder, 1986; Asmussen & Liston, 1998). The main center of diversity is the Eastern Mediterranean region, with smaller centers in North and South America. (Kupicha, 1983; Simola, 1986; Asmussen and Liston, 1998).

Lathyrus species are found in a diversity of habitats in which comprise pastures, meadows, sand dunes, roadsides, open woods, fields, marshes, forest margins, seashores and slopes. Many *Lathyrus* species have a climbing or sprawling habit using simple or branched tendrils. The genus display a typical bee-pollinated papilionoid flower, which may be, orange, purple, violet, yellow, bluish, red or white colors (Asmussen and Liston, 1998).

Lathyrus is the largest genus among the economically important tribe *Fabeae*. Its members are used as orneamentals, food and fodder crop, soil nitrifiers, dune stabilizers, important agricultural weeds, and model organisms for genetic and ecological researches (Kenicer 2008). Furthermore, most species are tolerable the moderate salinity, waterlogging and drought conditions (Lal et al., 1986; Campbell et al., 1994). *Lathyrus undulatus* Boiss., *L. aureus* (Stev.) Brandza, *L. vernus* (L.) Bernh., *L. grandiflorus* Sibth. & Sm. *and L. rotundifolius* Willd., are trading significant orneamentals (Fedchenko, 1948; van der Maesen & Somaatmadja, 1989). *L. cicera* L., *L. aphaca* L. and *L. ochrus* (L.) DC. are draught resistant herb (Holm et al., 1979). *L. sativus* L. *and L. tingitanus* L. are used as a food and fodder (Mathur et al., 1999). *L. tuberosus* L. is cultivated for its edible tubers throughout northern Europe (Lawson, 1852; Johnson & Sowerby, 1862; Norton, 1996). *L. hirsutus* L., is used in the southern United States for pasture, hay winter cover and soil improvement. *L. japonicus* Willd., *L. sativus* L., *L. latifolius* L., *L. niger* (L.) Bernh., *L. sylvestris* L. and *L. odaratus* are used as a model organism in early experimentations in genetics such as, early studies of genetic linkage, complementary factor inheritance (Bell et al., 1978; Rees & Narayan, 1989; Ehrlen & Eriksson, 1995; Lambein et al., 1999).

Before the classification of Godron (1848), botanists had accepted the two Linnaean genera, *Lathyrus* and *Orobus* L. Godron identified six sections within *Lathyrus*: *Eulathyrus* Ser., *Cicercula* (Medic.) Gren & Godr., *Clymenum* (Adans.) DC., *Orobus* (L.) Gren. & Godr., *Nissolia* (Adans.) Reichb. and *Aphaca* (Adans.) Reichb. . To include those members of section *Orobus* which lacked tendrils, Boissier (1872) reconstituted *Orobus* as a distinct genus. The remaining species which owned tendrils were placed in section *Orabastrum* (Taub.) Boiss. of *Lathyrus*.

Bässler (1966) revised the all perennial members of section *Orobus sensu* Godron. He recognized six sections within subgenus *Orobus*: *Orobus*, *Lathyrostylis* (Griseb.) Bässler (as *Platystylis* (Sweet) Bässler), *Orobon* Tamamsch., *Pratensis* Bässler, *Eurytrichon* Bässler. and *Neurolobus* Bässler.

Davis (1970) divided genus *Lathyrus* into eleven sections, namely *Orobus*, *Platystylis* (as *Lathyrostylis*), *Pratensis*, *Neurolobus*, *Orobon*, *Orabastrum*, *Lathyrus* L., *Cicercula*, *Aphaca*, *Nissolia* and *Clymenum*.

Czefranova (1971) divided the Asiatic and European (Eurasian) species of the genus *Lathyrus* into six subgenera; *Orobus, Lathyrus, Clymenum, Nissolia, Cicercula and Aphaca.* Subgenus *Orobus* contained five sections; *Lathyrobus (Tamamsch.) Czefr., Orobus, Pratensis, Eurytrichon* and *Neurolobus.* Subgenus *Lathryrus* contained three sections *Orobon, Orobastrum* and *Lathyrus.*

Kupicha (1983) divided the genus into thirteen sections; *Orobus, Lathyrostylis, Pratensis, Neurolobus, Orobon, Orobastrum, Viciopsis* Kupicha, *Linearicarpus* Kupicha, *Lathyrus, Aphaca, Nissolia, Clymenum* and *Notolathyrus* Kupicha.

These classifications are based mainly on morphological characters, which are interpreted in a traditional taxonomic way without applying explicit phenetic or cladistic methods of analyses (Asmussen & Liston, 1998).

In addition to using morphological characters, there are several molecular studies on *Lathyrus* which are based on enzyme electrophoresis (Chowdhury & Slinkard 2000, Chtourou-Ghorbel et al., 2001, Ben Brahim et al., 2002) molecular markers such as RAPD, RFLP, AFLP, SSR, ISSR and ITS analyses (Croft et al. 1999, Chtourou-Ghorbel et al., 2001, Badr et al., 2002, Belaid et al., 2006, Lioi et al., 2011).

These molecular studies are limited geographic or taxonomic scope. They mainly focus on Mediterranean taxa, especially within section *Lathyrus* which is a group composed of many economically and ecologically important species.

There are two studies Kenicer (2005) and Asmussen and Liston (1998) which are based on chloroplast DNA restriction site analyses that are more or less included all representatives of the *Lathyrus* taxa. Their results are generally showed an agreement with the sectional classification of Kupicha (1983) (Table 1).

Table 1 - Comparison of the most recent four sectional classifications of *Lathyrus*

Davis 1970 (based on taxonomic decisions)	Kupicha 1983 (based on taxonomic decisions)	Asmussen & Liston 1998 (based on RFLP data)	Kenicer et al. 2005 (based on ITS and cpDNA sequence data)
	Notalathyrus	*Orobus*	*Notalathyrus*
Orobus	*Orobus*		*Orobus*
Lathyrostylis as Platystylis	*Lathyrostylis*	*Lathyrostylis*	*Lathyrostylis*
Pratensis	*Pratensis*	*Pratensis*	*Pratensis*
Aphaca	*Aphaca*	*Aphaca*	*Aphaca*
	Neurolobus	*Neurolobus*	*Neurolobus*
Orobon	*Orobon*	*Lathyrus*	
Lathyrus	*Lathyrus*		*Lathyrus*
Cicercula		*Cicercula*	
Orobastrum	*Orobastrum*	*Orobastrum*	
	Linearicarpus	*L. sphaericus* Retz.	*L. sphaericus*
		L. angulatus L.	*L. angulatus*
	Viciopsis		
Nissolia	*Nissolia*	*Nissolia*	*Nissolia*
Clymenum	*Clymenum*	*Clymenum*	*Clymenum*
		L. gloeospermus Warb. & Eig	

1.2 The Genus *Lathyrus* L. in Turkey

Boissier (1872) was the first taxonomist who studied various Turkish taxa of *Lathyrus* along with many others genera known from the area covered by his famous book of *Flora Orientalis.* He recognized six sections: *Orobastrum*, *Eulathyrus*, *Cicercula*, *Aphaca*, *Nissolia*, and *Clymenum* in the genus *Lathyrus*.

In the Flora of Turkey, Davis (1970) collected 58 Turkish species under ten sections: *Orobus, Platystylis, Orobastrum, Pratensis, Orobon, Lathyrus, Cicercula, Aphaca, Nissolia*, and *Clymenum*.

Doğan et al. (1992) studied with 54 *Lathyrus* species in which they recognized basically nine sections: *Orobus, Lathyrostylis, Aphaca, Nissolia, Orobon, Gorgonia* M.Dogan, *Clymenum, Cicercula* and *Lathyrus* based on numerical analyses of floral and vegetative characters. According to their results, these sections were grouped under two subgenera, namely subgenus *Lathyrus* and subgenus *Orobus.*

Today, the genus *Lathyrus* is represented by 62 species (78 taxa formed by means of infraspecific taxa). These taxa are belonged to 10 sections and 22 of them are endemic for Turkey (Davis, 1970; Davis et al., 1988; Genç & Şahin, 2008; Genç, 2009).

Members of the genus *Lathyrus* in and around Bolu province are from the sect. *Orobus*, sect. *Platystylis*, sect. *Pratensis*, sect. *Lathyrus*, sect. *Cicercula*, sect. *Nissolia* and sect. *Aphaca* (Table 2).

Table 2 - The studied taxa found in Bolu province

Sections	**Taxa**
Orobus (L.) Gren. & Godr.	*Lathyrus aureus* (Stev.) Brandza
	Lathyrus venetus (Miller) Wohlf.
Platystylis (Sweet) Bässler	*Lathyrus brachypterus* Cel. var. *brachypterus* *
	Lathyrus digitatus (Bieb.) Fiori
Pratensis Bässler	*Lathyrus pratensis* L.
	Lathyrus laxiflorus (Desf.) O. Kuntze subsp. *laxiflorus*
	Lathyrus czezczottianus Bässler *
Lathyrus L.	*Lathyrus tuberosus* L.
	Lathyrus undulatus Boiss.*
Cicercula (Medic.) Gren. & Godr.	*Lathyrus cicera* L.
	Lathyrus hirsutus L.
Nissolia (Adans.) Reichb.	*Lathyrus nissolia* L.
Aphaca (Adans.) Reichb.	*Lathyrus aphaca* L. var. *aphaca* L.
	Lathyrus aphaca L. var. *biflorus* Post

*the endemic taxa

1.3 Aim of the Study

The aims of this study covering *Lathyrus* taxa in Bolu province are;

1. to determine the morphological variations within and between the taxa,
2. to group the taxa numerically using both morphological characters and molecular markers, and,
3. to compare the similarities and dissimilarities of these groups.

2. MATERIALS AND METHODS

2.1 Morphological Studies

2.1.1 Collection and Identification of Materials

According to the standard techniques (Woodland, 1997) large number of specimens were collected from in Bolu province between 2012 and 2013. The collected specimens were dried and preserved in Plant Biosystematics Laboratory, Department of Biology, Faculty of Arts and Science, Abant İzzet Baysal University, Bolu, using with the standard techniques given by Davis & Heywood (1973). Many herbarium specimens were prepared and kept in AIBU herbarium.

At the beginning of the field study, in order to get pre-information about locations of *Lathyrus* species growing in Bolu province, some floristic and vegetational studies are used as a reference, such as Davis (1970), Akman & Ketenoğlu (1979), Ekim & İlarslan (1982), Turgut (1996), Uçar (1996), Sümer (2002), Türker & Güner (2002), İkinci & Güner (2006).

Collected plant specimens' geographic locations (including GPS and altitude records) and photos taken during the field studies are given in Table 3, and Figure 1 and 2, respectively.

The specimens were identified and checked using some Floras such as *Flora of Turkey* (Davis, 1970), *Flora of the USSR* (Komarov et al., 1968), *Flora of Europaea* (Tutin et al. 1968).

Study materials are composed of 14 species belonging to 7 sections of *Lathyrus*. In this study 6 taxa represented by 2 populations, such as *L. brachypterus* var. *brachypterus*, *L. digitatus*, *L. pratensis*, *L. czezczottianus*, *L. hirsutus* and *L. nissolia* thus, 20 taxa were studied in total. Each population was represented by 10 samples. Totally 200 samples used for morphological analyses of the study.

Table 3 - Sampled populations with their coordinates, altitude, population codes and station numbers

No.	Taxa	Population code	Altitude (m)	Coordinates	
				N	E
1	*L. aureus*	AUR	975	40° 40.293	31° 38.121
2	*L. venetus*	VEN	895	40° 43.046	31° 30.592
3	*L. brachypterus* var. *brachypterus*	BRA 1	1416	40° 33.306	31° 40.221
4	*L. brachypterus* var. *brachypterus*	BRA 2	1497	40° 34.480	32° 24.888
5	*L. digitatus*	DIG 1	864	40° 42.926	31° 31.085
6	*L. digitatus*	DIG 2	1398	40° 35.389	31° 16.534
7	*L. pratensis*	PRA 1	845	40° 42.487	31° 30.313
8	*L. pratensis*	PRA 2	1346	40° 39.183	32°21.068
9	*L. laxiflorus* subsp. *laxiflorus*	LAX	157	40° 25.428	30° 57.342
10	*L. czezczottianus*	CZC 1	1743	40° 36.169	31° 08.398
11	*L. czezczottianus*	CZC 2	1342	40° 39.184	32° 21.068
12	*L. tuberosus*	TUB	993	40° 47.117	32° 01.066
13	*L. undulatus*	UND	1094	40° 36.512	31° 08.398
14	*L. cicera*	CIC	1485	40° 44.402	32° 25.184
15	*L. hirsutus*	HIR 1	880	40° 44.111	32° 20.894
16	*L. hirsutus*	HIR 2	994	40° 47.119	32° 00.552
17	*L. nissolia*	NIS 1	875	40° 42.560	31° 30.462
18	*L. nissolia*	NIS 2	1487	40° 34.402	32° 25.186
19	*L. aphaca* var. *aphaca*	APH	847	40° 42.484	31° 30.325
20	*L. aphaca* var. *biflorus*	BIF	862	40° 42.912	31° 31.068

Figure 1 - Photographical representation of studied *Lathyrus* taxa I: A) *L. venetus*, B) *L. brachypterus* var. *brachypterus*, C) *L. undulatus*, D) *L. aphaca* var. *aphaca*, E) *L. aureus* F) *L. digitatus*

Figure 2 - Photographical representation of studied *Lathyrus* taxa II: A) *L. czeczottianus* B) *L. pratensis*, C) *L. laxiflorus* subsp. *laxiflorus*, D) *L. tuberosus*, E) *L. nissolia*, F) *L. cicera* G) *L. hirsutus*, H) *L. aphaca* var. *aphaca*

2.1.2 Determination of the Characters

Totally 25 characters from leaves, stems and flowers were used in this study. Thirteen of them were qualitative and 12 were quantitative. The characters were determined and selected from different researches and sources such as those of Davis (1970), Kupicha (1983), Ertekin (1991), Doğan et al. (1992), Leht (2009). In Figure 3, leaflets belonging to *L. aureus* and *L. digitatus*, represented as an example of leaflet length and width characters. Measured flower characters presented example of *L. digitatus* flower in Figure 4 and used list of quantitative and qualitative characters shown in Table 4 and Table 5 respectively.

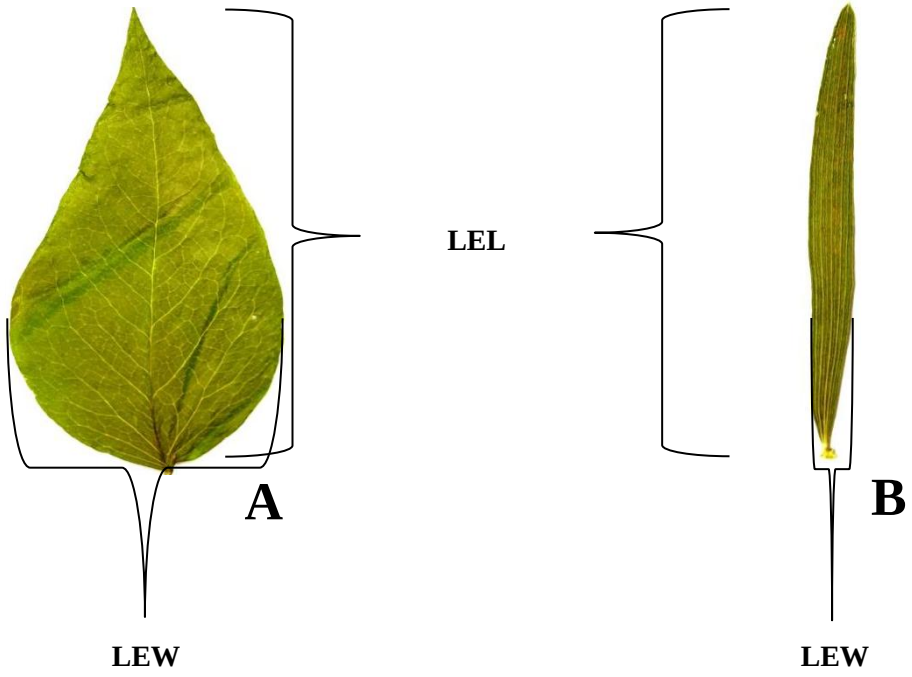


Figure 3 - Morphological leaflet characters with character codes A) *L. aureus* leaflet, B) *L. digitatus* leaflet (see Table 4)

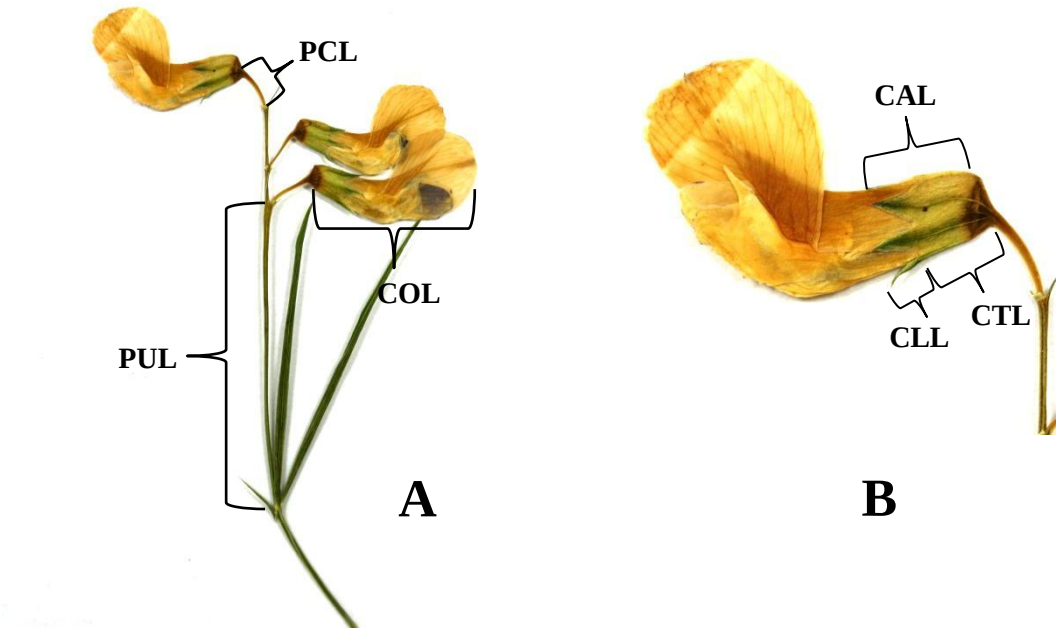


Figure 4 - Representation of measured flower characters with their character codes on *L. digitatus* (see Table 4). A) peduncle length (PUL), corolla length (COL) and Pedicel length (PCL). B) calyx tube length (CTL), calyx lobe length (CLL) and calyx length (CAL)

Table 4 - Quantitative characters used in the analysis

Character Name	**Code**
Leaflets length (cm)	LEL
Leaflets width (cm)	LEW
Petiole length (cm)	PEL
Petiolule length (cm)	PLL
Stipule length (cm)	STL
Stipule width (cm)	STW
Peduncle length (cm)	PUL
Pedicel length (cm)	PCL
Corolla length (cm)	COL
Calyx length (cm)	CAL
Calyx tube length (cm)	CTL
Calyx lobe length (cm)	CLL

Table 5 - Qualitative characters used in the analysis

Characters	**Character states**	**Codes of character states**
Life duration (LDU)	Annual	0
	Perennial	1
Stem wing (SWW)	Winged	0
	Not winged	1
Stem middle part (SMP)	Angular	0
	Round	1
Leaves ending (LFE)	Absent (NA)	0
	Aristate	1
	Tendrilous	2
Leaflets occurence (LST)	Absent (NA)	0
	Present	1
Leaflets shape (LSH)	Absent (NA)	0
	Lanceolat	1
	Ovate	2
	Linear	3
	Elliptic	4
	Linear-Lanceolate	5
	Linear-Elliptic	6
Leaflets venation (LEV)	Absent (NA)	0
	Parallele	1
	Pinnate	2
Stipule type (SST)	Filiform	0
	Semi-sagitate	1
	Unequally sagitate	2
Stipule shape (SSP)	Ovate	0
	Lanceolat	1
	Lanceolat-subulat	2
	Linear	3
	Ovate to lanceolat	4
	Ovate to suborbicular	5
Inflorescence type (FST)	Soliter (always 1)	0
	Racemous (1+)	1
Calyx hair (CLH)	Glabrous	0
	Hairy	1
Calyx teeth (CLT)	Very unequal	0
	Unequal	1
	Equal	2
Standard color (SCL)	Blue	0
	Cream	1
	Orange	2
	Purple	3
	Violet	4
	Lavender blue	5
	Yellow	6
	Pinkish red	7
	Brick red	8
	Deep pink	9
	Bluish purple	10

2.1.3 Measurements of the Characters

During the measuring of these selected characters, each population was represented by 10 samples. Each character was measured five times on the same plant. Then they were coded. Totally (200 samples x 25 characters x 5 times) 25.000 data matrix was obtained.

2.1.4 Calculating Means and Boxplots

Arithmetic means of all samples were calculated for each quantitative character (Appendix II). Then, these means of samples turned into boxplot graphs for each measured character (Appendix IV). The boxplot graphs were drawn with Minitab 16. statistical program (Minitab® 16.).

2.1.5 Grouping and Coding Character States

In contrast to qualitative data (Appendix III), during the Principal Component Analysis (PCA) processes, the raw quantitative data are standardized by Minitab 16. program, in order to reduce the effects of different measurement scales in different characters. To do this, the mean and the standard deviation of each character were used. In three steps the raw data was prepared for analyzing. Firstly, graphical representation of the boxplots is processed on means values of each character. Secondly, character boxes are taken in the same groups whether they overlap to each other or not. This is performed by separating them with breaklines into discontinuities on the plot. Finally, each group was accepted as a single state of the characters (Appendix IV) according to Almeida and Bisby (1984).

2.1.6 Construction of CA and PCA Figures in Morphological Study

After combining the quantitative and qualitative data, CA and PCA figures were drawn by using STATISTICA version 8.0. During the CA process, Euclidean distance was chosen as a type of distance since it strengthen tree diagram by geometric distance. According to phenetic approach in character weight that, each character has the same weight in species' evolution for this reason unweighted pair-group method using aritmetic averages (UPGMA) method applied for analyses (Sneath and Sokal, 1973).

2.2 Molecular Studies

2.2.1 Sampling Method

Young leaves used as a source of DNA material in which was collected from 20 populations for molecular studies. Particularly, fresh and clean leaves were selected, against to contamination during the DNA isolation or PCR. The leaves were preserved in plastic bags which were filled with orange silica gel and dried for the DNA isolation. Leaf materials were taken from one individual for each population.

2.2.2 DNA Isolation

In this study genomic DNA was isolated from silica gel-dried leaf material by using a DNA isolation kit (Nucleospin for Plant II). The quantification of genomic DNA was determined by electrophoresis on 1.5% agarose gel (Figure 5).

The modified kit protocol is as follows;

- About 60 mg dried leaf material was powdered with liquid nitrogen in morter and then transferred to Eppendorf tubes.
- For each sample 400-450 µl buffer PL1 (lysis buffer) was added and then 10 µl RNase was added to the same tubes.
- Mixture was incubated in a water bath at 65 °C for 20-30 minutes.
- Tubes were shaken gently every 10 minutes.
- The tubes were taken from water bath and added 75 µl Buffer PL3 (precipitation buffer).
- The mixture was incubated on ice for 10 minutes.
- The tubes were centrifuged at 13,000 RPM for 10 minutes.
- Supernatant was transferred to new tubes containing filter.
- The tubes were centrifuged at 11,000 RPM for 4 minutes.
- 450 µl PC Buffer (binding buffer) was added.
- The tubes were shaken gently by upside and down 5 times.
- 600 µl of the mixture was taken and put into new tubes containing filter.
- The tubes centrifuged at 11,000 RPM for 3 minutes and discarded the flow-through.
- 400 µL PW1 Buffer (wash buffer) was added then the tubes were centrifuged at 11,000 RPM for 3 minutes and discarded the flow-through.
- 600 µl PW2 Buffer (wash buffer) was added into the tubes and were centrifuged at 11,000 RPM for 3 minutes and discarded the flow-through.
- 200 µl PW2 Buffer (wash buffer) was added again then the tubes were centrifuged at 11000 RPM for 4 minutes and discarded the flow-through.

- New Eppendorf tubes replaced and spin colums were placed in tubes. Then 50 µl PE Buffer (elution buffer) was added and incubated in a water bath at 65°C for 10 minutes.
- The tubes were taken from water bath and centrifuged at 11000 RPM for 2 minutes to elute.
- Extracted genomic DNAs were kept at 4 °C.

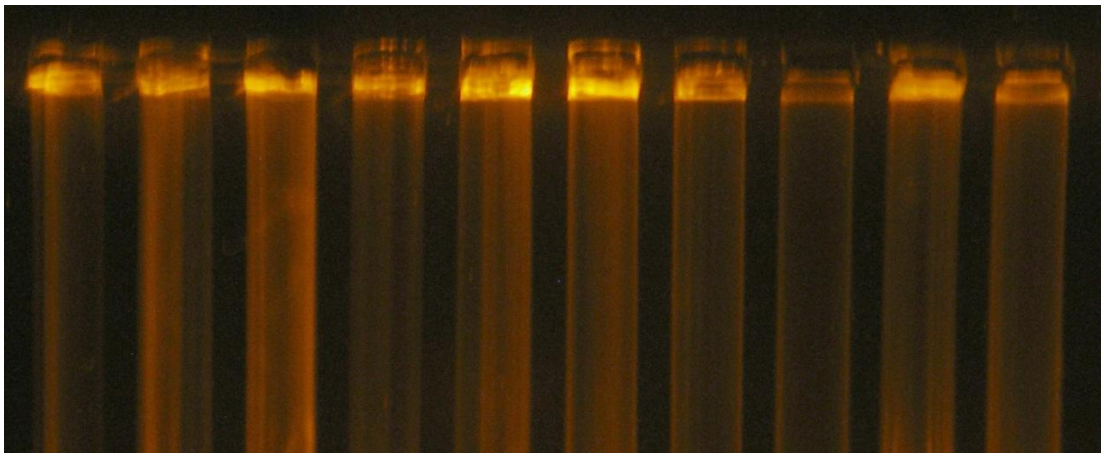


Figure 5 - After isolation of genomic DNA, its quality was checked by electrophoresis

Special codes (Table 6, 7) were given to isolated DNAs and sections because they were useful in molecular analysis (PCR process, in phenograms). Also the same codes were used in morphological analysis (Boxplots graphs, phenogram) so, each population had unique code which were used both morphological and molecular study.

Table 6 - Code list of studied *Lathyrus* taxa

Species	**Code**	**Species**	**Code**
L. aureus	AUR	*L. venetus*	VEN
L. brachypterus	BRA1	*L. tuberosus*	TUB
L. brachypterus	BRA2	*L. undulatus*	UND
L. digitatus	DIG1	*L. cicera*	CIC
L. digitatus	DIG2	*L. hirsutus*	HIR1
L. pratensis	LAX	*L. hirsutus*	HIR2
L. pratensis	PRA1	*L. nissolia*	NIS1
L. laxiflorus	PRA2	*L. nissolia*	NIS2
L. czezczottianus	CZC1	*L. aphaca var. aphaca*	APH
L. czezczottianus	CZC2	*L. aphaca var. biflorus*	BIF

Table 7 - Shorten names of Sections'

Section name	**Codes**
Sect. *Orobus*	OR
Sect. *Platystylis*	PL
Sect. *Pratensis*	PR
Sect. *Lathyrus*	LA
Sect. *Cicercula*	CI
Sect. *Nissolia*	NI
Sect. *Aphaca*	AP

2.2.3 Determination of RAPD and ISSR Primers

According to previous studies, Croft et al. (1999), Chowdhury and Slinkard (2000), Chtourou-Ghorbel et al. (2002), about 12 RAPD (Random Amplified Polymorphic DNA) primers were selected. And 6 ISSR (Inter Simple Sequence Repeats) primers were selected, (Belaid et al. 2006, Kafkas et al. 2006), in order to indicate polymorphism and also give reproductive results in this study. The list of selected primers and their open sequences is given in Table 8, 9.

Table 8 - RAPD primers list with their sequences

Primers	**Sequence (5′→ 3′)**
OPA-04	TGAGCCTCAC
OPJ-12	GTCCCGTGGT
OPJ-13	CCACACTACC
OPJ-18	TGGTCGCAGA
OPJ-09	AATCGGGCTG
OPC-19	GTTGCCAGCC
OPD-03	GTCGCCGTCA
OPP-10	TCCCGCCTAC
OPW-04	CAGAAGCGGA
OPAD-11	CAATCGGGTC
UBC353	TGGGCTCGCT
UBC368	ACTTGTGCGG

Table 9 - ISSR primer list with their sequences

Primers	**Sequence (5′→ 3′)**
UBC816	CACACACACACACACAT
UBC861	ACCACCACCACCACCACC
PRIMER1	TCTCTCTCTCTCTCTCTCCTA
PRIMER2	AGAGAGAGAGAGAGAGAGAGC
PRIMER3	CTCTCTCTCTCTCTCTCTCT
PRIMER4	AGAGAGAGAGAGAGAGAGAG

2.2.4 PCR and Electrophoresis Processes

The amplification of these primers with *Lathyrus* taxa DNA was carried out by means of a PCR. DNA amplification was performed in a Thermal Cycler (Techne-[3]Prime).

The RAPD amplification reactions were carried out in a 25 μL mix containing 25 ng of genomic DNA, 1X PCR buffer ($(NH_4)SO_4$), 2.0 mM $MgCl_2$, 1.0 mM of each dNTP (dATP, dCTP, dGTP, dTTP), 1.0 μM primer, 1.0 U of *Taq* polymerase. A PCR cycle program for RAPD was as follows: initial denaturation at 93 °C for 5 min, and then 45 cycles at 92 °C for 1 min (denaturation), 35 °C for 2 min (primer hybridization), and 72 °C for 3 min (elongation), and a final elongation at 72 °C for 10 min.

The ISSR amplification reactions performed as follows: 25 ng of genomic DNA, 2.0 mM of $MgCl_2$, 1.0 mM of each dNTP, 1.0 μM of ISSR primer, 1.0 U of *Taq* polymerase, and 1X buffer ($(NH_4)SO_4$) in a final volume of 25 μL. A PCR cycle program for ISSR was as follows: initial denaturation at 94 °C for 7 min, then 35 cycles of 45 s at 94 °C (denaturation), 1 min at appropriate primer hybridization temperature (50-60 °C), 2 min at 72 °C (elongation), and a final elongation at 72 °C for 7 min.

DNA gel electrophoresis are usually performed for determination of the presence and size of the PCR products. At the beginning of electrophoresis, agarose solution is boiled until clear in order to dissolve agarose. Then melted agarose is spilled into a form equipped with removable comb. The agarose polymerizes formed a flexible gel when cooled. Combs are removed carefully from the gel. The gel is placed in electrophoresis chamber. Electrophoresis buffer (1X TBE) is added to cover the gel to a depth of at least 1 mm. After this process, 0.5 μg/mL ethidium bromide added to the buffer.

The PCR products are placed in the wells with loading buffer and run under the influence of an electrical field through the gel at a neutral pH. DNA ladder and control samples were placed in the two of the wells before PCR products run. Inclusion of a DNA ladder (100-10.000 bp) makes it easy to determine the sizes of unknown PCR products. Blank sample is provided to negative control which was contained water instead of DNA during PCR.

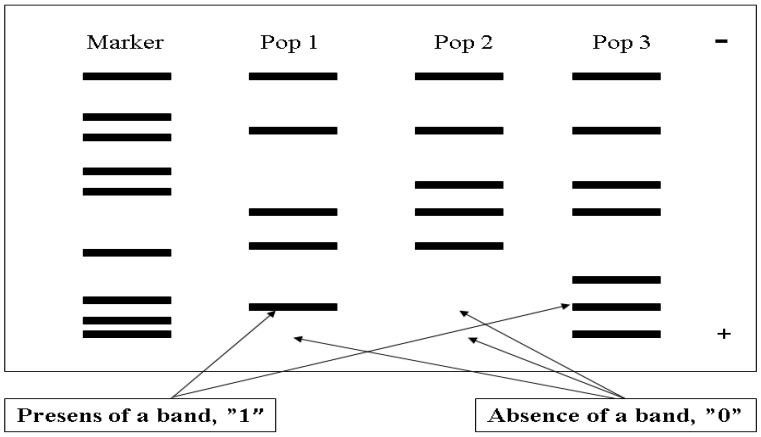
PCR products move from anode pole to cathode pole. Because of the small DNA fragments have less frictional drag, they move rapidly. In contrast to this, larger DNA fragments have more frictional drag so their mobility is slower. Finally, according to their sizes, PCR products distributed to large fragments are near the top, and small fargments are near the bottom of the gel.

In this study, both RAPD and ISSR PCR products, are separated by DNA gel electrophoresis is as follows with minor modifications;

1. DNA Loading Buffer (1.0 µl) added to (5.0 µl) of PCR products prior to electrophoresis.
2. 6.0 µl loaded from this mixture to each well.
3. Electrophoresis process occurred;
    a. For RAPD: in 1.5 % agarose gel for 1 h 5 min. at 100 Volt.
    b. For ISSR: in 1.5 % agarose gel for 1h 15 min. at 90 Volt.
4. The gel images were photographed with a gel documentation system (Ultralum-Teknis).

2.2.5 Coding Band States

The sizes of DNA fragments provided by RAPD and ISSR PCR for each primer were established in each population separately. After this process, data matrix was constructed containing the size of the DNA fragments which produced for each populations. Then the data matrix turned into a binary data matrix by scoring absence (0) or presence (1) of RAPD and ISSR bands (Figure 6).



A

Population1	Population2	Population3
1	1	1
1	1	1
0	1	1
1	1	1
1	1	0
0	0	1
1	0	1
0	0	1

B

Figure 6 - Construction of a binary data A) pattern of the gel bands, B) coded data

2.2.6 Construction of CA and PCA Figures in Molecular Study

According to RAPD and ISSR binary data, cluster analysis (CA) and principal component analysis (PCA) were carried by using STATISTICA version 8.0. (Statistica Statsoft Inc. 2007).

In order to compare molecular result with morphological result, the same CA and PCA analyses were carried out. Similarly, Euclidean distance was chosen as type of distance and UPGMA method selected during CA process. Two phenograms were presented by using molecular data. First phenogram was constructed by using only RAPD binary data. Second phenogram was constructed by combined RAPD and ISSR binary data. ISSR data was not used solely, because of not enough number of bands were obtain with the studied markers.

3. RESULTS

3.1 Results of Morphological Study

3.1.1 Identification Key of Studied *Lathyrus* Taxa

According to the morphological observation, the identification key of studied *Lathyrus* taxa was prepared using measured characters given below:

1 Leaflets absent

2 Leaf rachis ending in a tendrils; stipules large and biauriculate.

3 Peduncles always 1-flower, 1 x as long as stipules ***L. aphaca* var. *aphaca***

3 Peduncles 2-flowers, 2-3 x as long as stipulutes ***L. aphaca* var. *biflorus***

2 Leaf rachis ending in arista; stipules minute ***L. nissolia***

1 Leaflets present

4 Leaflets always 1 paired

5 Rachis with tendril

6 Flower color yellow, stem unwinged ***L. pratensis***

6 Flower color pinkish or purplish, stem winged

7 Root with tuber ***L. tuberosus***

7 Root wihout tuber

8 Legumes with glabrous sutures ***L. undulatus***

8 Legumes with hairy sutures ***L. hirsutus***

5 Rachis without tendril

9 Corolla lavender blue, leaflets lanceolate, corolla blue ***L. czezczottianus***

9 Corolla violet, leaflets ovate-elliptic ***L. laxiflorus* subsp. *laxiflorus***

4 At least median leaves usually with 2-5 pairs of leaflets

10 Leaflets ovate, stipules ovate-oblong

11 Flowers orange ***L. aureus***

11 Flowers purple ***L. venetus***

10 Leaflets linear, stipules lanceolate

12 Flower solitary, flowers brick red ***L. cicera***

12 Flowers in racemes; flowers cream or blue

13 Flowers cream ***L. brachypterus* var. *brachypterus***

13 Flowers blue ***L. digitatus***

3.1.2 Variations Among Studied *L. hirsutus* Populations

Another finding is also presented here, according to the morphological studies. In the study, there were two populations from L. hirsutus; pop1 or HIR1 collected from Gerede, pop2 or HIR2 from near Lake of Yeniçağa. The two population members showed some variations. Both members of populations were compared according to the different country Flora's and the results given in Table 10 and Figure 7.

Table 10 - Comparision of studied *L. hirsutus* populations according to different Countries Flora's

Floras / Characters	Flora of Turkey Davis 1970	Flora of Europe Tutin et al. 1968	Flora of U.S.S.R Komarov et al. 1968	Present Study	
				HIR1 (Gerede)	HIR2 (Yeniçağa)
Stem (cm)	40-60	20-120	20-50	10-20	50-70
Leaflet length (mm)	30-60	15-80	25-70	17-26	47-51
Stipule length (mm)	-	10-18	10-12	4-6	12-14
Tendrils (sect.)	3 or pinnately branched tendrils	–	branched tendrils	1-2(-3)	3
Number of flowers	1-3	1-3(-4)	–	always 1	1-2
Corolla color	bluish purple	pale blue	blue-violet	deep pink	bluish purple
Corolla length (mm)	10-14	7-15-(-20)	10-13	8-10	10-12
Number of seeds	5-7	5-10	-	3(-4)	5-6(-7)

***L. hirsutus* (HIR1) Gerede**	***L. hirsutus* (HIR2) Yeniçağa**
 **A**	 **B**

Figure 7 (continued)

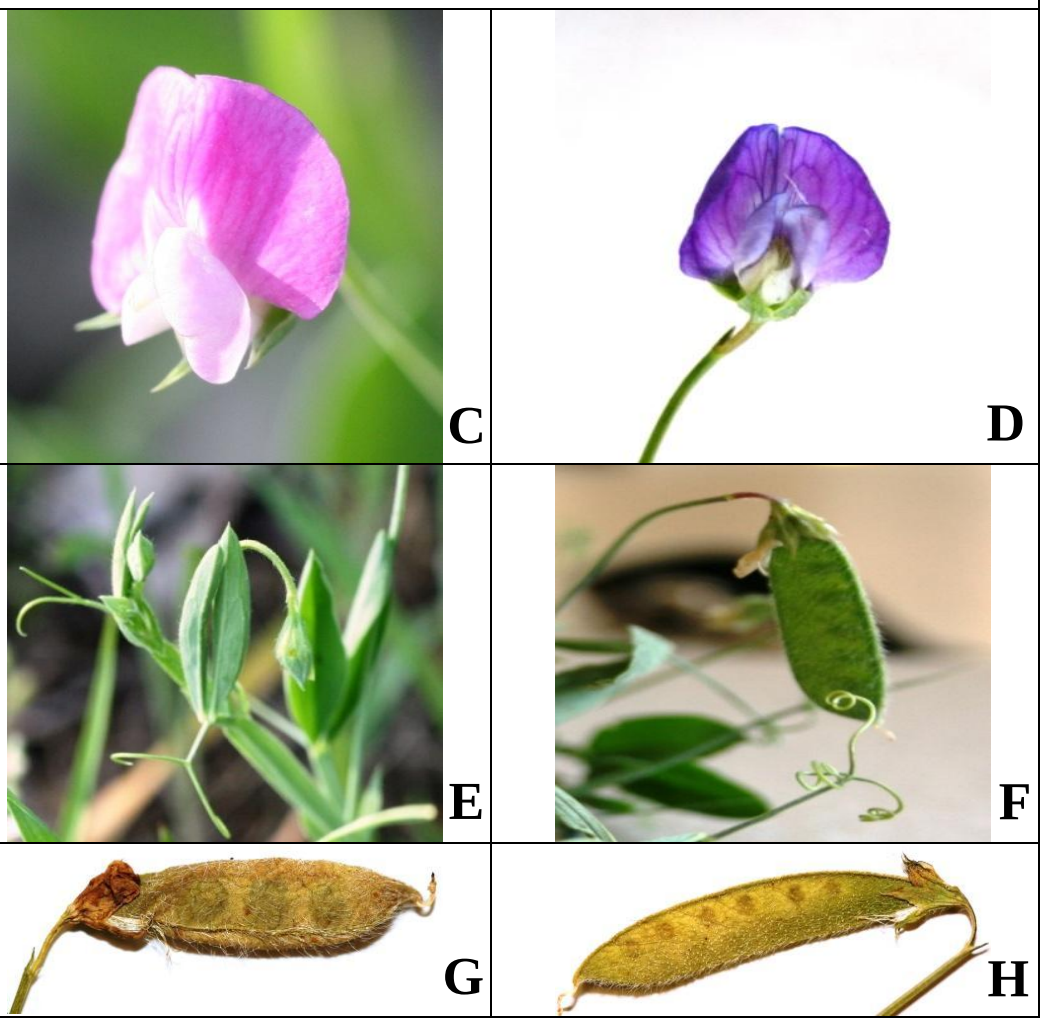


Figure 7 - Comparision of *L. hirsutus* populations collected from different locations. A-B; Stem length, C-D; Corolla color, E-F; Tendril sect., G-H; Number of seeds

3.1.3 CA and PCA Analyses

In order to construct the phenogram of cluster analysis (CA), 20 *Lathyrus* taxa were measured with 25 quantitative and qualitative characters. The obtained data were used for constracting phenograms by STATISTICA version 8.0 program, according to the UPGMA (unweighted pair-group average) algorithm based upon Euclidean distances. The phenogram was given in Figure 8.

In the Figure 8 firstly, *L. nissolia* (NIS1, NIS2), *L. pratensis* (PRA1, PRA2) and *L. digitatus* (DIG1, DIG2) which were sampled from two separate populations, showed similar characteristics and combined to each other. On the other hand, *L. czeczottianus* (CZC1, CZC2) and *L. brachypterus* (BRA1, BRA2) taxa, also they were sampled from two separate populations, combined at the level of 1.00 phenon line indicating some differencies. *Lathyrus hirsutus* populations (HIR1, HIR2) showing more differences as they combined at 1.75 phenon line. *Lathyrus cicera* linked to HIR1 and HIR2 group from upper part at phenon line 2.75. At phenon line 3.75, seven clusters comprised all the studied sections: *Cicercula, Lathyrus, Nissolia, Aphaca*, *Pratensis*, *Orobus* and *Platystylis* (as *Lathyrostylis*). Each sectional cluster contained their own sectional members. Differently, at phenon line 4.50, six clusters observed. The section *Cicercula* and section *Lathyrus* represented as a single section at this level. At phenon line 7.00, the 20 *Lathyrus* taxa formed two main clusters. The First main cluster contained the members of five sections: *Cicercula*, *Lathyrus*, *Nissolia*, *Aphaca* and *Pratensis*. The main second cluster covered the members of two sections: *Orobus* and *Platystylis* (as *Lathyrostylis*) (Figure 8).

Morphological data were also used to show groupings of studied *Lathyrus* members by the principal component analysis (PCA), given in Figure 9. Section *Nissolia*, *Aphaca* and *Orobus* populations were clearly separated from each other. The section *Platystylis* (as *Lathyrostylis*) members were located in the positive side of the y-axis, on the other hand section *Cicercula* populations were located in the negative side of the y-axis. The two members of *Lathyrus* section, *L. tuberosus* and *L. undulatus* divided by the x-axis. The section *Pratensis* members; *L. laxiflorus* and *L. czeczottianus* were located closely to each other than the two populations of *L. pratensis* which were close to negative side of the y-axis. Similarly to Cluster Analysis results (Figure 8), the two populations of the same species, such as *L. nissolia* (NIS1, NIS2), *L. pratensis* (PRA1, PRA2) and *L. digitatus* (DIG1, DIG2) each were overlapped at the same points in graph of PCA (Figure 9).

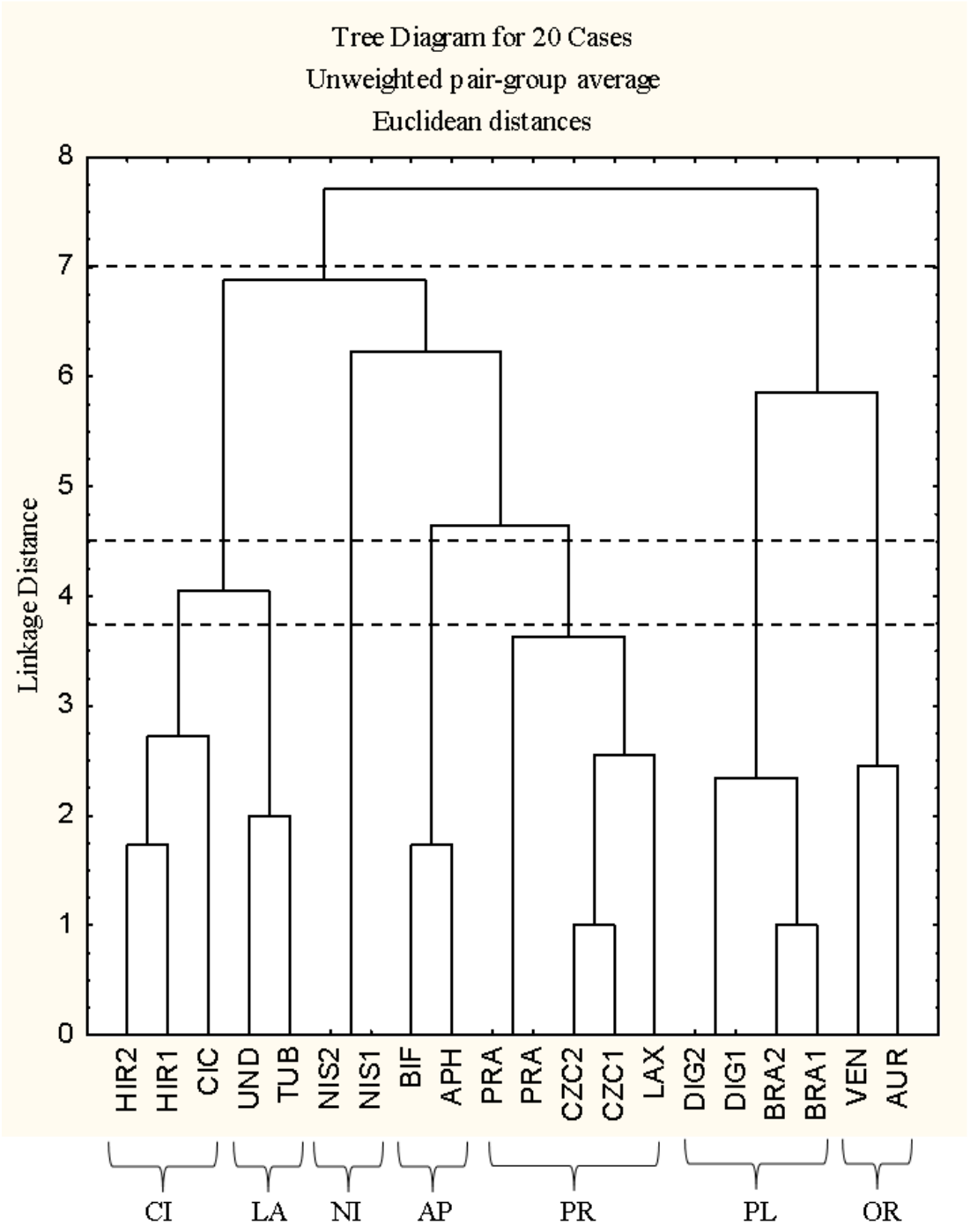


Figure 8 - Phenogram (Tree Diagram) resulting from Cluster Analysis with UPGMA based on morphological characters

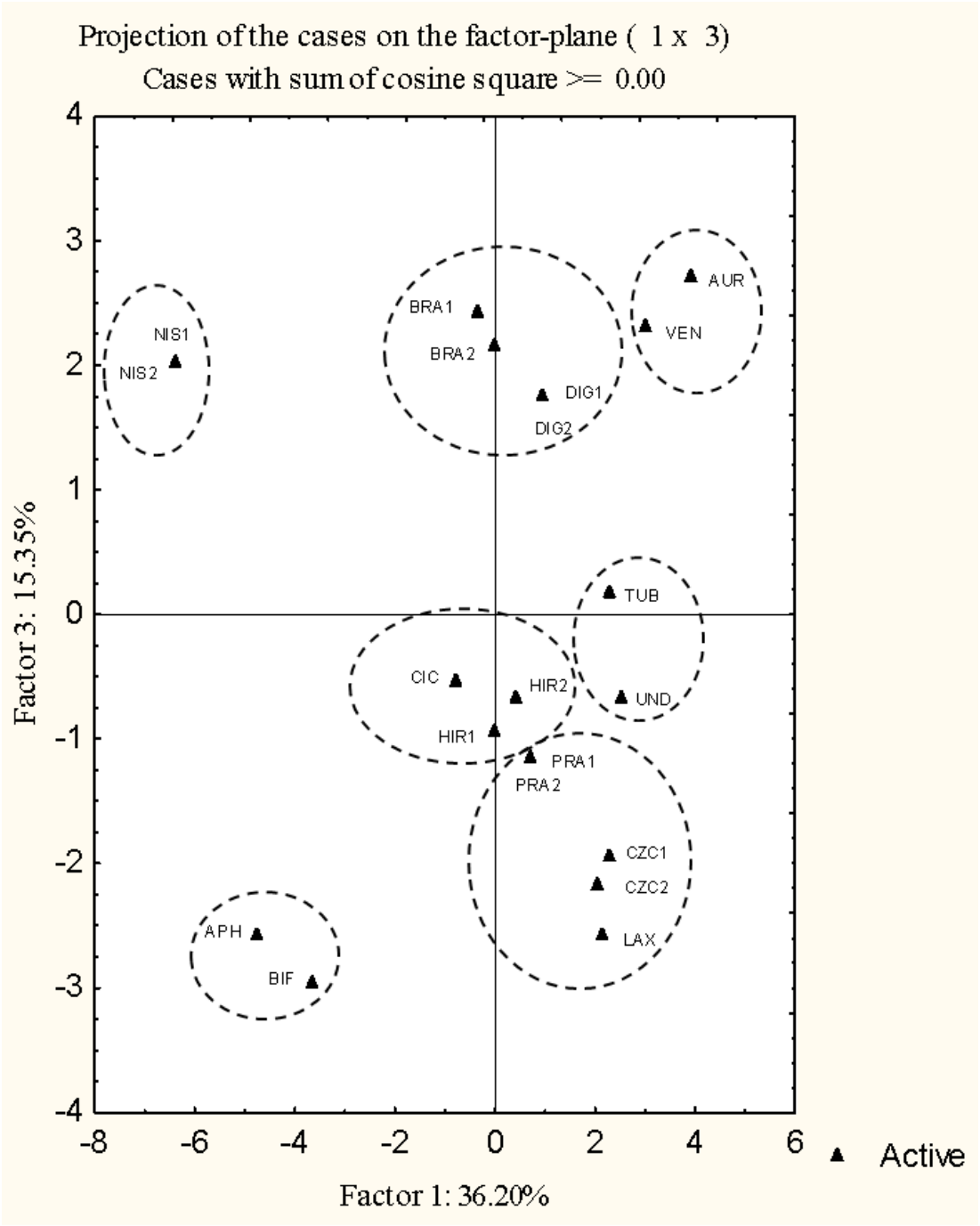


Figure 9 - Graphical representation of Principal Component Anlaysis (PCA) based on morphological data

3.2 Results of Molecular Study

3.2.1 RAPD and ISSR Gel Patterns

In order to compare the results of mophological with molecular results, CA and PCA analyses were also made. To determine the genetic relationships of 20 *Lathyrus* taxa, 18 (12 RAPD and 6 ISSR) primers were tested. About 16 primers, out of the 18, gave polymorphism based on the quality and reliability of their amplification with all the taxa. A total of 320 and 92 bands were produced by 12 RAPD and 4 ISSR markers, respectively. These bands were scored as absence and presence for all studied taxa.

In order to score the RAPD and ISSR products, 20 *Lathyrus* taxa were run together for each primer. RAPD products were given from Figure 10 to Figure 21. ISSR products were given from Figure 22 to Figure 25.

Numbers and codes which were using in Figures are given as follows; 1: HIR2, 2: HIR1, 3: BIF, 4: APH, 5: LAX, 6: CIC, 7: UND, 8: TUB, 9: VEN, 10: AUR, 11: NIS2, 12: NIS1, 13: PRA2, 14: PRA1, 15: CZC2, 16: CZC1, 17: DIG2, 18: DIG1, 19: BRA2, 20: BRA1, C: Control.

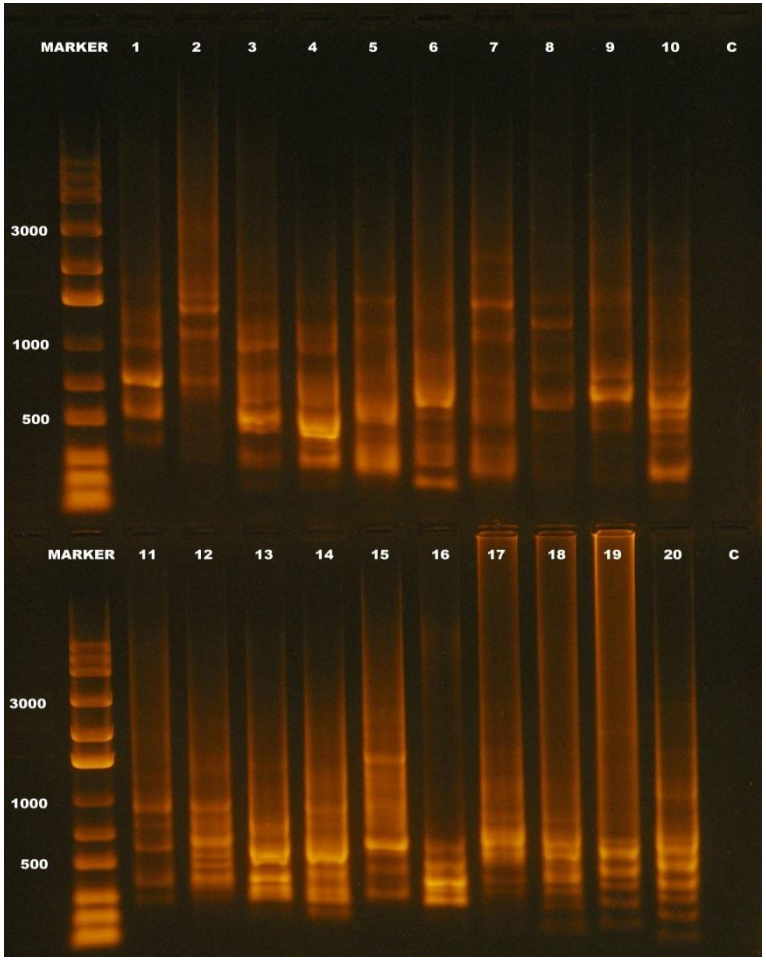


Figure 10 - All populations with OPA-04 primer

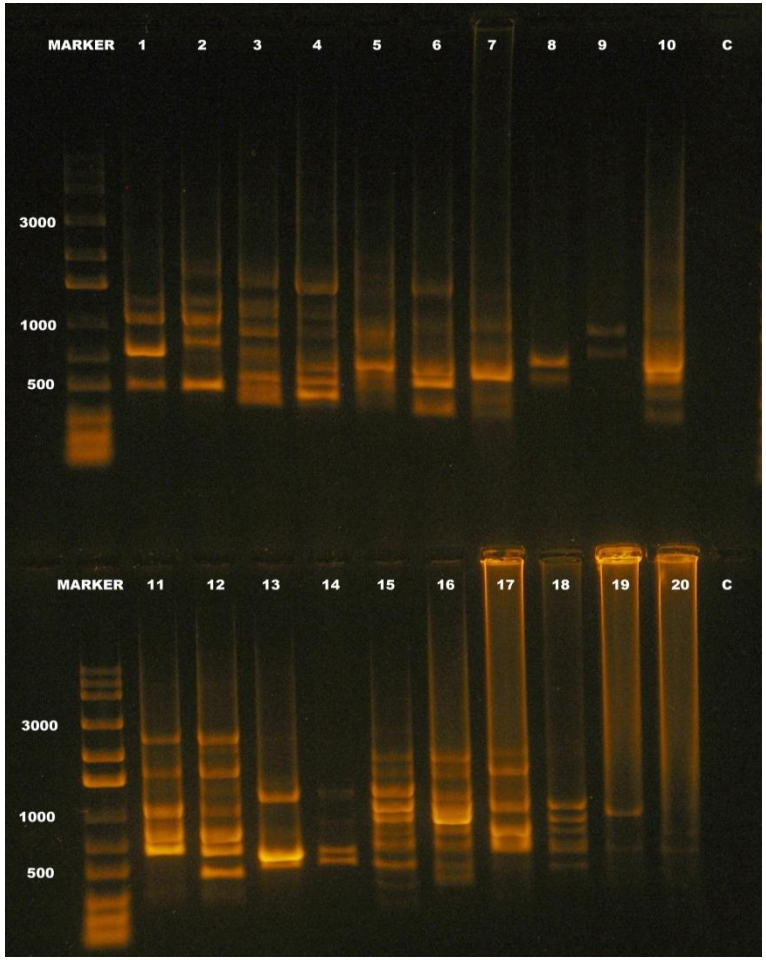


Figure 11 - All populations with OPJ-12 primer

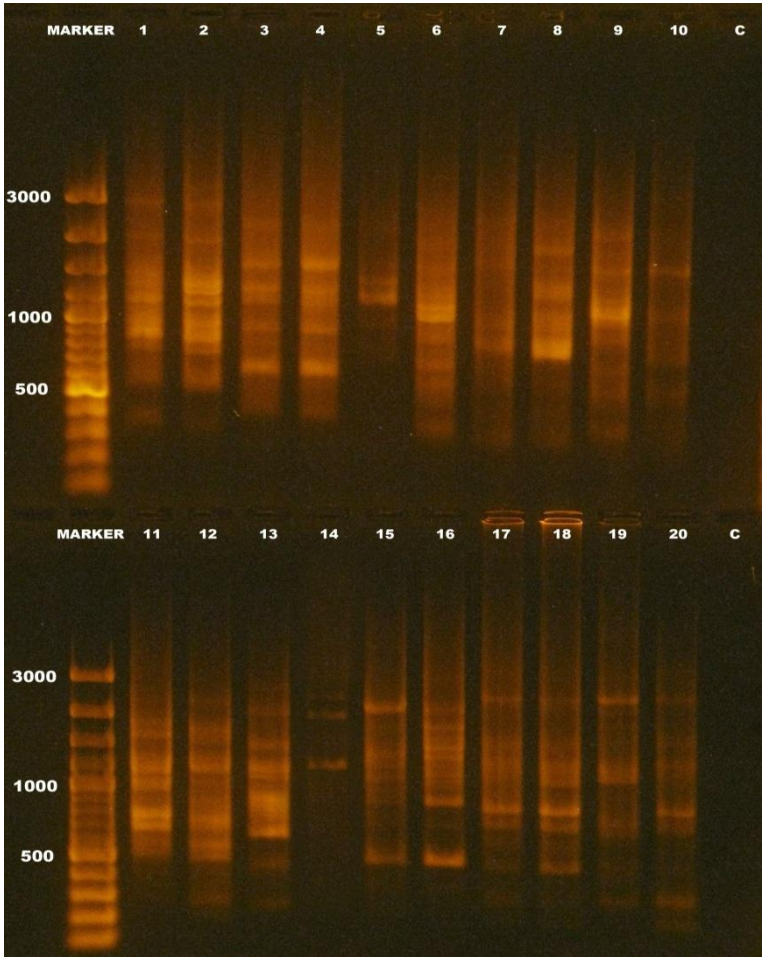


Figure 12 - All populations with OPJ-13 primer

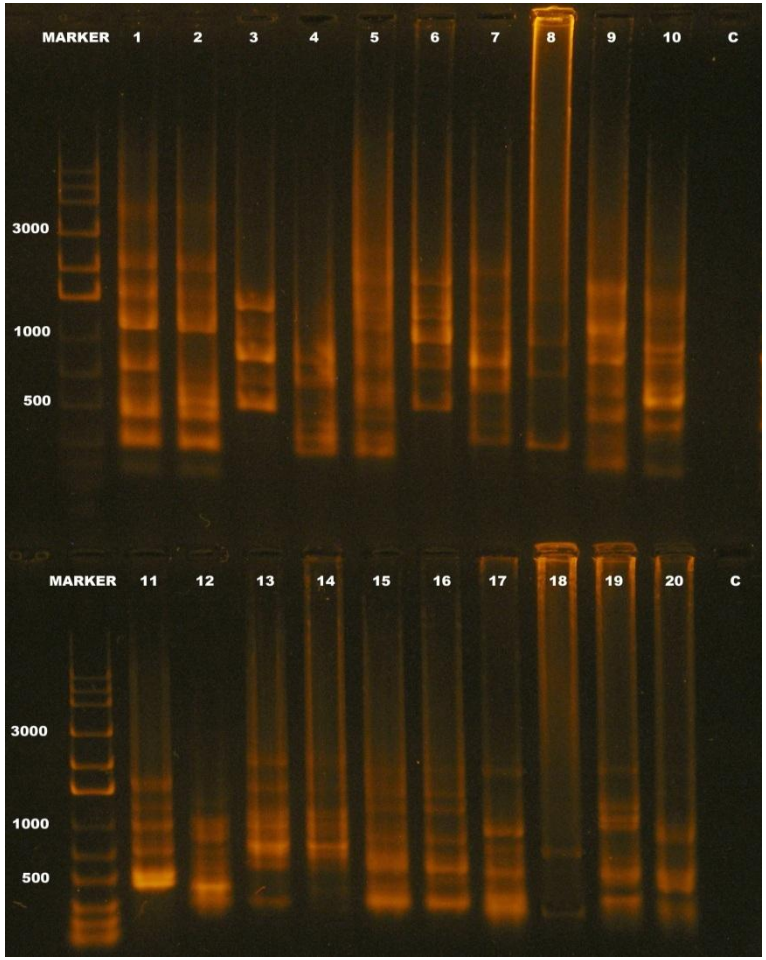


Figure 13 - All populations with OPJ-18 primer

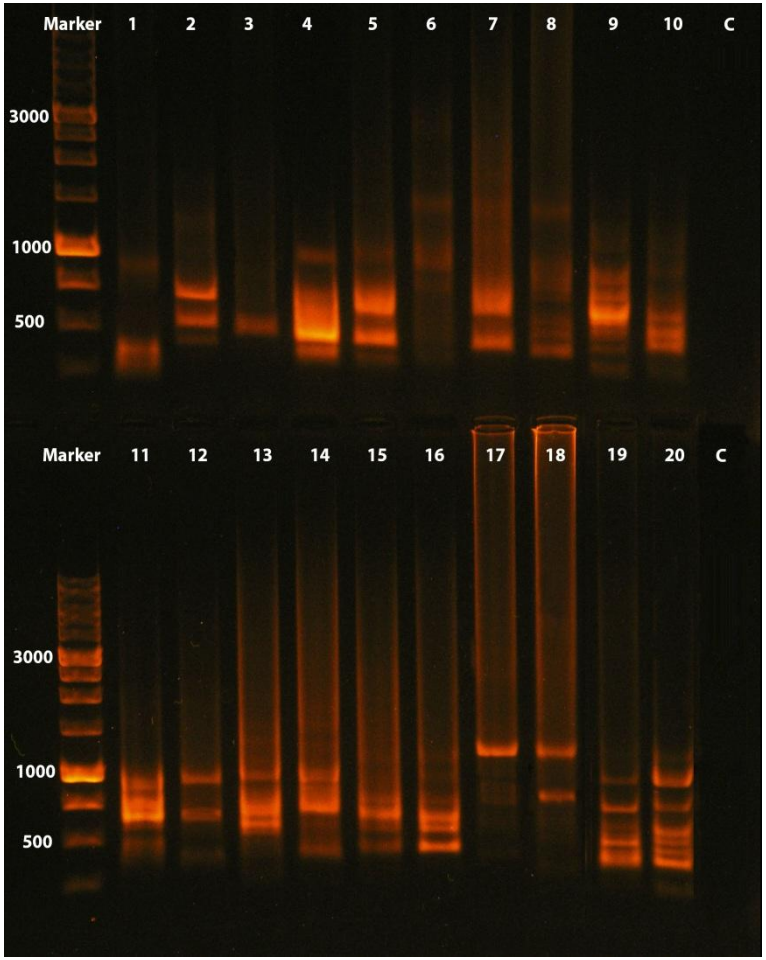


Figure 14 - All populations with OPJ-09 primer

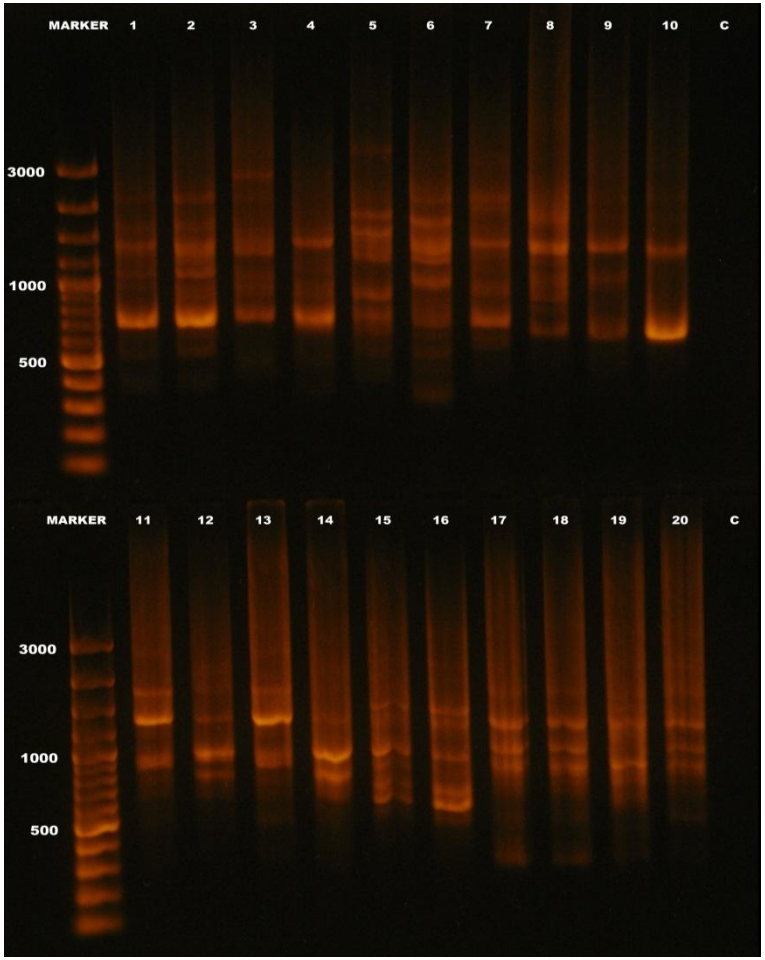


Figure 15 - All populations with OPC-19 primer

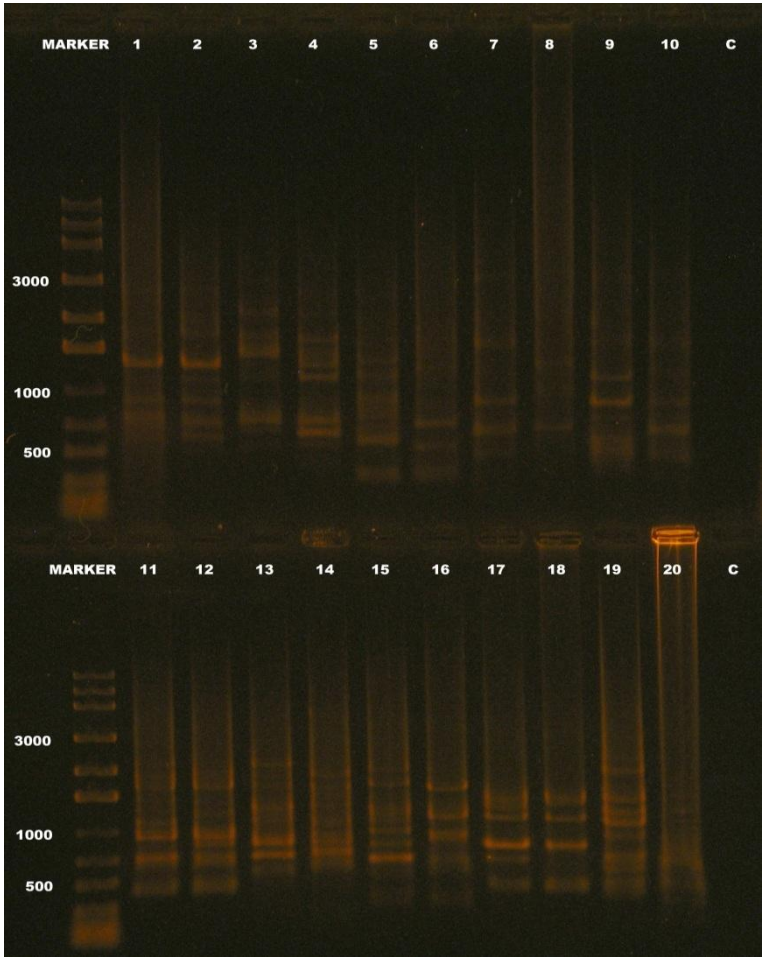


Figure 16 - All populations with OPD-03 primer

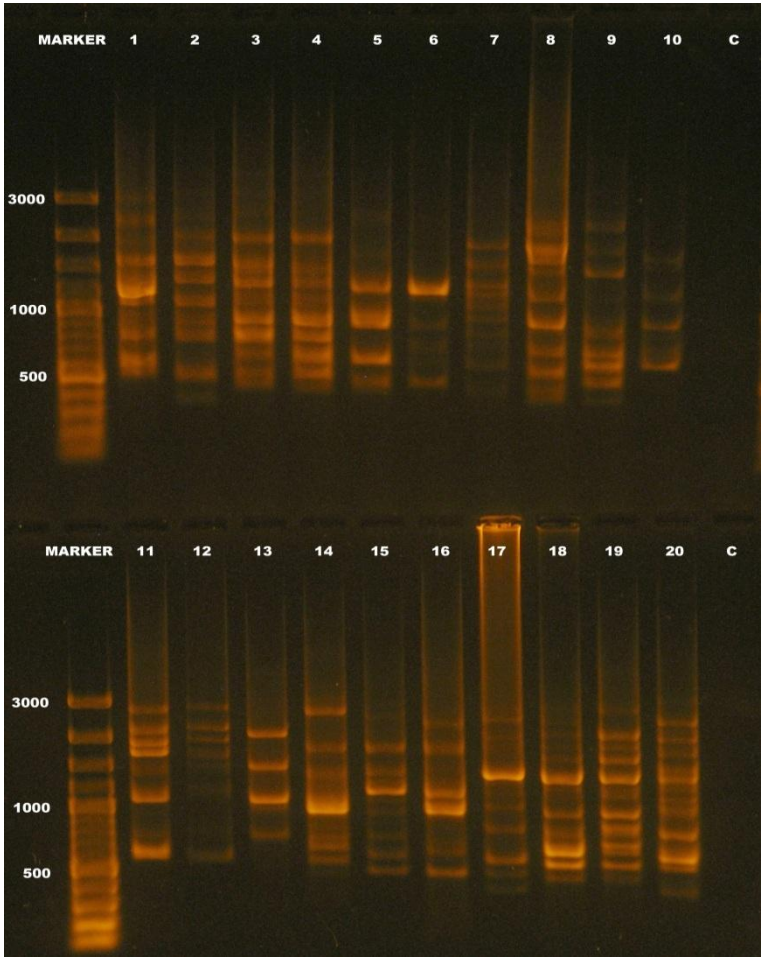


Figure 17 - All populations with OPP-10 primer

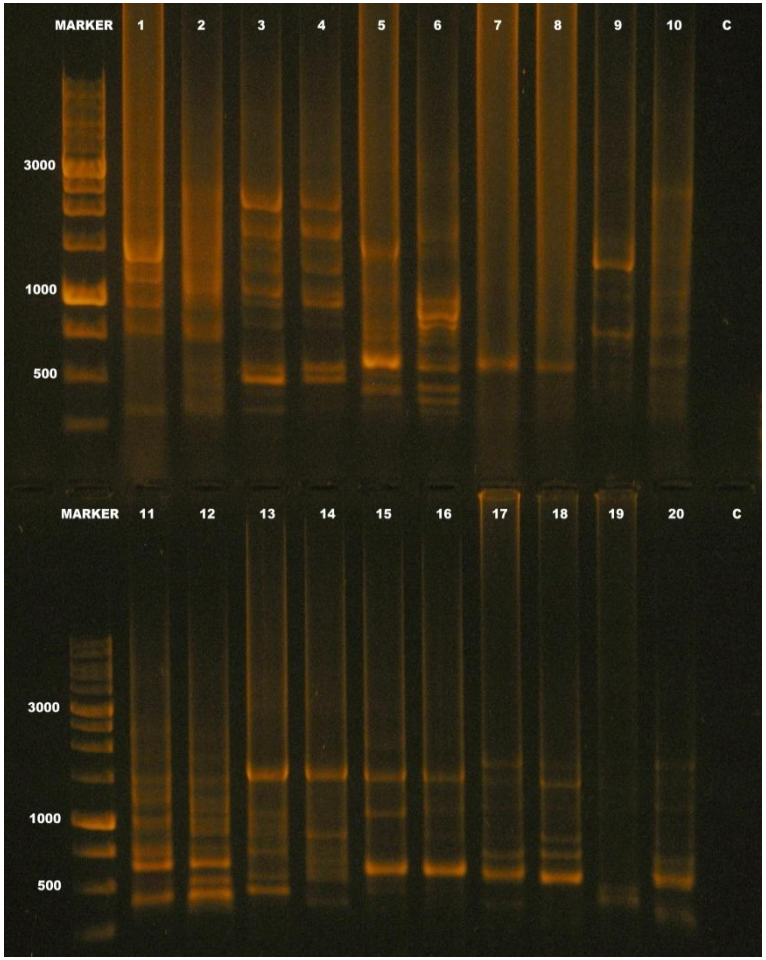


Figure 18 - All populations with OPW-04 primer

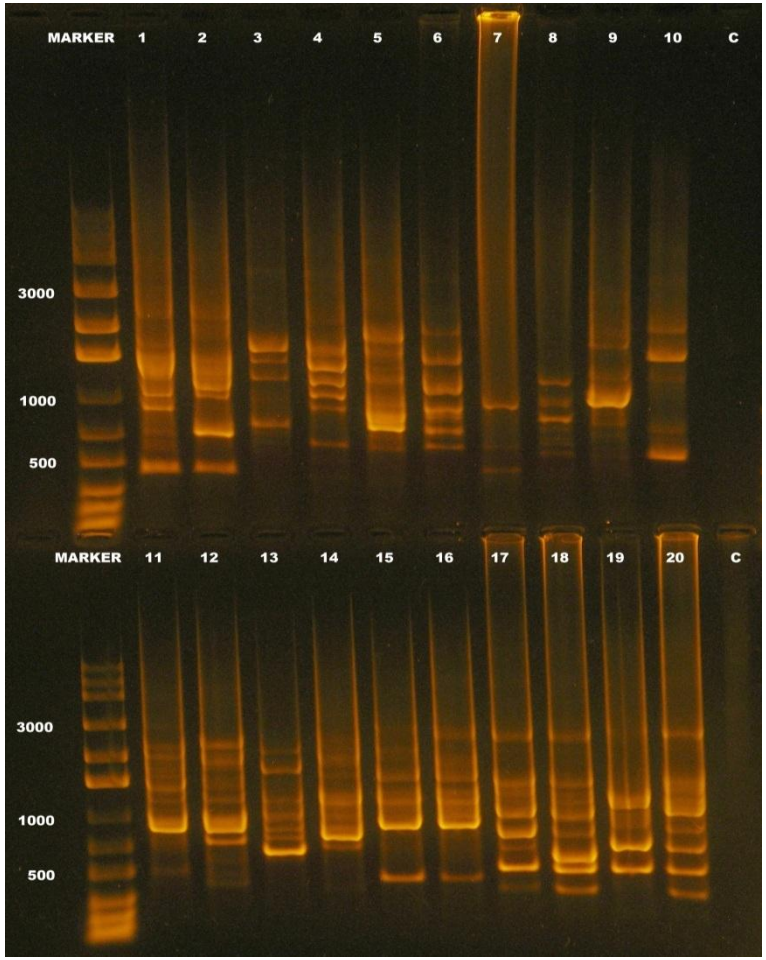


Figure 19 - All populations with OPAD-11 primer

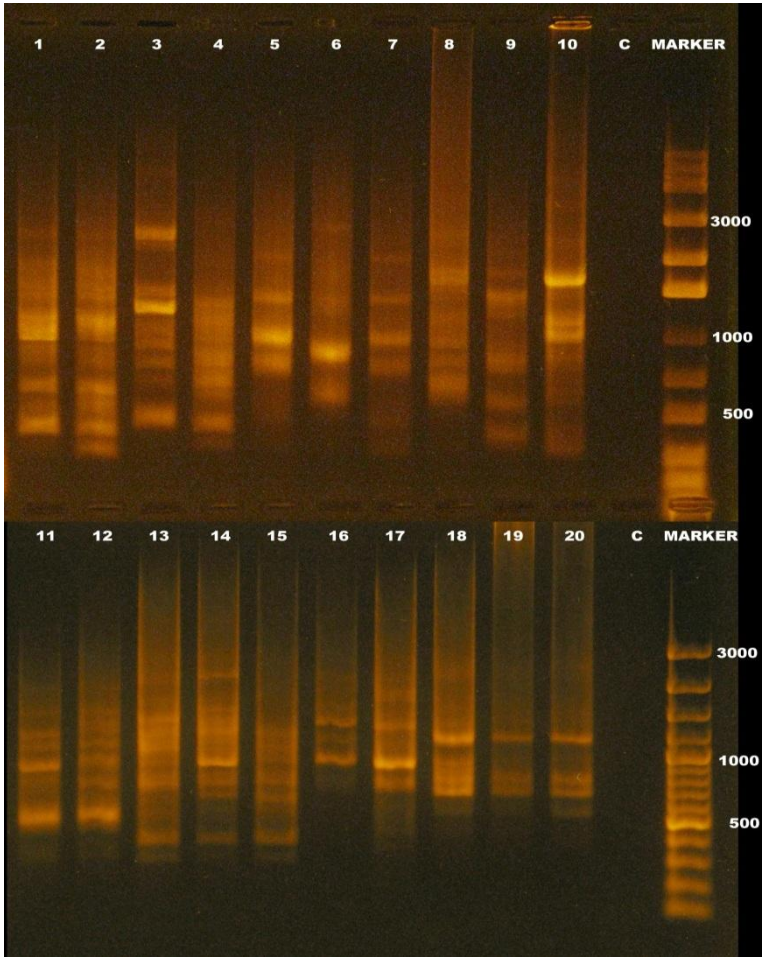


Figure 20 - All populations with UBC353 primer

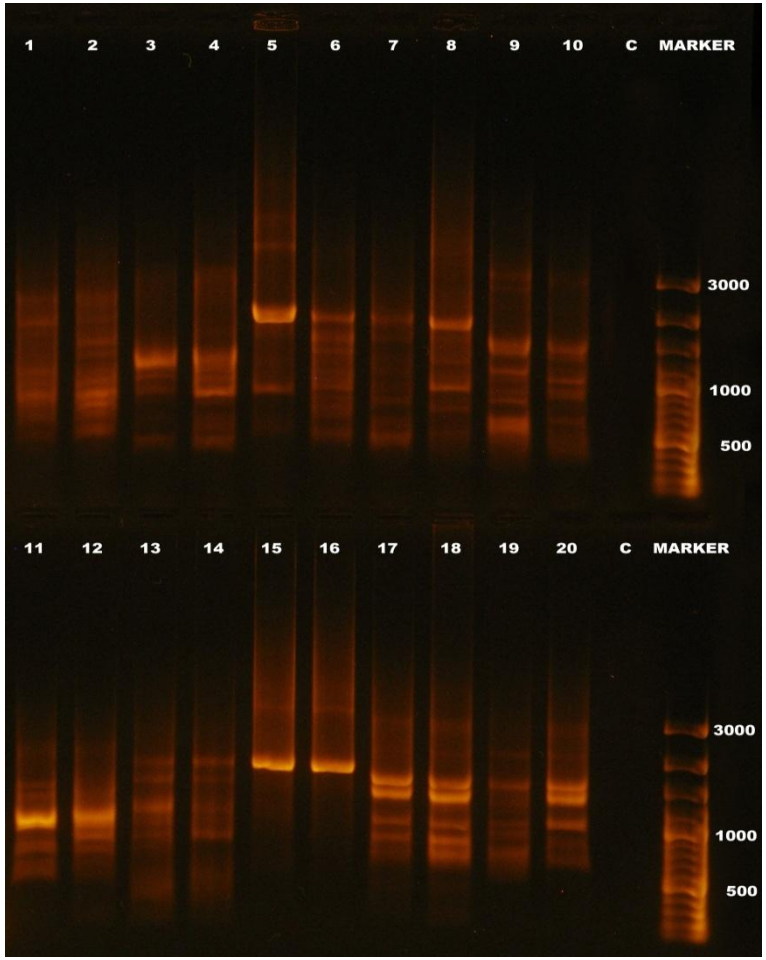


Figure 21 - All populations with UBC368 primer

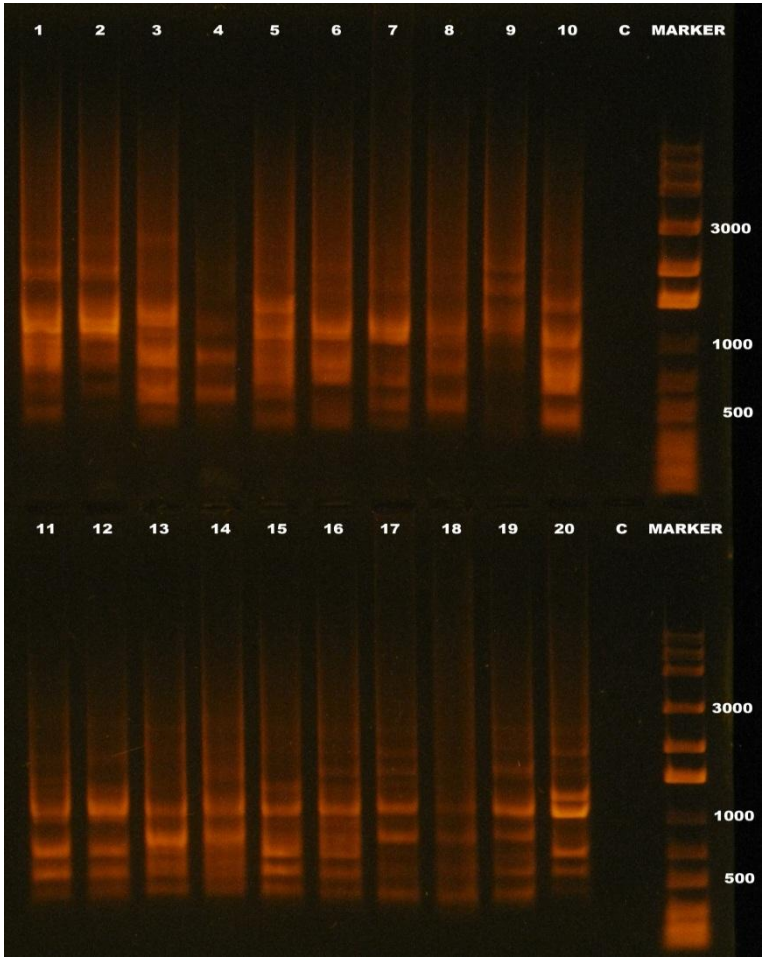


Figure 22 - All populations with UBC816 primer

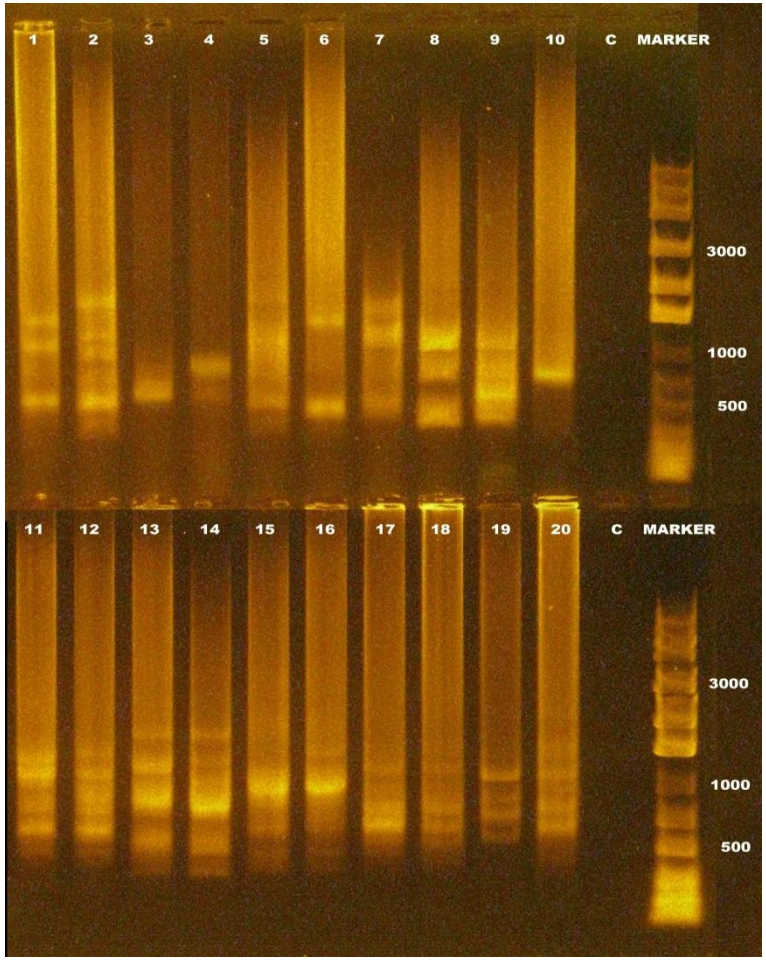


Figure 23 - All populations with UBC861 primer

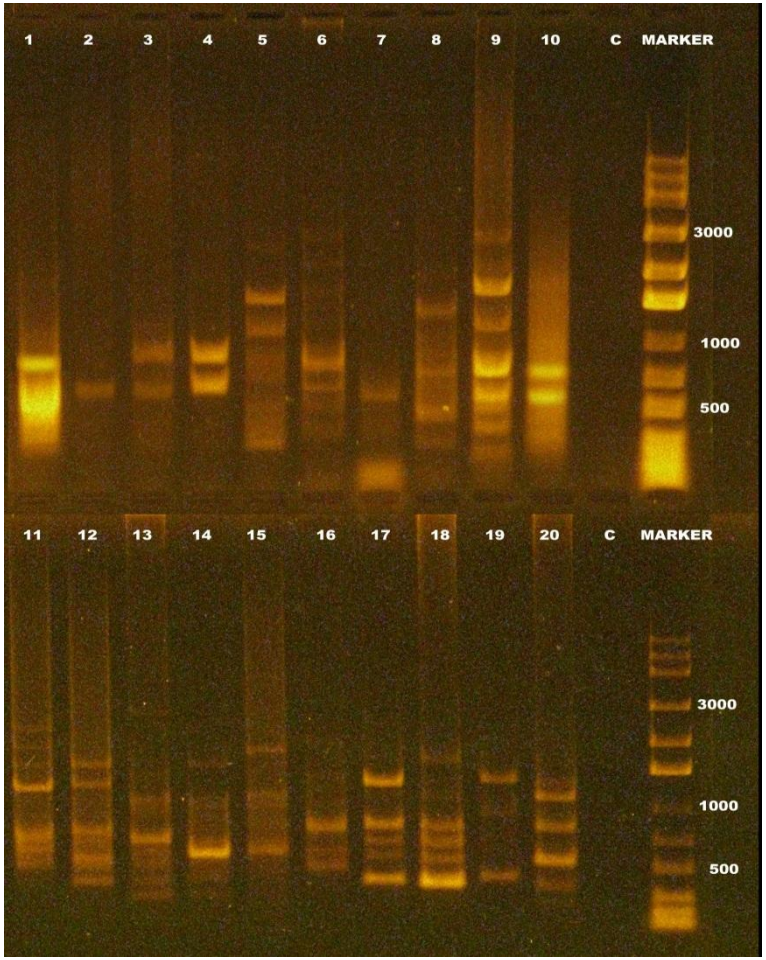


Figure 24 - All populations with Primer 1

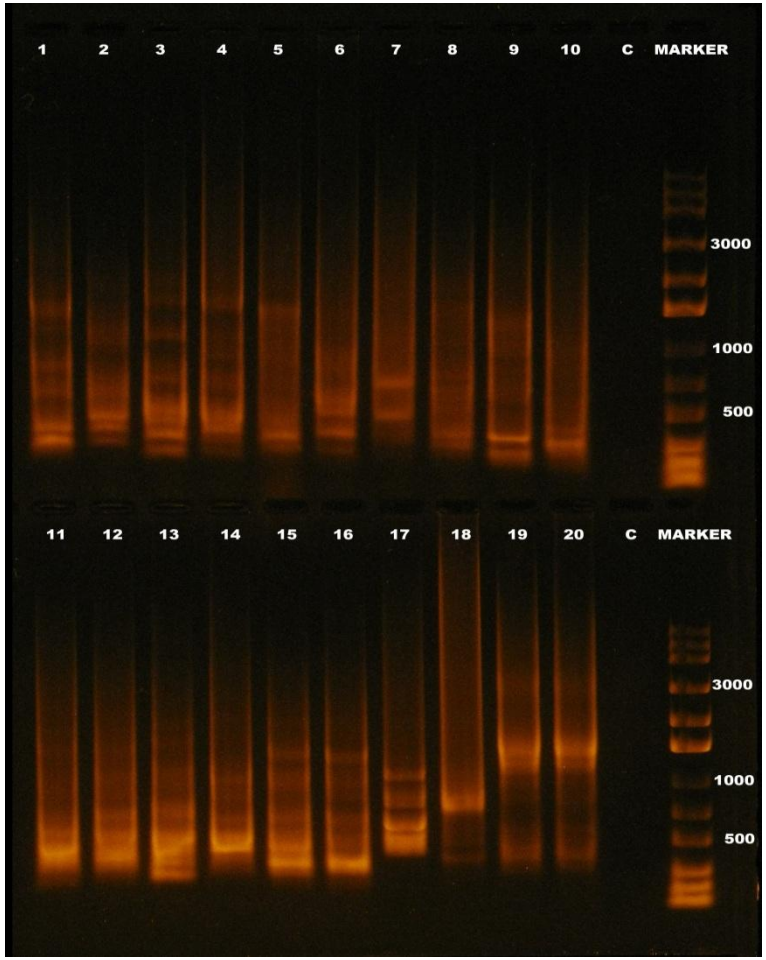


Figure 25 - All populations with Primer 2

3.2.2 CA and PCA Analyses

Two phenograms were constructed according to RAPD and ISSR binary data which are obtained from their gel patterns. During construction of phenograms, STATISTICA version 8.0 program (selected UPGMA unweighted pair-group average algorithm based upon Euclidean distances) were used. First phenogram (Figure 26) was constructed by using only RAPD data. Second phenogram (Figure 27) was constructed by combining RAPD and ISSR data. Due to the low polymorphism and few primers, the separate phenogram did not draw with using only ISSR binominal data.

In Figure 26, there occurred seven separate clusters according phenon line 9.25. These groups represented seven sections which are; *Nissolia*, *Orobus*, *Platystylis* (as *Lathyrostylis*), *Pratensis*, *Aphaca*, *Lathyrus* and *Cicercula*. At the phenon line 10.50, *Lathyrus* taxa divided into two groups. The first group contains taxa of *Nissolia*, Orobus, *Platystylis*, *Pratensis* and *Aphaca* sections, and the second group includes sections *Lathyrus* and *Cicercula* members.

In the combined results, Figure 27, at phenon line 10.10, seven clusters were separated as a sectional level; sections *Nissolia*, *Orobus*, *Platystylis* (as *Lathyrostylis*), *Pratensis*, *Aphaca*, *Lathyrus* and *Cicercula*. There ocurred six clusters according to phenon line 10.30 because the sections *Cicercula* and *Lathyrus* are clustered as a single section. At the phenon line 11.50, *Lathyrus* taxa divided into three subgroups. The section *Nissolia* was a first subgroup. The sections Orobus, *Platystylis*, *Pratensis* and *Aphaca* populations were placed as a second subgroup and the last subgroup includes section *Lathyrus* and section *Cicercula* populations. Differently from Figure 26, BRA1 population linked firstly to *L. digitatus* subgroup (DIG1, DIG2) then BRA2 linked this group from upper part in Figure 27.

In the morphological CA result the two populations of the same taxon (NIS1, NIS2; DIG1, DIG2; PRA1, PRA2) were overlapped at the same point (Figure 8) but according to the molecular RAPD and combined (RAPD+ISSR) CA results these taxa were showing some differences and placed as two different subclusters under the main sectional clusters (Figure 26, 27).

In Figure 28, all *Lathyrus* members were separated by PCA analysis which were constructed by using RAPD and ISSR binary data. At the section level, sections *Nissolia*, *Pratensis* and *Aphaca* populations were located in the positive side of the y-axis. On the other hand, sections *Cicercula*, *Lathyrus*, *Orobus* and *Platystylis* populations were located in the negative side of the y-axis. Differently to morphological PCA result (Figure 9), two populations of the same taxa (NIS1, NIS2; DIG1, DIG2; PRA1, PRA2) were not overlapped with each other, indicating genetical differences (Figure 28).

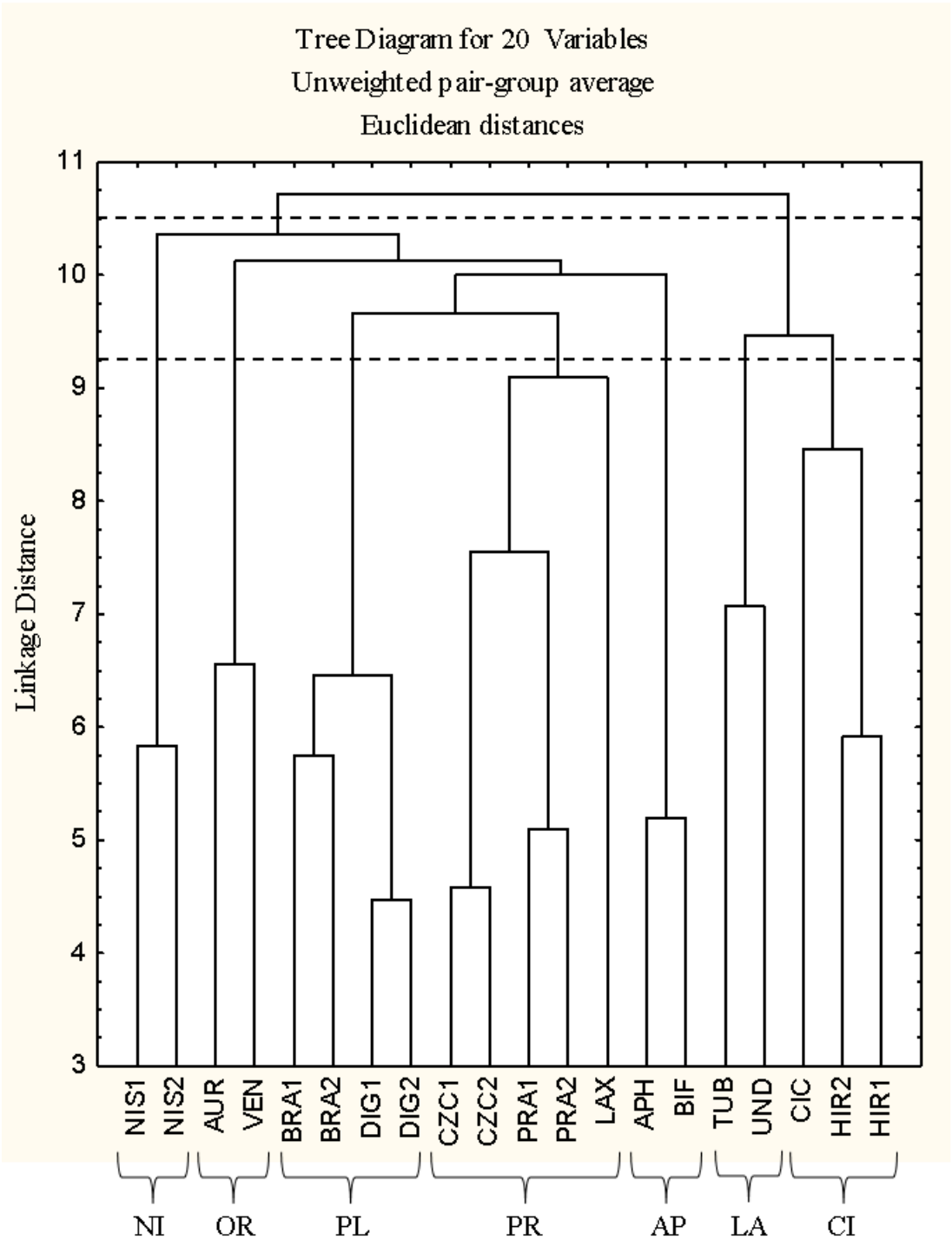


Figure 26 - Phenogram (Tree Diagram) resulting from Cluster Analysis with UPGMA based on RAPD markers

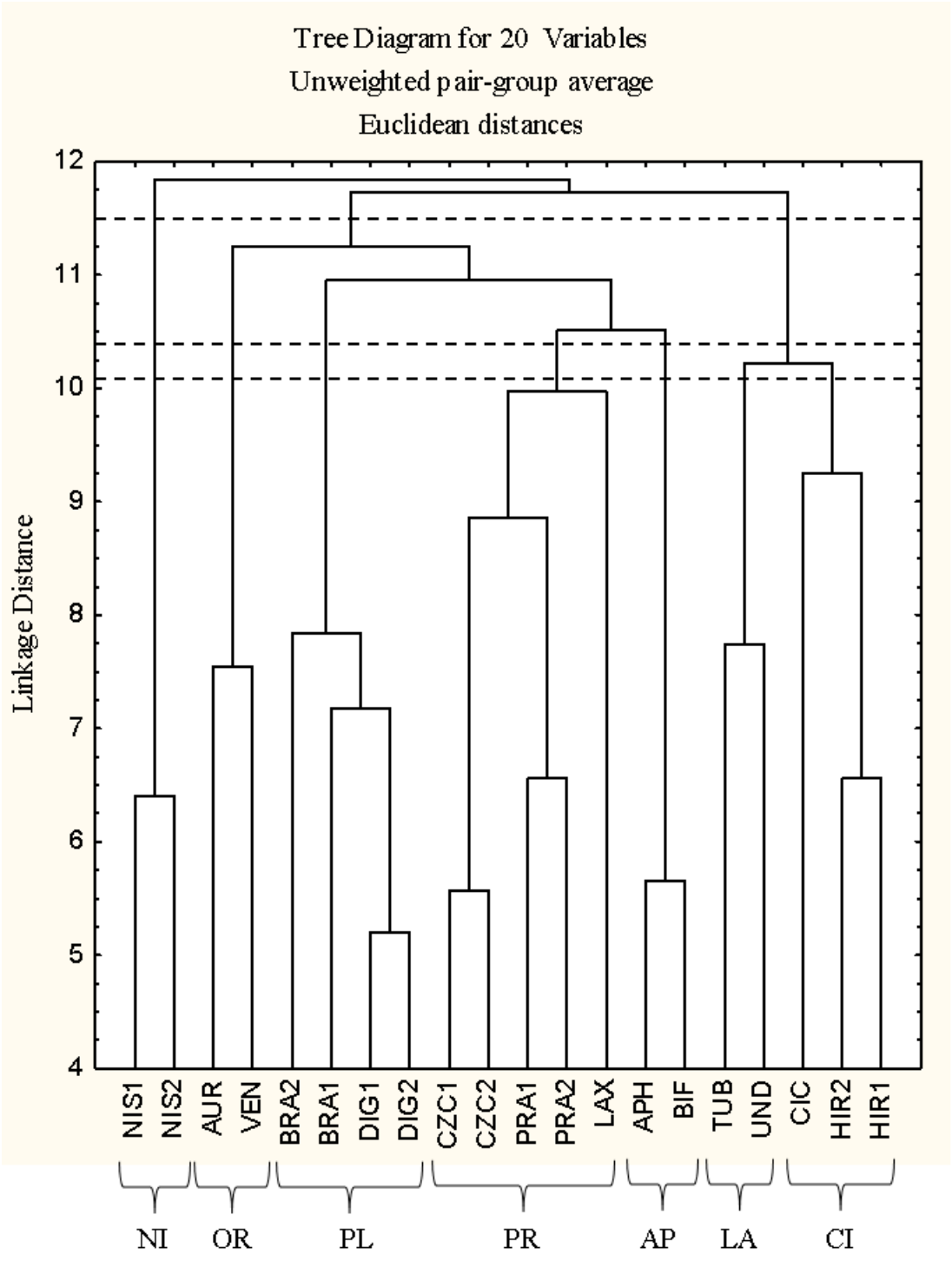


Figure 27 - Phenogram (Tree Diagram) resulting from Cluster Analysis with UPGMA based on RAPD with ISSR together

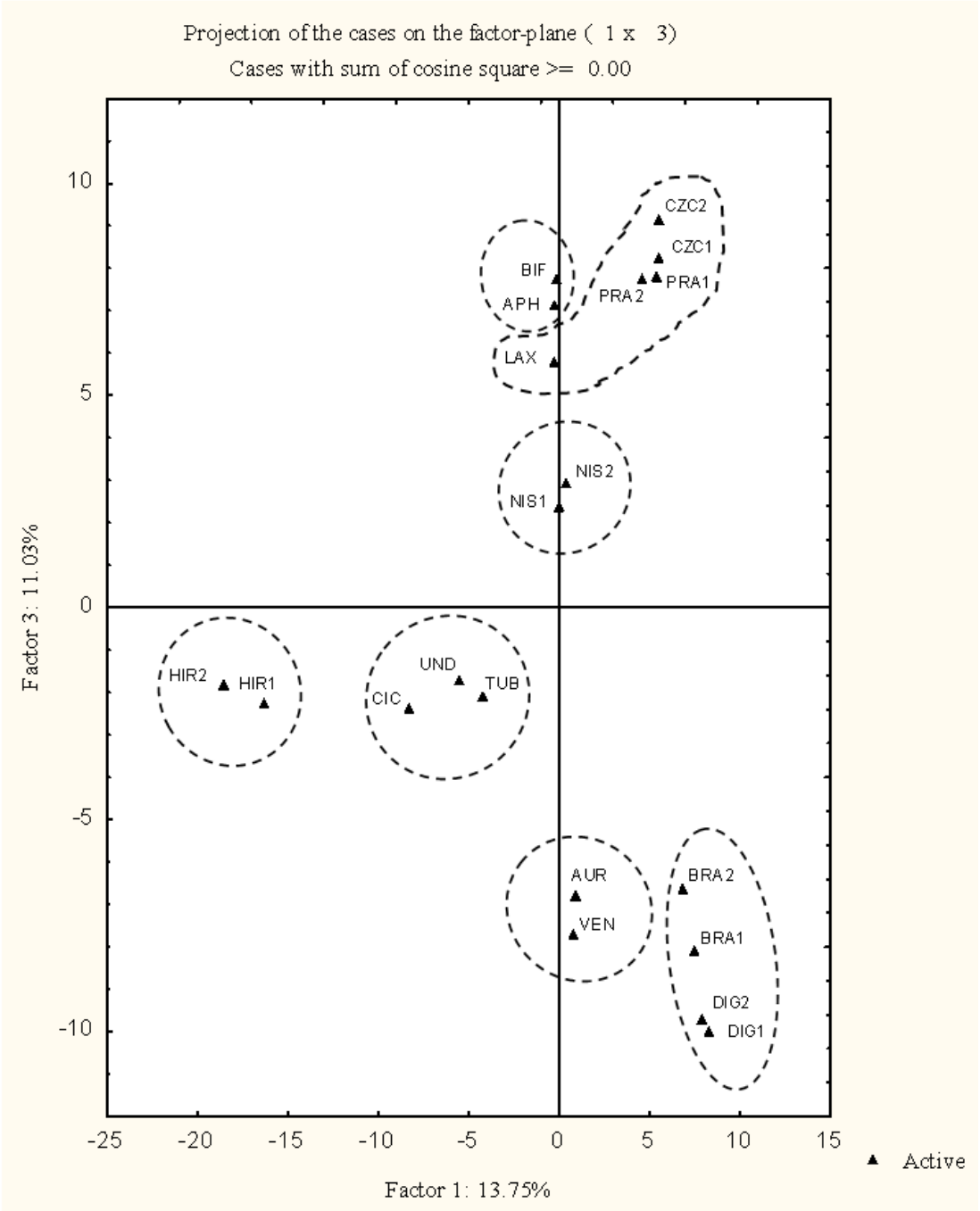


Figure 28 - Graphical representation of Principal Component Anlaysis (PCA) based on molecular data (RAPD+ISSR)

4. DISCUSSION

In this study, morphological and molecular phenetic classifications of the genus *Lathyrus* L. were prensented on the specimens grown in the Bolu Province. Twenty different *Lathyrus* taxa were identified in Bolu Province. Six of them were obtained from two separate populations that are *L. hirsutus*, *L. nissolia*, *L. pratensis*, *L. czeczottianus*, *L. digitatus* and *L. bracypterus* var. *brachypterus*. Totally 20 *Lathyrus* taxa were numerically analysed by using morphological and molecular data.

According to morphological and molecular cluster analyses (CA) results (Figure 8, 26 and 27), 7 major clusters are represented all sections, *Cicercula* (*L. hirsutus*, *L. cicera*); *Lathyrus* (*L. undulatus*, *L. tuberosus*); *Nissolia* (*L. nissolia*); *Aphaca* (*L. aphaca* var. *aphaca*, *L. aphaca* var. *biflorus*); *Pratensis* (*L. pratensis*, *L. czeczottianus*, *L. laxiflorus* subp. *laxiflorus*); *Platystylis* (as *Lathyrostylis*) (*L. digitatus*, *L. brachypterus* var. *brachypterus*); and *Orobus* (*L. venetus*, *L. aureus*).

According to the Turkish Flora (Davis, 1970), two species and one var. of *brachyptreus* are endemic for Turkey among these *Lathyrus* taxa. They are *L. undulatus, L. czeczottianus* and *L. brachypterus* var. *brachypterus*, on the other hand *L. undulatus* is a new record for Bolu Province.

In morphometric studies, because of some morphological differences, two populations of *L. hirsutus* (HIR1 and HIR2) showed a high variation at population level, also molecular results supported this variation within *L. hirsutus* populations. *L. hirsutus* from Gerede (HIR1) could not identified according to the identification keys given in the Flora of Turkey (Davis 1970). Instead, the Flora of U.S.S.R (Komarov 1968) were used for the identification of this taxon. Some quantitative characters ranges such as (leaflet length, leaflet width, stipul length and stipule width) and especially a qualitative character, corolla color of *L. hirsutus* showed some important variations from those of the characters given in the description of the species in the Flora of Turkey (Davis, 1970). These additional differences provided some contrubutions to the Flora by this study. Comparision of morphological characteristics of these *L. hirsutus* populations (HIR1 and HIR2), according to different Flora descriptions are presented in Table 10 and their photographs given in Figure 7.

Briefly, in the results of morphological and molecular analyses, the phenograms mainly support the results of Kupicha (1983), Asmussen & Liston (1998), and Kenicer et al. (2005). Among these studies, the last two studies are based on phylogenetic data. In their studies, members of the section *Cicercula* placed into the *Lathyrus* section. In contrast of their results, this study supported that sections *Cicercula* and *Lathyrus* retained as two separate sections (Figure 8, 26 and 27). This result, most probably, arose from their distinctiveness in phenograms and phylogenetics interpretations. Moreover, Sections *Aphaca* and *Pratensis* retained as separate because of their morphological distinctiveness (Figure 8). This finding agrees with the conclusion of Kupicha (1983), Asmussen & Liston (1998) and Kenicer et al. (2005). The results of Principal Component Analyses (PCA) (Figure 9 and 28) also separated the sections which were supported by the cluster analyses (CA) results.

5. CONCLUSION

As a result of this study, *Lathyrus* taxa from Bolu province are grouped into 7 sections which are *Cicercula, Nissolia, Aphaca, Pratensis, Platystylis* (as *Lathyrostylis*) *and Orobus.* This finding is supported by both morphological and molecular data. In this sense, the results were found to be quite similar to the section classifications of Kupicha (1983), Asmussen & Liston (1998), and Kenicer et al. (2005). Present study is limited, because it is based on the Bolu taxa only. However these phenetic results obtained by morphological and molecular methods appear to give some preliminary results for the biosystemtics studies of the *Lathyrus* species in Turkey.

The following suggestions based on the results of this study may be proposed: A comprehensive study covering all of the *Lathyrus* species in Turkey seems to be necessary to construct a satisfactory phenetic classification using both morphological and molecular data. Such kind of a biosystematic study about *Lathyrus* taxa may strengthen the reliability of their classification. Because of molecular and especially morphological differences obtained in this study, *L. hirsutus* (HIR1) from Gerede region might be taken into account as being a new subspecies.

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APPENDIX I - GENERAL INFORMATION ON *Lathyrus* TAXA

Table 11 - General features of species of genus *Lathyrus* L. from Davis (1970)

	Taxa	**Habitat**	**Range**	**Endemism**	**Flowering Period**
1	*Lathyrus aureus*	Forest, scrub	15-2000	Non-endemic	5-7
2	*Lathyrus venetus*	Forest, grazed land	600-950	Non-endemic	5-6
3	*Lathyrus brachypterus* var. *brachypterus*	Pastures, rocky slopes	1500-2000	Endemic	4-7
4	*Lathyrus digitatus*	*Quercus* woods, macchie, rocky slopes, scree	200-1550	Non-endemic	4-6
5	*Lathyrus pratensis*	Water meadows, streamsides, bushy places	0-2300	Non-endemic	6-7
6	*Lathyrus laxiflorus* subsp. *laxiflorus*	Forest, scrub, shady banks	0-1900	Non-endemic	5-7
7	*Lathyrus czezczottianus*	Open forests, rocky slopes, eroded banks	1150-2200	Endemic	6-7
8	*Lathyrus tuberosus*	Water meadows, grassy banks, fallow fields	1000-2150	Non-endemic	6-7 (8)
9	*Lathyrus undulatus*	Deciduous forest, hedges, roadsides	0-600	Endemic	4-6
10	*Lathyrus cicera*	*Quercus* scrub, *Pinus brutia* forest, rocky slopes, vineyards, corn & fallow fields	5-2000	Non-endemic	(3) 4-5
11	*Lathyrus hirsutus*	Bushy & grassy places, cultivated land	0-1000	Non-endemic	5-7
12	*Lathyrus nissolia*	*Pinus nigra* forest, *Quercus* scrub, grassy places, marshes	0-1900	Non-endemic	5-7
13	*Lathyrus aphaca* var. *aphaca*	Fields, marshy ground, disturbed steppe, roadsides	0-100	Non-endemic	4-5
14	*Lathyrus aphaca* var. *biflorus*	Fields, marshy ground, disturbed steppe, roadsides	0-1900	Non-endemic	4-6 (7)

Table 12 - Information on distribution of species of genus *Lathyrus* L. from Davis (1970)

NO	Taxa	Phytogeographical distribution	Distribution in Turkey	General Distribution
1	*L. aureus*	Euxine	N.W. Turkey, N. Anatolia, Inner Anatolia	Bulgaria, Romania, Crimea, Caucasia, Greece?
2	*L. venetus*	Euro-Sib.	N. Anatolia	Corsica, Itay, Balkans, C. Europe, C. & S. Russia
3	*L. brachypterus* var. *brachypterus*	Ir.-Tur	C. & S. Anatolia	Turkey
4	*L. digitatus*	E. Medit.	C. Anatolia	W. Italy, Greece, Crimea, Cyprus
5	*L. pratensis*	Euro-Sib.	N., Inner & S. W. Anatolia, Islands	Europe, N.W Africa, Abyssinia, Lebanon, N.Iraq, Caucasia, C. Asia, Himalayas
6	*L. laxiflorus* subsp. *laxiflorus*	Unk. or Multi.	Mainly Turkey-in-Europe, Outer Anatolia (common in N), Islands	S. Italy, Balkans, Crimea, Caucasia, N. & N. W. Iran, W. Syria
7	*L. czezczottianus*	Unk. or Multi.	Inner Anatolia, N. Anatolia	Turkey
8	*L. tuberosus*	Euro-Sib.	Inner Anatolia, E. Anatolia	Europe, Caucasia, W. Iran, Siberia, C. Asia
9	*L. undulatus*	Euxine ?	N. W. Turkey	Turkey
10	*L. cicera*	Unk. or Multi.	Outer & C. Anatolia, E. Anatolia, Islands	Greece, Crimea, N. Africa, Cyprus, W. Syria, Syrian Desert, N. Iraq, N. & N.W. Iran, Transcaucasia, C. Asia
11	*L. hirsutus*	Unk. or Multi.	N. Turkey	S. & C. Europe, Crimea, Lebanon, N. Africa, Caucasia, N. Iran, Transcaspia
12	*L. nissolia*	Unk. or Multi.	Widespread	W., C., S. Europe, Crimea, Caucasia, N. Iraq, N. W. Africa
13	*L. aphaca* var. *aphaca*	Unk. or Multi.	N. W. Turkey	W., C., S. Europe, N. Africa, S. W., C. Asia
14	*L. aphaca* var. *biflorus*	Unk. or Multi.	Widespread except in N. E. Anatolia	W., C., S. Europe, N. Africa, S. W., C. Asia

Table 13 - Specimen locations with their date of collection

NO	Taxa	Collection Date	Grid	County
1	*Lathyrus aureus*	06.08.2012	A3	Gölcük
2	*Lathyrus venetus*	05.11.2012	A3	Campus
3	*Lathyrus brachypterus* var. *brachypterus*	06.19.2012	A3	Kıbrıscık
4	*Lathyrus brachypterus* var. *brachypterus*	06.15.2012	A4	Gerede
5	*Lathyrus digitatus*	05.14.2012	A3	Campus
6	*Lathyrus digitatus*	06.10.2012	A3	Abant Lake
7	*Lathyrus pratensis*	05.28.2012	A3	Campus
8	*Lathyrus pratensis*	06.15.2012	A4	Gerede
9	*Lathyrus laxiflorus* subsp. *laxiflorus*	05.18.2012	A3	Sünnet Lake
10	*Lathyrus czezczottianus*	06.13.2012	A3	Abant Lake
11	*Lathyrus czezczottianus*	06.15.2012	A4	Gerede
12	*Lathyrus tuberosus*	06.22.2012	A4	Yeniçağa
13	*Lathyrus undulatus*	06.13.2012	A3	Mudurnu
14	*Lathyrus cicera*	06.15.2012	A4	Gerede
15	*Lathyrus hirsutus*	06.15.2012	A4	Gerede
16	*Lathyrus hirsutus*	06.22.2012	A4	Yeniçağa
17	*Lathyrus nissolia*	05.23.2012	A3	Campus
18	*Lathyrus nissolia*	06.15.2012	A4	Gerede
19	*Lathyrus aphaca* var. *aphaca*	05.30.2012	A3	Campus
20	*Lathyrus aphaca* var. *biflorus*	05.14.2012	A3	Campus

APPENDIX II - ARITHMETIC MEANS WITH STANDART ERROR OF QUANTITATIVE DATA

Table 14 - Arithmetic means with standart error of quantitative data

TAXA	LEL	LEW	PEL	PLL	STL	STW	PUL	PCL	COL	CAL	CTL	CLL
AUR	7.217 ±0.54	3.696 ±0.29	1.123 ±0.21	0.082 ±0.01	1.351 ±0.23	0.417 ±0.08	6.523 ±0.55	0.355 ±0.05	1.597 ±0.09	0.871 ±0.07	0.501 ±0.03	0.366 ±0.05
VEN	6.519 ±0.84	3.159 ±0.46	2.457 ±0.49	0.087 ±0.01	1.329 ±0.34	0.850 ±0.18	5.964 ±0.73	0.167 ±0.03	1.194 ±0.05	0.906 ±0.06	0.545 ±0.05	0.363 ±0.03
BRA1	3.275 ±0.38	0.121 ±0.01	0.328 ±0.07	0.000 ±0.00	0.607 ±0.13	0.050 ±0.00	4.809 ±0.92	0.349 ±0.03	1.325 ±0.11	0.531 ±0.01	0.354 ±0.02	0.175 ±0.02
BRA2	3.555 ±0.33	0.150 ±0.03	0.325 ±0.04	0.000 ±0.00	0.721 ±0.06	0.114 ±0.01	4.650 ±0.24	0.415 ±0.04	1.347 ±0.16	0.647 ±0.03	0.418 ±0.02	0.229 ±0.02
DIG1	4.721 ±0.81	0.300 ±0.04	0.076 ±0.02	0.000 ±0.00	0.907 ±0.25	0.066 ±0.02	4.281 ±0.55	0.394 ±0.04	1.632 ±0.14	0.603 ±0.05	0.389 ±0.03	0.211 ±0.02
DIG2	4.105 ±0.37	0.348 ±0.06	0.100 ±0.00	0.000 ±0.00	0.855 ±0.22	0.097 ±0.00	4.276 ±0.55	0.439 ±0.04	1.952 ±0.19	0.668 ±0.04	0.424 ±0.04	0.244 ±0.02
PRA1	1.883 ±0.11	0.435 ±0.05	1.564 ±0.09	0.000 ±0.00	1.866 ±0.19	0.504 ±0.04	5.923 ±0.43	0.196 ±0.03	1.424 ±0.08	0.691 ±0.02	0.360 ±0.01	0.328 ±0.01
PRA2	1.618 ±0.16	0.356 ±0.06	1.554 ±0.16	0.000 ±0.00	1.736 ±0.07	0.362 ±0.07	6.251 ±1.12	0.181 ±0.05	1.057 ±0.07	0.673 ±0.05	0.393 ±0.04	0.285 ±0.02
LAX	2.867 ±0.21	1.363 ±0.14	1.610 ±0.23	0.000 ±0.00	2.185 ±0.20	1.130 ±0.10	4.922 ±0.92	0.250 ±0.03	1.357 ±0.08	1.000 ±0.10	0.391 ±0.05	0.618 ±0.04
CZC1	3.484 ±0.35	0.675 ±0.06	1.593 ±0.14	0.000 ±0.00	2.139 ±0.19	0.394 ±0.09	5.314 ±0.52	0.339 ±0.03	1.680 ±0.14	1.204 ±0.07	0.436 ±0.03	0.756 ±0.06
CZC2	2.750 ±0.47	0.546 ±0.09	1.016 ±0.21	0.000 ±0.00	2.294 ±0.51	0.477 ±0.12	5.475 ±0.56	0.272 ±0.03	1.984 ±0.08	1.108 ±0.07	0.382 ±0.04	0.720 ±0.05
TUB	4.343 ±0.14	1.479 ±0.05	1.076 ±0.02	0.139 ±0.02	1.669 ±0.09	0.161 ±0.01	5.861 ±0.46	0.521 ±0.03	1.568 ±0.06	0.631 ±0.03	0.368 ±0.02	0.272 ±0.02
UND	4.878 ±0.21	2.219 ±0.26	1.348 ±0.28	0.149 ±0.02	1.804 ±0.20	0.159 ±0.04	5.886 ±0.39	0.521 ±0.10	1.941 ±0.12	0.756 ±0.04	0.448 ±0.03	0.308 ±0.04
CIC	4.933 ±0.20	0.160 ±0.00	0.903 ±0.04	0.050 ±0.00	1.329 ±0.06	0.097 ±0.00	3.486 ±0.28	0.382 ±0.05	1.107 ±0.15	0.732 ±0.06	0.316 ±0.03	0.414 ±0.04
HIR1	2.144 ±0.29	0.513 ±0.03	0.690 ±0.13	0.066 ±0.02	0.590 ±0.07	0.093 ±0.01	3.959 ±0.74	0.287 ±0.03	0.938 ±0.04	0.644 ±0.02	0.278 ±0.02	0.371 ±0.03
HIR2	4.833 ±0.12	1.060 ±0.05	1.405 ±0.15	0.100 ±0.00	1.315 ±0.03	0.106 ±0.01	4.330 ±0.21	0.376 ±0.02	1.099 ±0.06	0.723 ±0.04	0.328 ±0.02	0.393 ±0.04
NIS1	0.000 ±0.00	0.000 ±0.00	0.000 ±0.00	0.000 ±0.00	0.100 ±0.00	0.020 ±0.00	2.648 ±0.24	0.248 ±0.02	0.839 ±0.06	0.452 ±0.02	0.290 ±0.02	0.161 ±0.02
NIS2	0.000 ±0.00	0.000 ±0.00	0.000 ±0.00	0.000 ±0.00	0.087 ±0.01	0.020 ±0.00	2.868 ±0.39	0.236 ±0.04	0.874 ±0.09	0.484 ±0.05	0.322 ±0.03	0.164 ±0.03
APH	0.000 ±0.00	0.000 ±0.00	0.000 ±0.00	0.000 ±0.00	1.020 ±0.26	0.666 ±0.12	1.519 ±0.36	0.267 ±0.02	1.095 ±0.09	0.696 ±0.02	0.291 ±0.02	0.405 ±0.02
BIF	0.000 ±0.00	0.000 ±0.00	0.000 ±0.00	0.000 ±0.00	1.752 ±0.16	1.136 ±0.09	2.364 ±0.30	0.335 ±0.02	1.067 ±0.08	0.689 ±0.03	0.300 ±0.01	0.384 ±0.01

APPENDIX III - CODED DATA OF QUALITATIVE CHARACTERS

Table 15 - Coded data of qualitative characters

Taxa	LEL	LEW	PEL	PLL	STL	STW	PUL	PCL	COL	CAL	CTL	CLL	LDU	SWW	LFE	LST	LSH	LEV	SSP	SST	FST	CLH	CLT	CCL	SMP
AUR	2	3	2	1	1	1	1	1	1	2	2	1	1	0	1	1	2	2	4	1	1	0	0	2	1
VEN	2	3	2	1	1	2	1	0	0	2	2	1	1	0	1	1	2	2	5	1	1	0	0	3	0
BRA1	1	1	1	0	1	0	1	1	0	0	1	0	1	0	1	1	3	1	1	1	1	0	0	1	0
BRA2	1	1	1	0	1	0	1	1	0	1	1	0	1	0	1	1	3	1	1	1	1	0	0	1	0
DIG1	1	1	1	0	1	0	1	1	1	1	1	0	1	0	1	1	3	1	1	1	1	1	1	0	1
DIG2	1	1	1	0	1	0	1	1	1	1	1	0	1	0	1	1	3	1	1	1	1	1	1	0	1
LAX	1	2	2	0	2	2	1	0	0	2	1	2	1	0	1	1	2	1	0	2	1	1	2	4	1
PRA	1	1	2	0	2	1	1	0	0	1	1	1	1	0	2	1	1	1	1	2	1	0	0	6	0
PRA	1	1	2	0	2	1	1	0	0	1	1	1	1	0	2	1	1	1	1	2	1	0	0	6	0
CZC1	1	1	2	0	2	1	1	1	1	2	1	2	1	0	1	1	1	1	1	2	1	1	2	5	1
CZC2	1	1	2	0	2	1	1	0	1	2	1	2	1	0	1	1	1	1	1	2	1	1	2	5	1
TUB	1	2	2	2	2	0	1	1	1	1	1	1	1	0	2	1	4	2	1	1	1	0	2	7	0
UND	1	2	2	2	2	0	1	1	1	1	1	1	1	1	2	1	4	1	1	1	1	1	2	7	1
CIC	1	1	2	1	1	0	1	1	0	1	0	1	0	1	2	1	5	1	1	1	0	0	2	8	0
HIR1	1	1	2	1	1	0	1	0	0	1	0	1	0	1	2	1	6	1	2	1	1	1	2	9	0
HIR2	1	2	2	1	1	0	1	1	0	1	0	1	0	1	2	1	6	1	2	1	1	1	2	10	0
NIS1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	2	7	0
NIS2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	2	7	0
APH	0	0	0	0	1	2	0	0	0	1	0	1	0	0	2	0	0	0	0	2	0	0	1	6	0
BIF	0	0	0	0	2	2	0	1	0	1	0	1	0	0	2	0	0	0	0	2	1	0	1	6	0

APPENDIX IV - BOXPLOTS AND CHARACTER CODING

A graphical summary of the distribution of a sample that shows its shape, central tendency, and variability. The default boxplot display presents the following:

1. Upper whisker - Extends to the maximum data point from the top of the box
2. Interquartile range box - Middle 50% of the data
   2.1. Top line - Q3 (third quartile). 75% of the data are less than or equal to this value.
   2.2. Middle line - Q2 (median). 50% of the data are less than or equal to this value.
   2.3. Bottom line - Q1 (first quartile). 25% of the data are less than or equal to this value.
3. Lower whisker - Extends to the minimum data point from the bottom of the box.

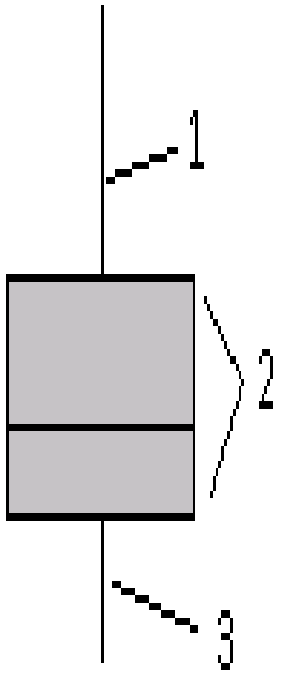

Figure 29 - Boxplots and character coding key

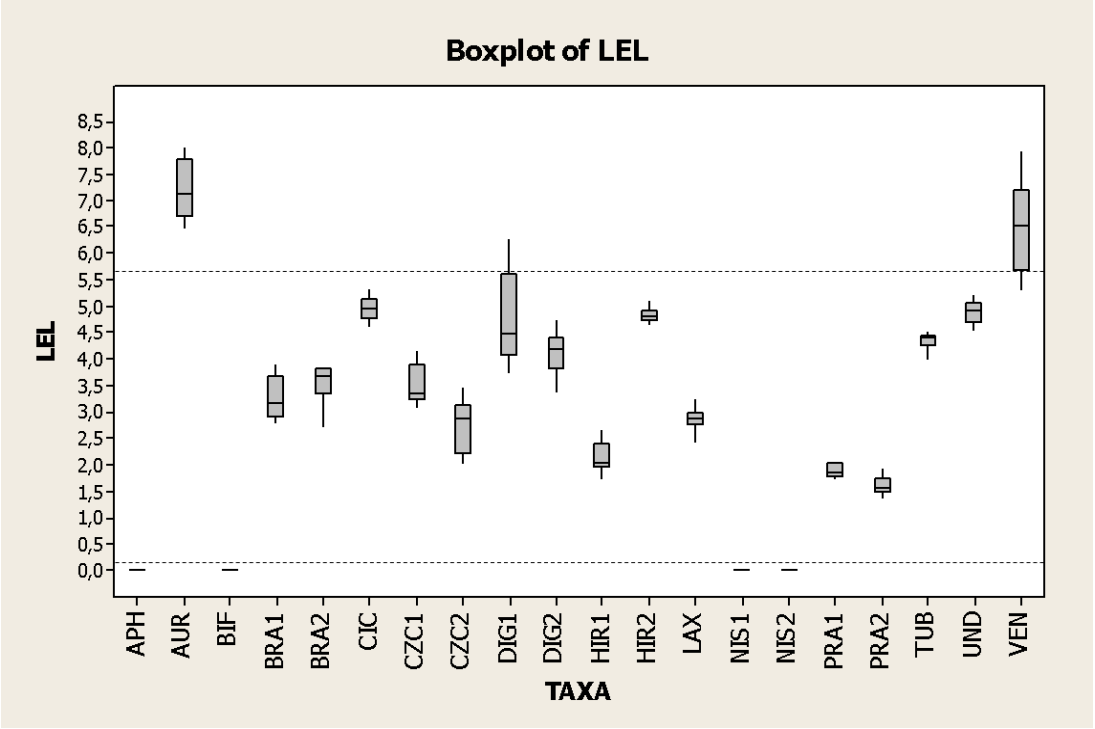

Figure 30 - Means with boxplots for leaflet length (LEL)

Table 16 - Scoring character states for leaflet length (LEL)

Group Number	Range	Codes
1	<0.10	0
2	0.10-5.63	1
3	5.63<	2

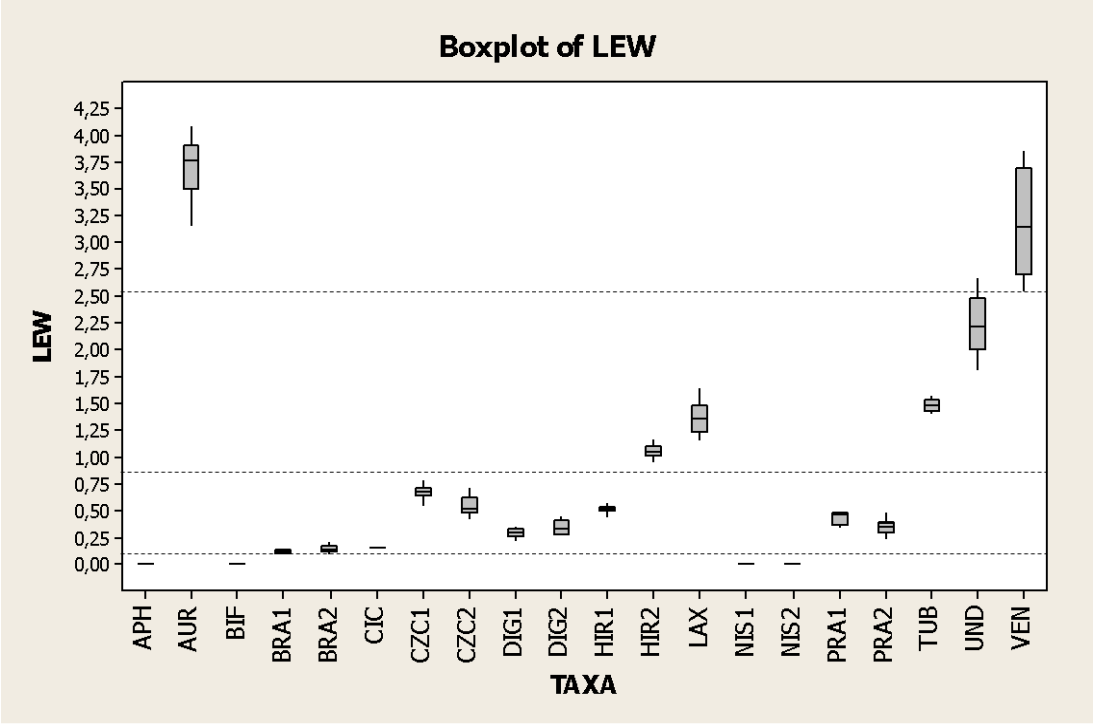

Figure 31 - Means with boxplots for leaflet width (LEW)

Table 17 - Scoring character states for leaflet width (LEW)

Group Number	Range	Code
1	<0.10	0
2	0.10-0.80	1
3	0.80-2.55	2
4	2.55<	3

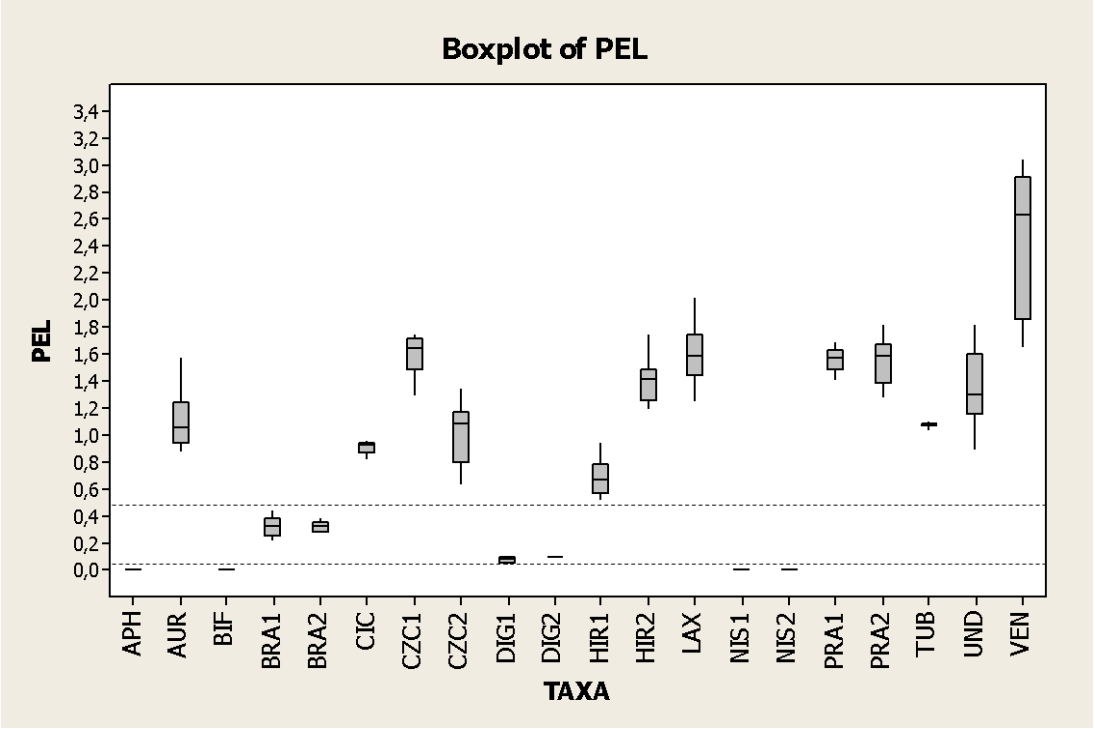

Figure 32 - Means with boxplots for petiole length (PEL)

Table 18 - Scoring character states for petiole length (PEL)

Group Number	Range	Code
1	<0.05	0
2	0.05-0.50	1
3	0.50<	2

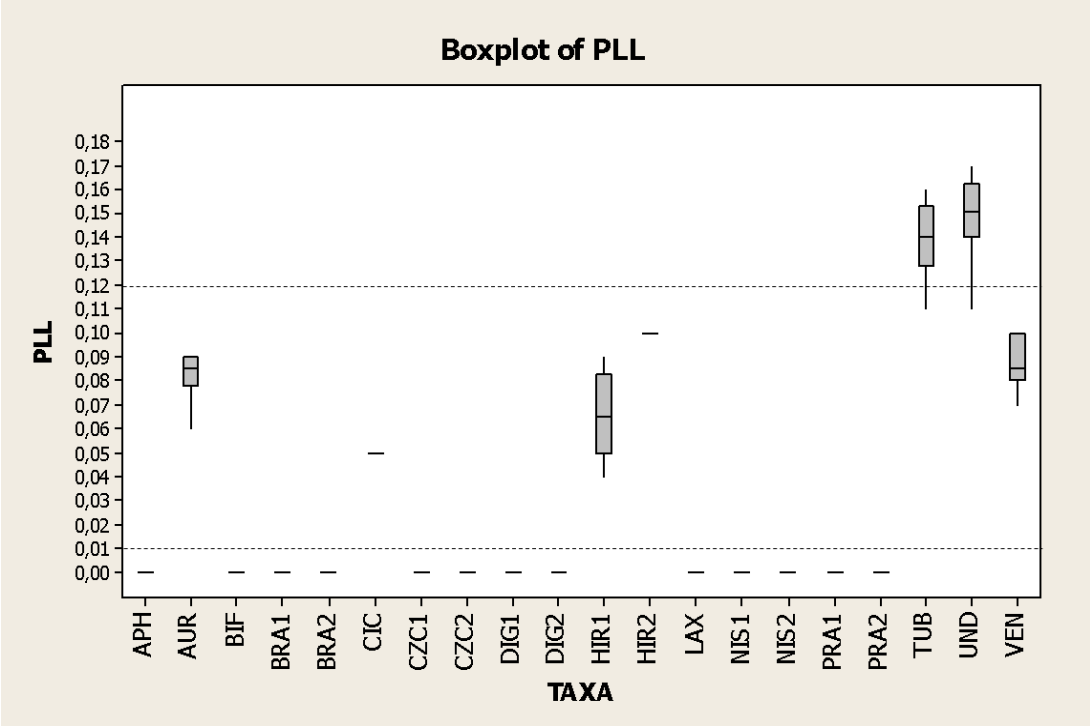

Figure 33 - Means with boxplots for petiolule length (PLL)

Table 19 - Scoring character states for petiolule length (PLL)

Group Number	Range	Code
1	<0.01	0
2	0.01-0.12	1
3	0.12<	2

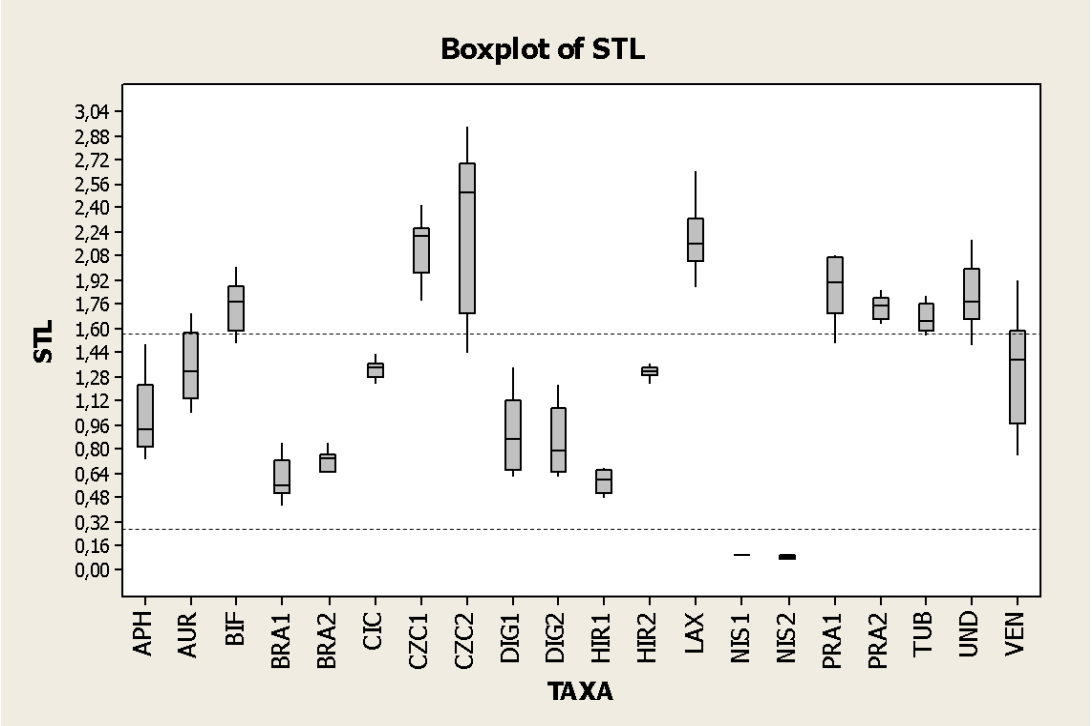

Figure 34 - Means with boxplots for stipule length (STL)

Table 20 - Scoring character states for stipule length (STL)

Group Number	Range	Code
1	<0.30	0
2	0.30-1.58	1
3	1.58<	2

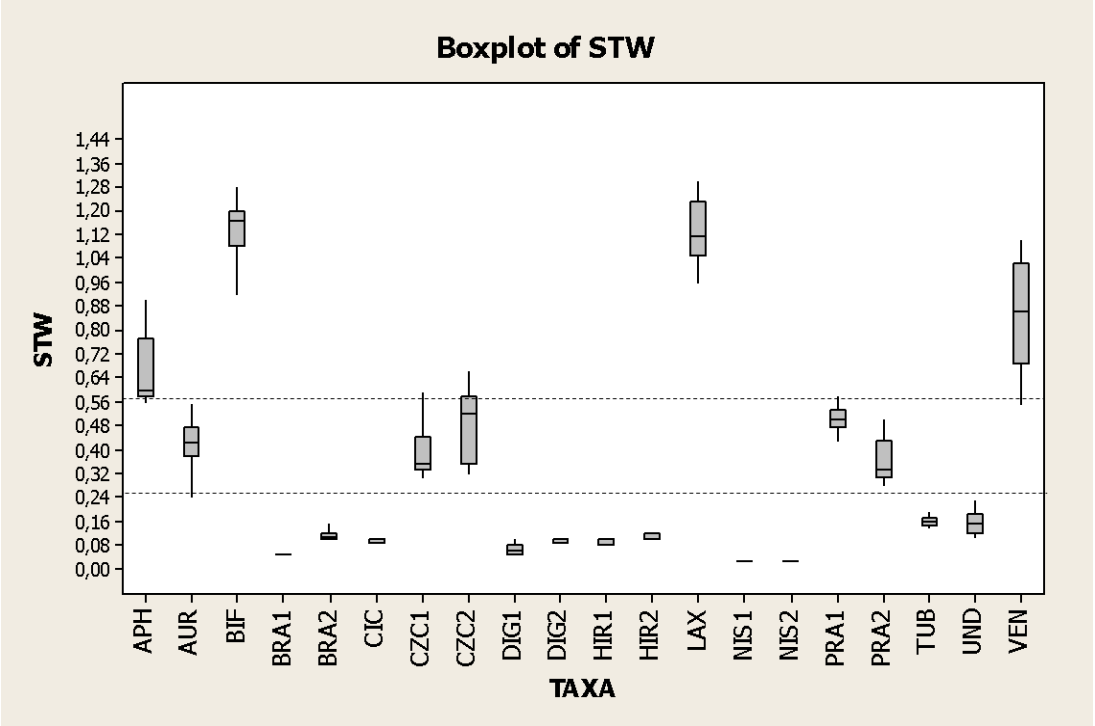

Figure 35 - Means with boxplots for stipule width (STW)

Table 21 - Scoring character states for stipule width (STW)

Group Number	Range	Code
1	<0.25	0
2	0.25-0.58	1
3	0.58<	2

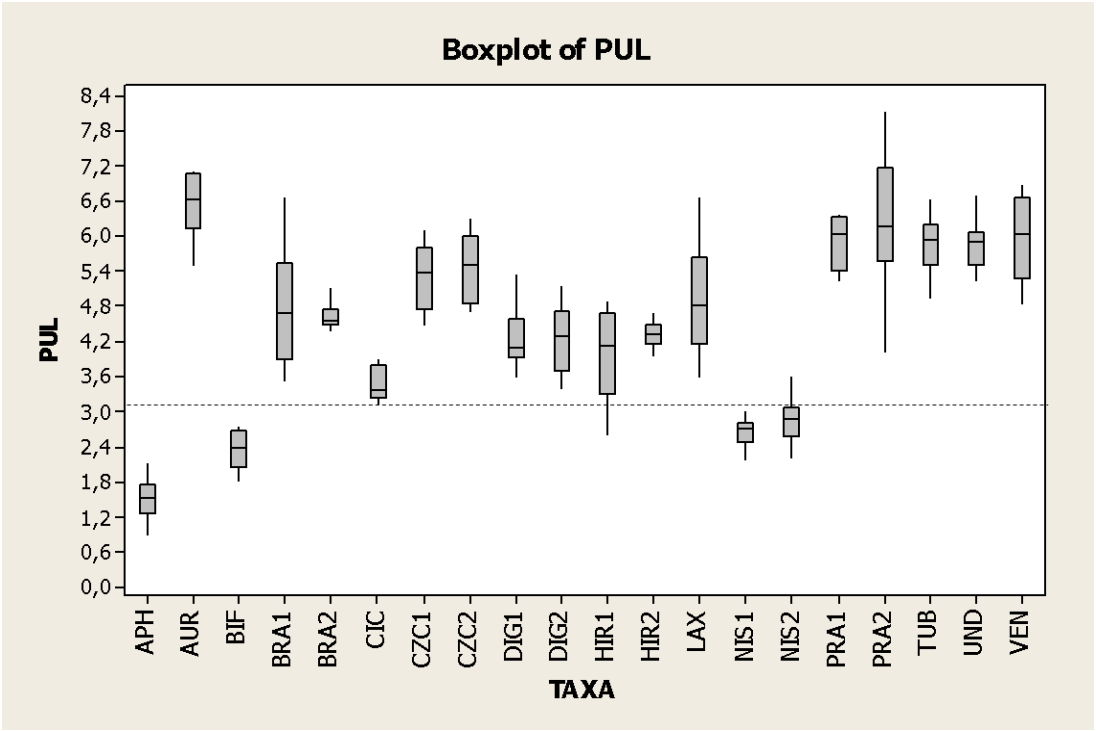

Figure 36 - Means with boxplots for peduncle length (PUL)

Table 22 - Scoring character states for peduncle length (PUL)

Group Number	Range	Code
1	<3.10	0
2	3.10<	1

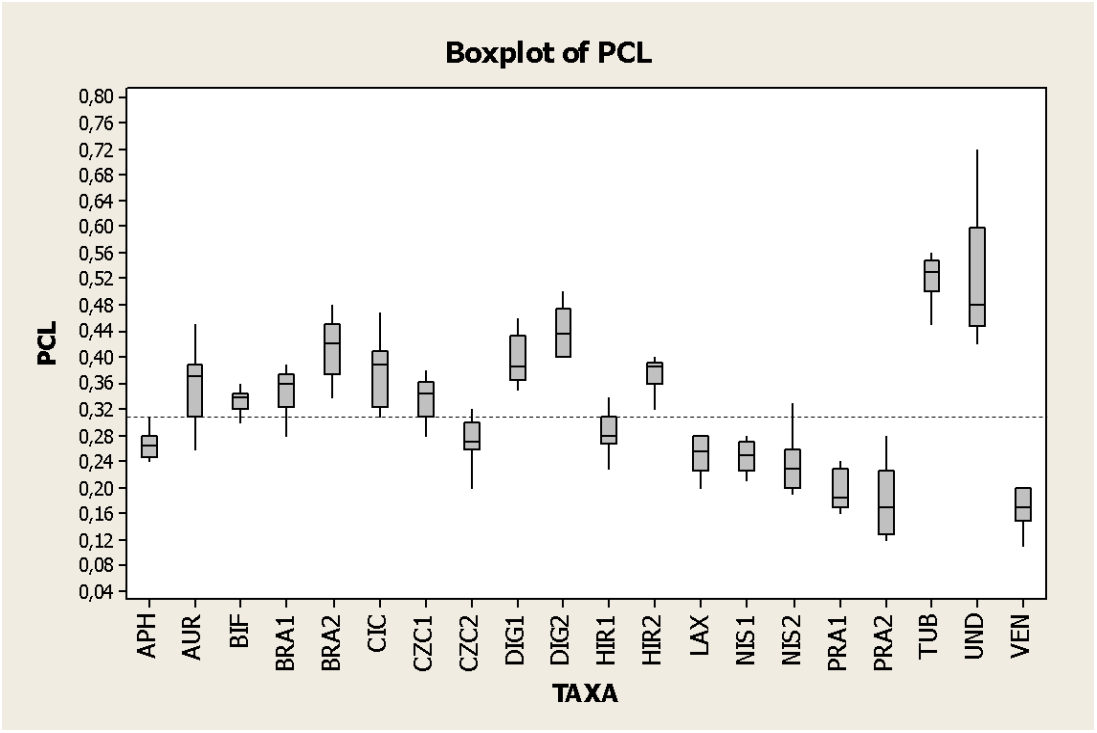

Figure 37 - Means with boxplots for pedicle length (PCL)

Table 23 - Scoring character states for pedicle length (PCL)

Group Number	Range	Code
1	<0.31	0
2	0.31<	1

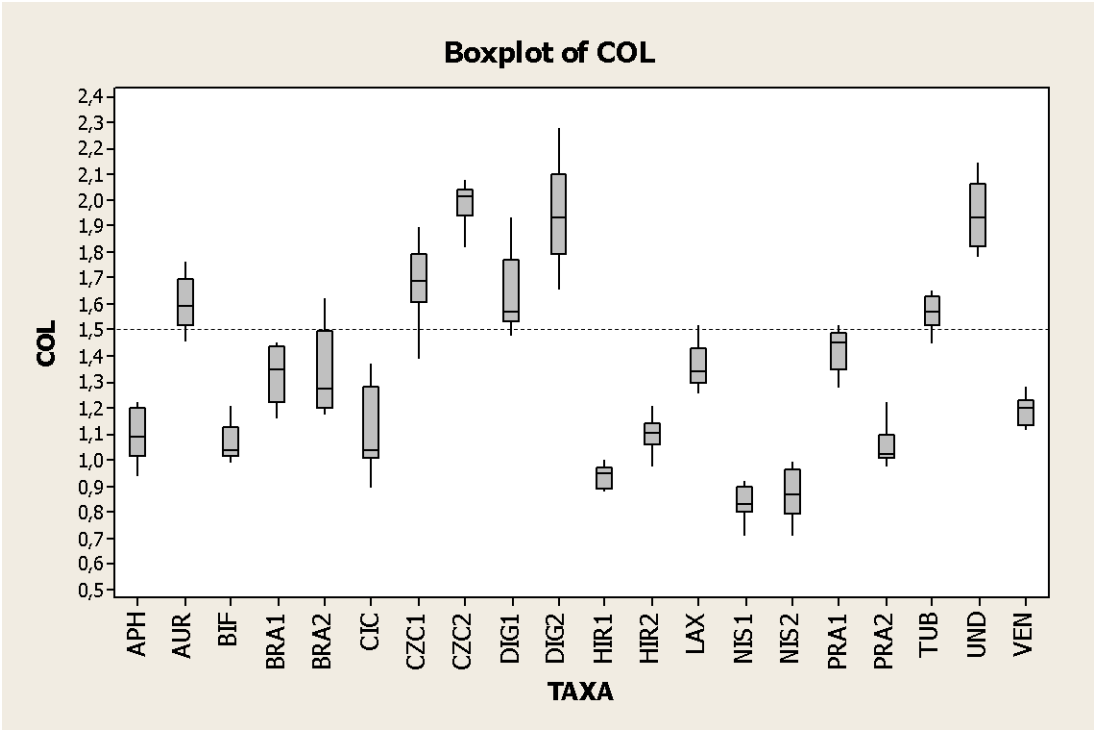

Figure 38 - Means with boxplots for corolla length (COL)

Table 24 - Scoring character states for corolla length (COL)

Group Number	Range	Code
1	<1.50	0
2	1.50<	1

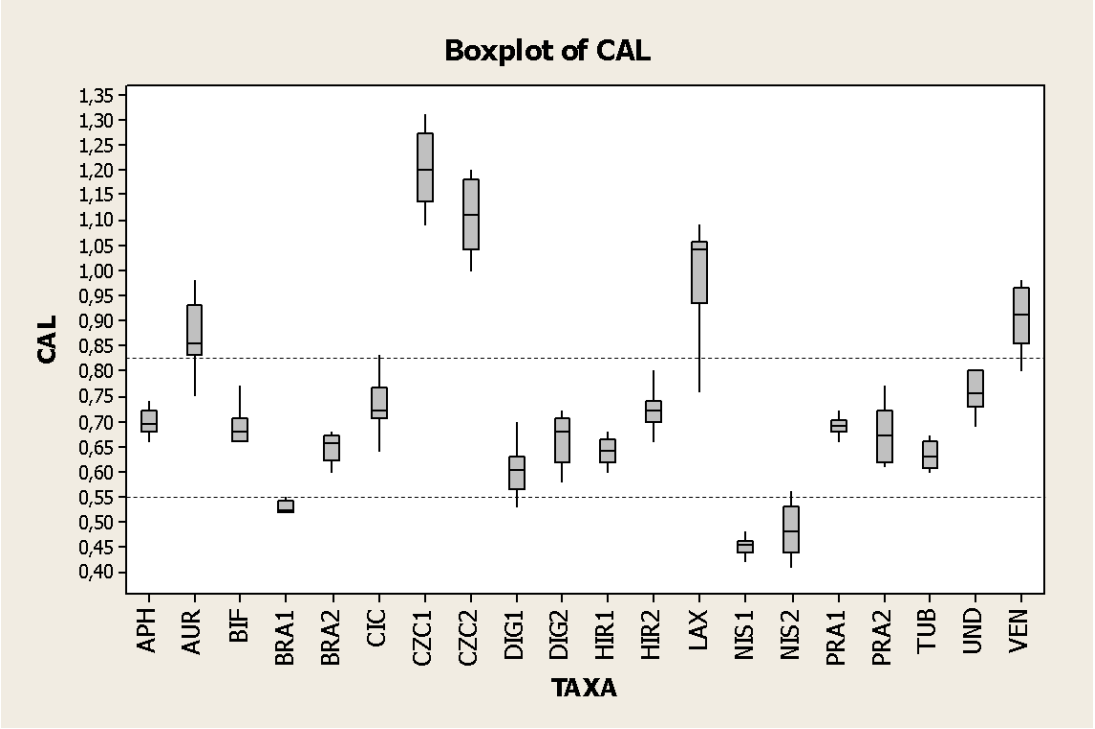

Figure 39 - Means with boxplots for calyx length (CAL)

Table 25 - Scoring character states for calyx length (CAL)

Group Number	Range	Code
1	<0.55	0
2	0.55-0.83	1
3	0.83<	2

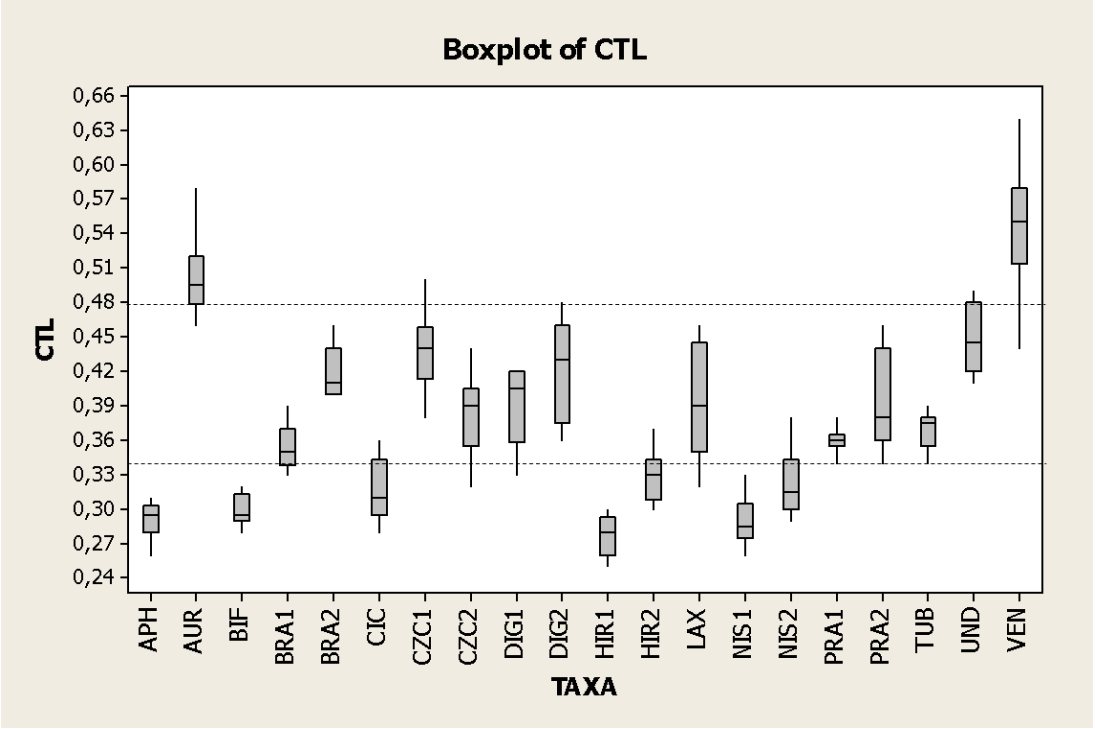

Figure 40 - Means with boxplots for calyx tube length (CTL)

Table 26 - Scoring character states for calyx tube length (CTL)

Group Number	Range	Code
1	<0.34	0
2	0.34-0.48	1
3	0.48<	2

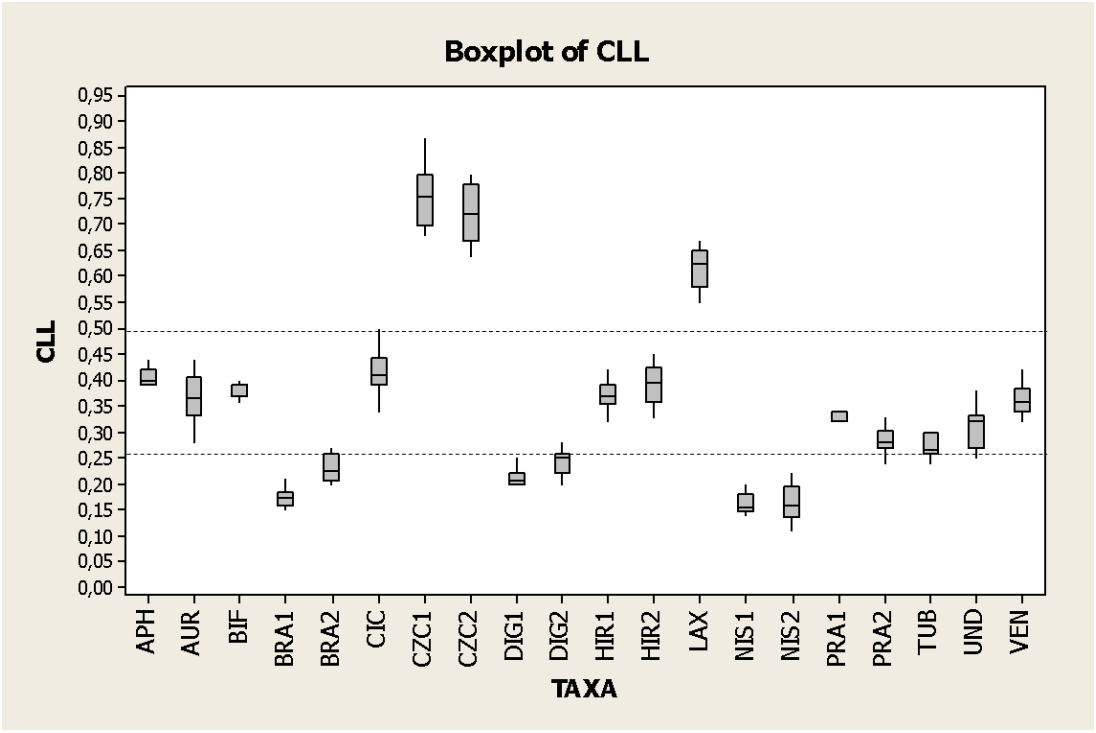

Figure 41 - Means with boxplots for calyx lobe length (CLL)

Table 27 - Scoring character states for calyx lobe length (CLL)

Group Number	Range	Code
1	<0.26	0
2	0.26-0.50	1
3	0.50<	2