A MICROMORPHOLOGICAL STUDY OF *LATHYRUS* L. (LEGUMINOSAE) SPECIES IN TURKEY

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RODIN MAZAHERI

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A MICROMORPHOLOGICAL STUDY OF *LATHYRUS* L. (LEGUMINOSAE) IN TURKEY

submitted by **RODIN MAZAHERI** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Biological Sciences Department, Middle East Technical University** by,

Prof. Dr. Gulbin Dural Unver
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Orhan Adali
Head of Department, **Biological Sciences** _____

Prof. Dr. Musa Doğan
Supervisor, **Biological Sciences Department, METU** _____

Examining Committee Members:

Prof. Dr. Zeki Kaya
Biological Sciences Department, METU _____

Prof. Dr. Musa Doğan
Biological Sciences Department, METU _____

Prof. Dr. Inci Togan
Biological Sciences Department, METU _____

Prof. Dr. Mecit Vural
Biological Sciences Department, Gazi University _____

Prof. Dr. Osman Ketenoğlu
Biological Sciences Department, Ankara University _____

Date: _____

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last Name: Rodin Mazaheri

Signature:

ABSTRACT

A MICROMORPHOLOGICAL STUDY OF *LATHYRUS* L. (LEGUMINOSAE) SPECIES IN TURKEY

Mazaheri, Rodin

PhD, Department of Biological Sciences

Supervisor: Prof. Dr. Musa Doğan

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This research study was carried out to determine the taxonomic value of micromorphological properties in the infrageneric delimitation of *Lathyrus* species found in Turkey. In the present study, first micromorphological characters of the leaf surface of 15 species (sect. *Lathyrostylis*) were analyzed by Light Microscope. Multivariate statistical approaches (for instance, Principal Component Analyses (PCA) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA)) were used to evaluate the results. Among the eleven characters employed, stomata index of abaxial leaf surface, epidermal cell shape, and cell wall pattern of adaxial leaf surface were found to be discriminative among the species.

Secondly, many more micromorphological characters of the leaf and calyx surfaces (32 in total) of 28 species (sect. *Lathyrostylis, Orobus, Orobon, Pratensis, Clymenum, Lathyrus*) were studied via Scanning Electron Microscope (SEM). UPGMA based on quantitative characters of leaf and calyx tend to disagree with existing phylogenetic estimates of relationships. Perhaps environmental factors highly affecting quantitative characters blur the phylogenetic relationships of the species.

However, based on qualitative data among 28 species of *Lathyrus*, 23 species have correctly been assigned to existing sections by the UPGMA. Meanwhile, character loading demonstrated seven characters to be the most important in differentiating and

grouping species and sections. These were: epidermal cell shape, cell wall pattern, stomata position of both leaf surfaces and trichome type on the abaxial leaf surface. These characters can be used as micro markers to classify *Lathyrus* species at the sectional level.

Also, to evaluate the exact relations of *L. boissieri, L. haussknechtii, L. roseus, L. clymenum, L. pratensis* with other species, further analyses is needed. *L. haussknechtii* is confirmed as a different species as was suggested recently by Çıldır, (2011).

Keywords: Micromorphology, *Lathyrus,* Leaf, Calyx, Multivariate analyses, Light Microscope, Scanning Electron Microscope (SEM), PCA, UPGMA, Turkey.

ÖZ

TÜRKIYE' DEKI *LATHYRUS* L. (LEGUMINOSAE) TÜRLERİNDE BİR MİKROMORFOLOJİK ÇALIŞMA

Mazaheri, Rodin

Doktora, Bioloji Bölümü

Tez Yöneticisi: Prof. Dr. Musa Doğan

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Bu araştırma Türkiye'de bulunan *Lathyrus* türlerinin cinsiçi sınıflandırmasında mikromorfolojik özelliklerin taksonomik değerini saptamak amacıyla yapılmıştır. Bu çalışmada ilk olarak, *Lathyrostylis* seksiyonuna ait olan 15 *Lathyrus* türunun yaprak yüzeylerindeki mikromorfolojik karakterler ışık mikroskopuyla incelenmiştir. Sonuçların değerlendirilmesinde, çok değişkenli istatistksel analizler (örneğin, PCA ve UPGMA) kullanılmıştır. Türlerin ayırt edilmesinde,11 karakter arasında, abaksial yaprak yüzeyinin stomata indeksi, adaksial yaprak yüzeyinin epiderm hücre şekli ve hücre duvar paterni önemli bulunmuştur.

Ikincisi olarak, yaprak ve kaliks yüzeylerindeki bulunan çok daha fazla sayıdakı mikromorfoloji karakterleri (toplam 32), *Lathyrostylis*, *Orobus*, *Orobon*, *Pratensis*, *Clymenum, Lathyrus* seksiyonlara ait olan 28 *Lathyrus* türu SEM yardımı ile incelenmiştir. Yaprak ve kaliksten elde edilen sayısal karakterlerin UPGMA'sı, var olan filogenetik ilişkilere uyum sağlamamaktadır. Belki çevresel faktorlerin sayısal karakterler üzerinde yüksek derecede filogenetik ilişkilerinin net olmamasına sonuç vermiştir.

Bununla birlikte, kalitatif datalara göre 28 tür arasında, 23 tür UPGMA ile var olan seksiyonlara doğru bir şekilde ayrılmıştır. Bu arada, türler ve seksiyonların karakter ağırlıklarına göre gruplandırılmasında, 7 karakterin en önemli karakter olduğu gösterilmiştir. Bunlar; yaprağın her iki yüzeyindeki epiderm hücre şekli, hücre duvar

paterni, stomata' nin yaprak yüzeyindeki pozisyonu ve yaprağın abaksial yüzeyindeki trikom tipleridir. Bu karakterler, *Lathyrus* türlerinde seksiyonel düzeyde, mikro marker olarak sınıflandırma amacı ile kullanılabilmektedir.

Ayrıca, *L. boissieri, L. haussknechtii, L. roseus, L. clymenum, L. pratensis'* in diğer türlerle olan kesin ilişkilerini değerlendirmek için daha fazla analiz gereklidir. *L. haussknechtii* farklı bir tür olarak , Çıldır' ın (2011) önerdiği gibi teyit edilmiştir.

Anahtar kelime: Mikromorfoloji, *Lathyrus*, Yaprak, Kaliks, Çok değişkenli analiz, Işık Mikroskop, SEM, PCA, UPGMA, Türkiye.

To My Dearest Parents,

For their endless love and support

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TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

CHAPTER 1

1. INTRODUCTION

1.1 The Family Leguminosae

Leguminosae (Fabaceae) is the third largest family of flowering plants after Asteraceae (the sunflower family) and Orchidaceae (the orchid family) (Polhill et al., 1981). As was explained by Smykal et al (2015), Lewis et al., (2005) stated that Leguminosae is divided into three subfamilies based on morphological characters: the mimosoid legumes (Mimosoideae) with 4 tribes and 3,270 species; Papilionoid legumes (Papilionoideae) with 28 tribes and 13,800 species; and Caesalpinioid legumes (Caesalpinioideae) with 4 tribes and 2,250 species. This family consists of approximately 727 genera, 36 tribes and 19,325 species.

Papilionoideae is one of the largest subfamilies of Leguminosae which covers *Lathyrus* L. (sweet peas) as the largest genus of the tribe Fabeae (Adans) DC (Kenicer et al., 2005). Fabeae was first described by Reichenbach (1832) on the basis of its abaxially pubescent styles, leaves with terminal tendrils, unusual stem vasculature supplying the stipules and several floral characters (Kenicer, 2007). The status of genus *Lathyrus,* which harbors all the species of interest in the present study within Leguminosae is depicted in **Figure 1-1.**

Five classification of Leguminosae were provided by Linnaeus, Persoon, Bentham, Taubert and Hutchinson during the period from 1753 to 1964 (Cronk, 1990).

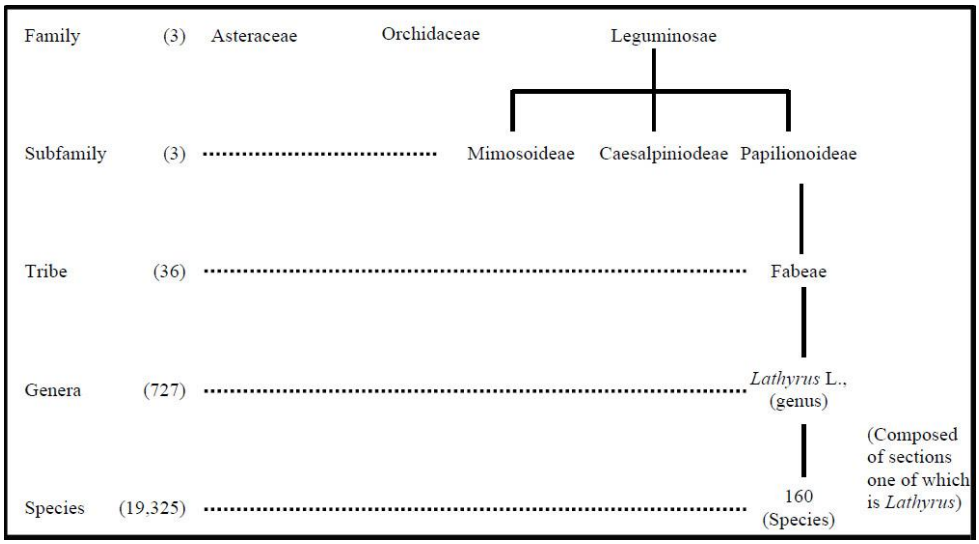

Figure 1-1 The state of *Lathyrus* in the Leguminosae, prepared on the basis of Kenicer et al (2005) and Lewis et al (2005) studies.

The family Leguminosae is represented by 71 genera and 1013 species in Turkey with 400 of these species being endemic (22 taxa are *Lathyrus* species). The rate of endemism is 39 % (Erik, Tarikahya, 2004).

In the last two decades molecular data has further contributed to our understanding of the systematics, phylogenetics, biogeography, and evolution of Leguminosae. For instance, nucleotide sequences of *rbcL* gene have been found to be a powerful genetic marker for the construction of phylogeny in green plants (Kass and Wink, 1995; Doyle et al., 1997). Also, *trnL* intron sequences of chloroplast have been used in the subfamily Caesalpinioideae, and this paraphyletic subfamily is now divided into several monophyletic groups according to *trnl* analyses (Bruneau et al., 2001). However, Papilionoideae has been confirmed as a monophyletic group by other scientists in more recent years (Lavin et al., 2005).

Leguminosae are distributed in many ecological regions throughout the world from deserts of high latitudes to seasonally dry or wet tropical forests of equatorial areas.

Recent large scale molecular phylogenies (derived from DNA sequences of the chloroplast regions *matK, rbcL* and *trnL*) were used to identify the major subgroups and clades of legumes (Schrire et al., 2005). Global distribution patterns of the clades revealed that they are clustered in four different biomes within which they are endemic. These four different biomes are listed below and shown in **Figure 1-2.**

a. succulent rich , grass poor , dry tropical forest and fire intolerant

b. succulent poor , grass rich , fire tolerant , woodland and savannah biome

c. tropical wet forest biome rain forest

d. temperate biome in both the northern and southern hemispheres

Different cladistic analyses suggest that those lineages confined to the succulent biome gave rise to sub-lineages occupying all other biomes, and evolutionary shifts between the rain forest and grass biomes are frequent, but shifts from temperate into tropical biomes are infrequent. Molecular phylogenetics suggests that rain forest clades, in general, may be the most recently derived ones among the legumes.

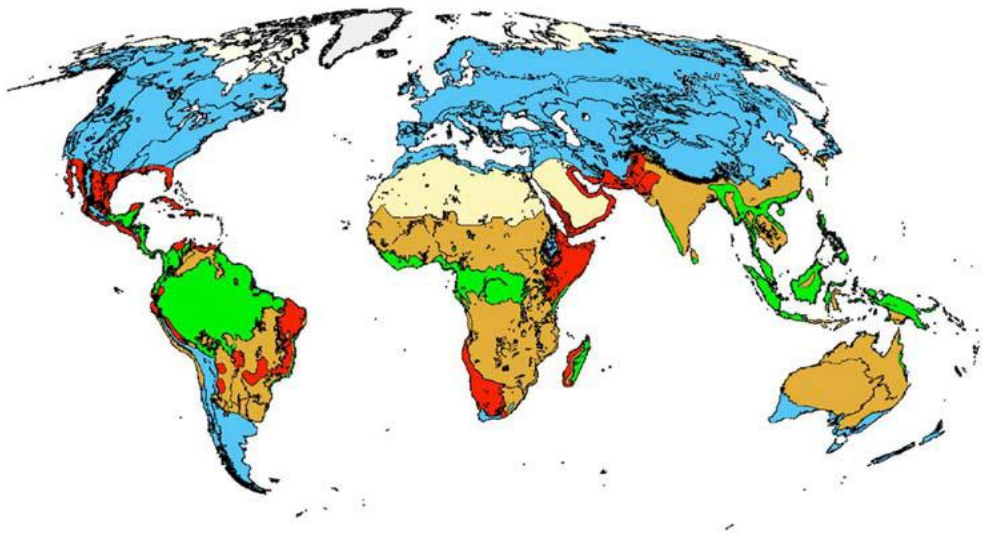

Figure 1-2 Global map with four different legume distribution patterns at the biome level (from Oliveria Filho et al., 2013 taken from Schrire et al., 2005).

The colour Red shows the distribution of the succulent biome, which occupies highly drought-prone tropical areas with succulent growth habits in plant families such as the Agavaceae, Bromeliaceae, Cactaceae and Euphorbiaceae. Brown indicates the grass-rich savannah biome, which occupies highly seasonal low latitude areas. Green depicts the tropical wet forest biome, which occupies the wettest end of the precipitation gradient at low latitudes. Blue represents the temperate biome, which occupies high elevations and latitudes (Oliveria Filho et al., 2013)

The tribe Fabeae has an almost worldwide distribution. It is considered one of the youngest tribes of legumes (Steele and Wojciechowski, 2003), and estimates based on rates of evolution in the *maturase k* chloroplast gene place the age of the crown node at 17.5 Mya in the mid-Miocene age (Lavin et al., 2005). Similarly, Schaefer et al., 2012 suggests a crown age of c. 23 – 16 Mya for Fabeae based on the Bayesian molecular clock and ancestral range analyses.

1.2 The Genus *Lathyrus*

1.2.1 Morphological Diversity of the *Lathyrus*

The name of *Lathyrus* is derived from (*la–thyris*), meaning ‘‘little form’’. Descriptions connoting ‘‘powerful ‘‘or ‘’vigorous’’ have been used since the ancient Greek times to characterize *Lathyrus.* Pre-Linnean botanists used *Lathyrus* to describe the tendrilous, bijugate plants now recognized as members of sect. *Lathyrus*. *Lathyrus* is mentioned in Figure **1-1** as described by Kenicer (2007).

Stems are exclusively herbaceous, and in some sections they are winged as shown in **Figure 1-3**. The stem wings are green and increase the photosynthetic area available to the plants. Leaves are typically paripinnate with paired stipules, pairs of leaflets and a terminal tendril that may be sometimes reduced to a simple arista. Most of the species have reticulate veins in the leaflets, but some members have parallel veins. In morphology-based classifications, leaf characters are strongly considered. Inflorescences are solitary or in racemes of up to thirty flowers with a typical Papilionoid legume flower. *Lathyrus* flowers (corolla) are seen in varying colors of yellow, orange, red, purple, violet, bluish tinged, or white which usually change with

the age of the plant. *Lathyrus* has a typical bee-pollinated Papilionoid flower (Asmussen and Liston, 1998).

The corolla has three types of petals; standard, wing and keel. The wing petals correspond to keel petals. The basal part forms a pivot against which the wings and keel are pushed when a pollinator lands on the flower.

Figure 1-3 Stems of *L. latifoliuos*

Most members of *Lathyrus* have more or less regular calyces. A flower with unequal calyx lobes typical of sect. *Orobus* like *L. aureus* is shown in **Figure 1-4**. The androecium is diadelphous with a single stamen at the adaxial part. Remaining stamens form a tube. Ovaries are linear and may be pubescent with simple hairs or glandular hairs. Styles are often linear, and in some sections species have a spathulate or twisted style. Stigmas are typically simple and in some members are divided into two separate pads or flaps. Fruits can be heavily ornamented with dense pubescence or wings along the sutures or valves on the entire pod. Fruits contain between 2-15 seeds (Kupicha, 1983; Kenicer, 2007).

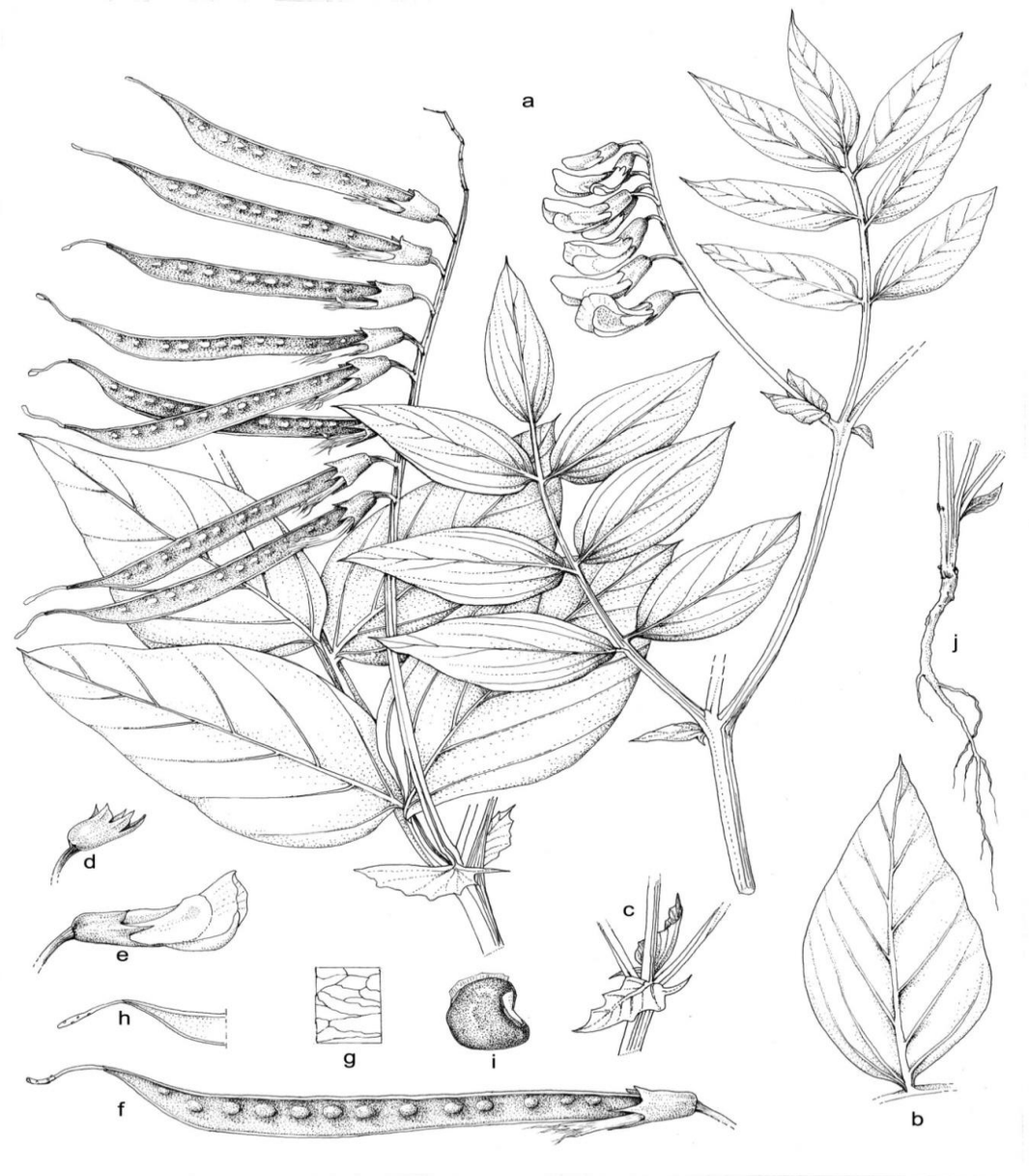

Figure 1-4 *L. aureus* (Stev.) Brandza (*Steven*) Bornm.: a) habit; b) leaflet; c) stipule; d) calyx; e) flower; f) pod; g) pod venation and hairiness; h) style; i) seed; j) rooting system
(Adopted from Shehadeh, 2011)

1.2.2 Background of Micromorphological Studies

Taxonomists obtain valuable information from genetic, biochemical and physiological features of different parts of the plant as well as micromorphological characters from the leaf epidermis. However, the usefulness of different characters in

revealing taxonomic level varies from taxon to taxon. Epidermal characters have proved to be useful not only in identifying fossil remains of Angiosperms but also in studying the relationship between taxa (Stace, 1965 a, 1984).

Since leaves play a vital role in photosynthesis, observed modifications in leaves help explain how structure, function, and metabolism are related in the plants. The leaves' epidermis is influenced by the needs of the plants in performing photosynthesis which in turn depends on arrangements of epidermal cells and other structures like stomata and cuticle (Mauseth, 2008). Stomatal characters have been regarded as playing significant roles in systematic and evolutionary studies (Metcalfe and Chalk, 1950; Stace, 1965; Barthlott, 1981). Despite the economic importance of legumes, stomatal characters have not been studied in many species (Tripathi and Mondal, 2012). The cuticle is the standard source of crucial information in leaf compression fossils, so epidermal characters may provide additional and decisive insight into taxonomic investigations (Kerp and Krings, 1999). Cuticle ornamentation and epicuticular wax composition may also serve as additional taxonomic characters (Ma et al., 2004).

Therefore, micromorphological research is useful in identifying characters in the vegetative phase, for instance, epidermal cell shape, cell wall pattern, and the presence of stomata in leaves (Srivastava et al., 2013). Also, other pertinent features like the distribution, density and forms of trichomes in the leaf epidermis of flowering plants have been suggested by Dickison (2000); Valkama et al (2003); Ma et al (2004) and Aliero et al (2006) as diagnostic character. Trichome types are useful in the identification of species and important in pharmacognosy, archeobotany, paleobotany, and agronomy (Rao and Ramaya, 1977; Werker, 2000).

There have been numerous studies on the foliar epidermis (as in the leaf) as one of the most important micromorphological taxonomic characters in different plants (Bhatia, 1984; Jones, 1986; Parveen et al., 2000; Scatena et al., 2005; Yang and Lin, 2005; Celka et al., 2006; Krajsek et al., 2006; Zou et al., 2008; Yasmin et al., 2009).

Also, the venation pattern of the leaf in dicotyledons is considered to be important by Simola (1968).

Conversely, in some species of the family Eriocaulaceae, root and stem characters seem to be more affected by environmental factors, but leaf characters offer much more value for delimiting taxonomic groups (Scatena et al., 2005). There is evidence for strong genetic control over epidermal characters, which are affected minimally by their environment (Cutler and Brandham, 1977).

Gunes, (2011) investigated the pollen morphology of *Lathyrus* species (section *Lathyrostylis*) in Turkey. A comparative study based on micromorphological traits of style between 26 species of Iranian and Turkish *Lathyrus* was conducted by Oskoueiyan et al (2011). Epidermal micromorphological traits of the petal and, sepal of some species of the genus *Lathyrus* have been studied by various researchers in the world (Stirton, 1981; Hammette et al., 1994; Christensen and Hansen, 1998; Ojeda et al., 2009) and some other studies based on anatomy, macro and micro morphological traits have been conducted in Turkey (Celep et al., 2011; Çıldır et al., 2012; Kahraman et al., 2013), but the leaf and calyx micromorphologies of the 28 species covered in this study are presented here for the first time.

1.2.3 Infrageneric Classification of *Lathyrus*

The classification of the genus *Lathyrus* has been discussed for about 260 years by various researchers. As explained by Doğan et al (1992), Linnaeus, (1753) recognized *Lathyrus sensu stricto* and *Orobus* L. as two distinct genera for the first time.

Kupicha, (1983) studied *Lathyrus* in a very detailed way. The only worldwide treatment of the genus *Lathyrus* based on morphological characters was considered in Kupicha‘s classification (without using phenetic or cladistic methods of analyses). Kupicha’s classification along with that of other recent researchers’ is presented in **Table 1-1**. She recognized 13 sections in the genus *Lathyrus*. The section *Orobastrum* was subdivided into three sections (*Orobastrum, linearicarpus* and *viciopsis*). The

section *Cicercula* is included in the section *Lathyrus* with the section *Eurytrichon* included in the section *Pratensis*. The section *Notolathyrus* is part of a new section of South American species. Most of the species (153) of *Lathyrus* are placed in *Orobus, Lathyrus, Notolathyrus and Lathyrostylis* and five monotypic sections, the section represented by a single species, (*Neurolobus, Nissolia, Orobastrum, Orobon, viciopsis*), and four small sections (*Aphaca, Clymenum, linearicarpus* and *Pratensis*) were also identified.

Boissier, (1872) was the first researcher to study the Turkish *Lathyrus* species as well as many other species from the area covered in *Flora Orientalis*. In Turkey, *Lathyrus* covers 67 taxa (58 species) under ten sections in the flora of Turkey by Davis (1970) which is given in **Table 1-2**. These ten sections are *Orobus, Platystylis, Orobastrum, Pratensis, Orobon, Lathyrus, Cicercula, Aphaca, Nissolia* and *Clymenum.* However, the number of taxa from Turkey has been increased to 78 taxa (66 species) belonging to 10 sections by other researchers (Davis et al, 1988; Guner et al., 2000). For example, *L. gloeospermus* (Ertekin and Saya, 1991), *L. inconspicuous* var. *stenophylla* (Ertekin, 1994) and, *L. grandiflorus* (Ertekin et al, 1997) were introduced in the flora of Turkey. Doğan et al., (1992) provided a "Numerical Taxonomic Study on Turkish *Lathyrus* (Leguminosae)" and placed *Lathyrus* species in nine sections (*Orobus, Platystylis, Aphaca, Nissolia, Orobon, Gorgonia, Clymenum, Cicercula, Lathyrus*) based on a phenetic analyses of vegetative and floral characters (the section *Gorgonia* was introduced for the first time). The exact number of sections is still unknown.

In order to classify plant species, phenetic approaches are used to evaluate morphological, micromorphological, or molecular based characters. Characters as operational taxonomical unit (OTUs) are ordered along planes in multidimensional space and then reduced to two or three planes (PCA, PCoA) or one dimension (UPGMA) for more effective comprehension to calculate relations. Most phenetic analyses in morphological and micromorphological based characters in *Lathyrus* have been employed in UPGMA or Neighbor Joining method by using Gower,

Euclidean and Jaccard coefficients (Doğan et al., 1992; Abou-El-Enain et al., 2007; Leht, 2009).

Table 1-1 Infrageneric classifications of *Lathyrus* based on the Morphology (Mor) or Molecular Markers (Mar) covering worldwide species (W) or species of Turkey (T).

L. sphaericus, L. angulatus, L. gloeospermus, all depicted in italics, belong to unresolved basal node and remain problematic.

Kupicha, 1983 (Mor, W)	**Doğan et al 1992 (Mor, T)**	**Asmussen & Liston 1998 (Mar , W)**	**Kenicer, 2007 (Mar , W)**
Notolathyrus		Orobus	Notolathyrus
Orobus	Orobus		Orobus
Lathyrostylis	Lathyrostylis	Lathyrostylis	Lathyrostylis
Pratensis		Pratensis	Pratensis
Aphaca	Aphaca	Aphaca	Aphaca
Neurolobus		Neurolobus	Neurolobus
Orobon	Orobon	Lathyrus	
Lathyrus			Lathyrus
	Lathyrus		
	Gorgonia		
	Cicercula	Cicercula	
Orobastrum	Clymenun	Orobastrum	
Linearicarpus		*L.sphaericus*	*L.sphaericus*
		L.angulatus	*L.angulatus*
Viciopsis			
Nissolia	*Nissolia*	Nissolia	Nissolia
Clymenum	Clymenum	Clymenum	Clymenum
		L.gloeospermus	*L.gloeospermus*

This symbol represents species which are not included in that study.

Although some micromorphological data has been considered in *Lathyrus,* detailed microtraits were not used in previous classifications. There is voluminous research on the classification of *Lathyrus* based on AFLP data (Badr et al., 2002), chloroplast

DNA characters (Asmussen and Liston, 1998) and other molecular analyses such as Croft et al., (1999); Kenicer et al., (2005); Schaefer et al., (2012).

Table 1-2 The list of *Lathyrus* species that are mentioned in Flora of Turkey (Davis, 1970). The species that are marked with asterisk are investigated in the present study.

Sect. Orobus	**Sect. Pratensis**	*40. L. cassius*
1. *L. aureus* *	*22. L. pratensis**	*41. L. gorgoni**
2. L. libani	*23. L. layardii*	*42. L. pseudo- cicera*
*3. L. vernus**	24. *L. laxiflorus**	*43. L. cicera**
4. L. venetus	*25. L. czeczottianus* *	*44. L. sativus**
5. L. niger *	**Sect. Orobon**	*45. L. blepharicarpus*
6. L. incurvus	26. *L. roseus**	*46. L. marmoratus*
7. L. palustris	**Sect. Lathyrus**	*47. L. stenophyllus*
Sect. Platystylis **	27. *L. tuberosus*	*48. L. lycicus*
8. *L. pallesence* *	28. *L. rotundifolius*	49. *L. phaselitanus*
9. L. brachypterus *	*29. L. undulatus**	*50. L. hirsutus*
10. L. karsianus *	*30. L. sylvestris*	*51. L. chrysanthus*
11. L. satdaghensis *	**Sect. Orobastrum**	*52. L. chloranthus*
12. L. nivalis	31. *L. saxatilis*	*53. L. trachycarpus*
*13. L. armenus**	*32. L. vinealis*	**Sect. Clymenum**
*14. L. cyaneus**	*33. L. sphaericus*	54. *L. clymenum**
*15. L. digitatus**	*34. L. inconspicuous*	*55. L. ochrus*
*16. L. tuktensis**	*35. L. tauricola*	**Sect. Nissolia**
*17. L. variabilis**	*36. L. woronowii*	56. *L. nissolia*
*18. L. spathulatus**	*37. L. setifoliuos*	**Sect. Aphaca**
*19. L. elongatus**	**Sect. Cicercula**	*57. L. aphaca*
*20. L. cilicicus**	38. *L. annuus**	*58. L. stenolobus*
*21. L. boissieri**	*39. L. hierosolymitanus*	

The name of sect. *Platystylis*, which is marked with two asterisks, is synonymous with *Lathyrostylis. L. annuus, L. gorgoni, L. cicera* and *L. sativus* are in section *Cicercula* due to Davis, (1970), but these species are considered in sect. *Lathyrus*, according to Kupicha, (1983); Kenicer et al., (2005) and Schaefer et al., (2012). Two of the species used in the present study and not mentioned in the Table 1-2 are *L. haussknechtii* and *L. atropotanus (*both belong to sect. *Lathyrostylis*). The first one was known as a variety of *L. brachypterus* according to Davis,

(1970) but is now accepted as a different species (Çıldır, 2011). Similarly, *L. atropatanus* is accepted as different species (Gunes and Cirpici, 2011) but is very similar to *L. nivalis*.

Asmussen and Liston, (1998) studied chloroplast DNA (cp DNA) restriction site data from 42 species. In general, suggested classification of this study agreed with the sectional classification of Kupicha, 1983. They suggested merging the sections of *Orobon* and *Orobastrum* with *Lathyrus.* Similarly, it was suggested that sections *Notolathyrus* and *Orobus* be combined. Kenicer et al., (2005) modified Kupicha's classification (13 sections) and accepted 11 sections.

Schaefer et al., (2012) analyzed the systematics of the tribe Fabeae based on plastid and nuclear DNA sequences. This study regarding the genus *Lathyrus* (which covers some of the species of the present study) indicated that in contrast to previous studies (Asmussen and Liston, 1998), *Lathyrus* is not a monophyletic genus and includes two genera *Pisum* and *Vavilovia* as can be seen from Figure 1-5. The *Lathyrus*, *Pisum* and *Vavilovia* species are shown with green, red and pink colours respectively in the related figure.

In the molecular marker based studies, maximum likelihood method and parsimony analyses have been used to construct UPGMA or Neighbor Joining tree using Dice and Jaccard coefficients (Asmussen and Liston, 1998; Croft et al., 1999; Badr et al., 2002; Kenicer et al., 2005; Schaefer et al., 2012).

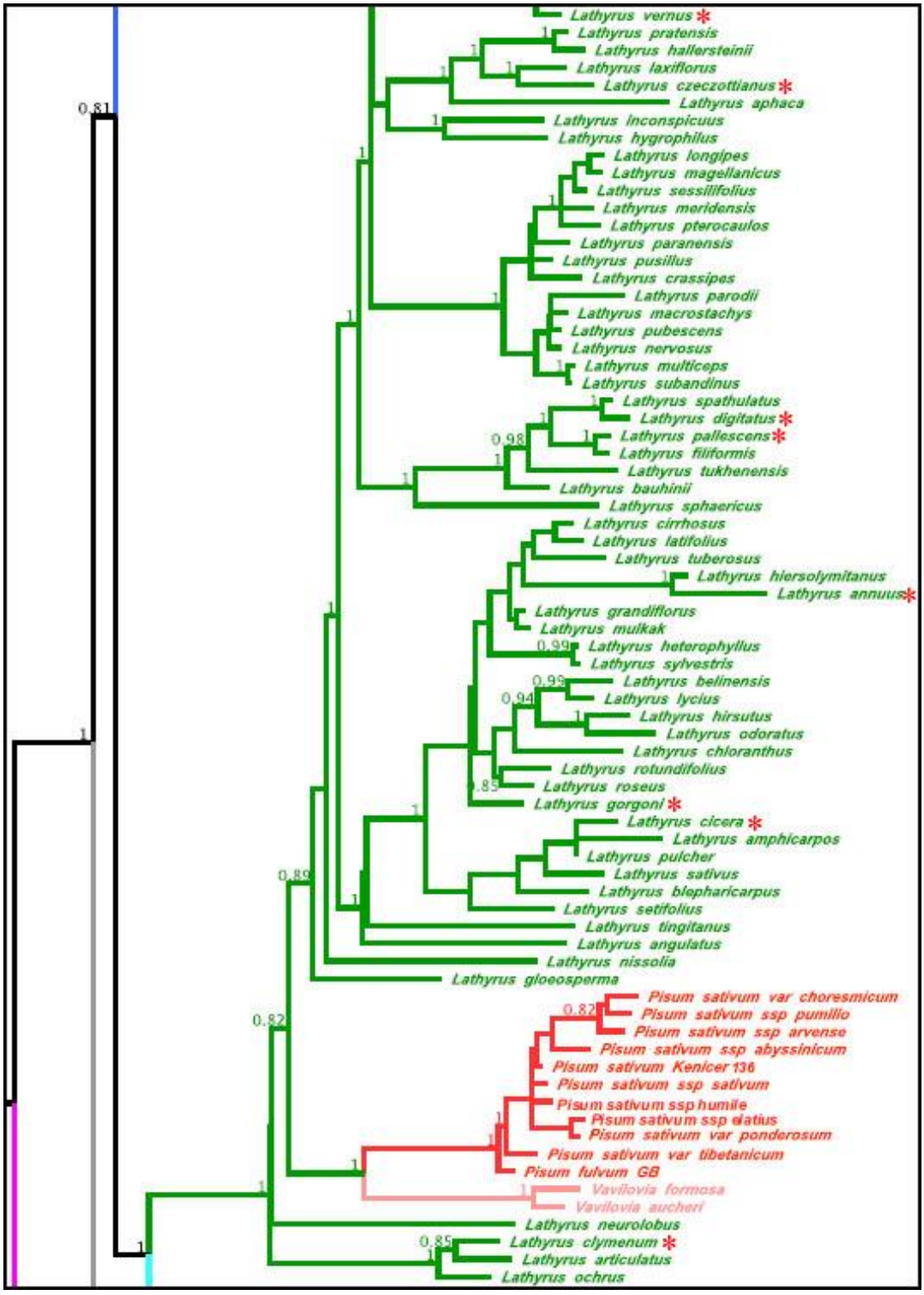

Figure 1-5 *Lathyrus* genus is not a monophyletic group, according to Schaefer et al., 2012.

1.2.4 Worldwide Distribution of *Lathyrus*

There are three lineages in relation to the worldwide distribution of *Lathyrus* (Kenicer, 2007): 1. The Trans Bering lineage 2. The Mediterranean 'Ancestral'lineage, and 3. The South American lineage (the Notolathyrus group). In accordance with the distribution of these groups as seen in **Figure 1-6**. *Lathyrus* is exclusively distributed in temperate regions everywhere in the northern hemisphere. The primary center of diversity of *Lathyrus* is in Western Eurasia, especially around the Eastern Mediterranean where more than a third of the species are native to Turkey (Davis, 1970). The secondary centers of diversity are in East Asia, North America and temperate areas of South America. Between the northern and southern hemispheres there is an interesting disjunction, and DNA data suggests that the South American species are more closely related to the species from western Eurasia than to their North American neighbors. This implies that long distance dispersal by humans had a major influence on the modern distribution of many species of *Lathyrus* (Kenicer, 2007).

A few species reached tropical East Africa, and of these *Lathyrus hygrophilus* Taubert is the only native one (ILDIS, 2002). Central Asia has very few *Lathyrus* species. In Siberia, there are a number of endemic species (*L. pisiformis*, *L. humilis* Fischer ex Seringe*)* which are cryptic species, yet some of the Siberian species have strong affinities to species found in western Eurasia (Kenicer, 2007).

Only two species, *L. palustris* and *L. japonicus* Willdenow are native to both the Old and New Worlds and are distributed across the temperate Northern Hemisphere. In North America, the greatest diversity is found in the west, especially in the Rocky Mountains and coastal Oregon and California (Kenicer, 2007).

Endemic species of *Lathyrus* are distributed on all continents except Australia and Antarctica (Kupicha, 1981). Fabeae were introduced very recently to Australia and Polynesia by European settlers (Schaefer et al., 2012).

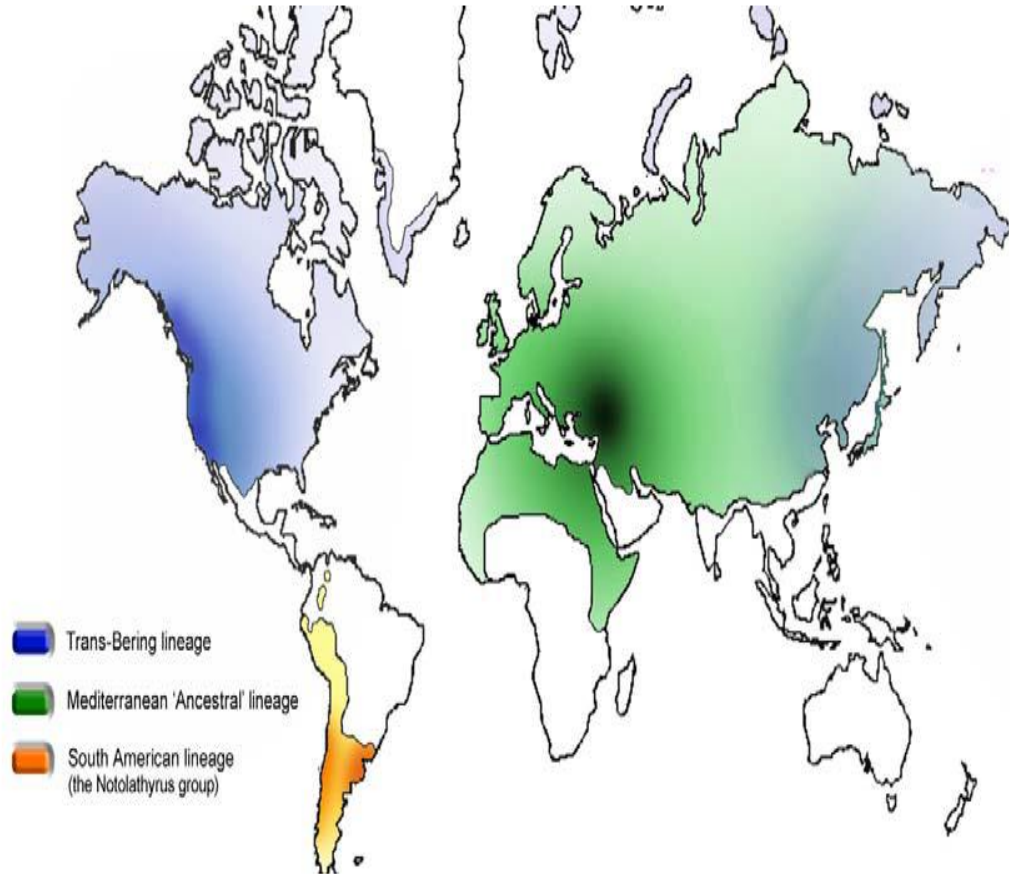

Figure 1-6 Worldwide distribution of *Lathyrus* species. Color density is proportional to the diversity of species in each area (Kenicer, 2007).

Turkey has the richest diversity of *Lathyrus*. It is the most important center of distribution of section *Lathyrostylis* (Gunes, 2011) as worldwide distribution demonstrates in **Figure 1-7**.

However, there has been significant genetic erosion due to changes in cultivation systems and the introduction of new varieties, as well as over grazing and erosion (Sabanci, 1996).

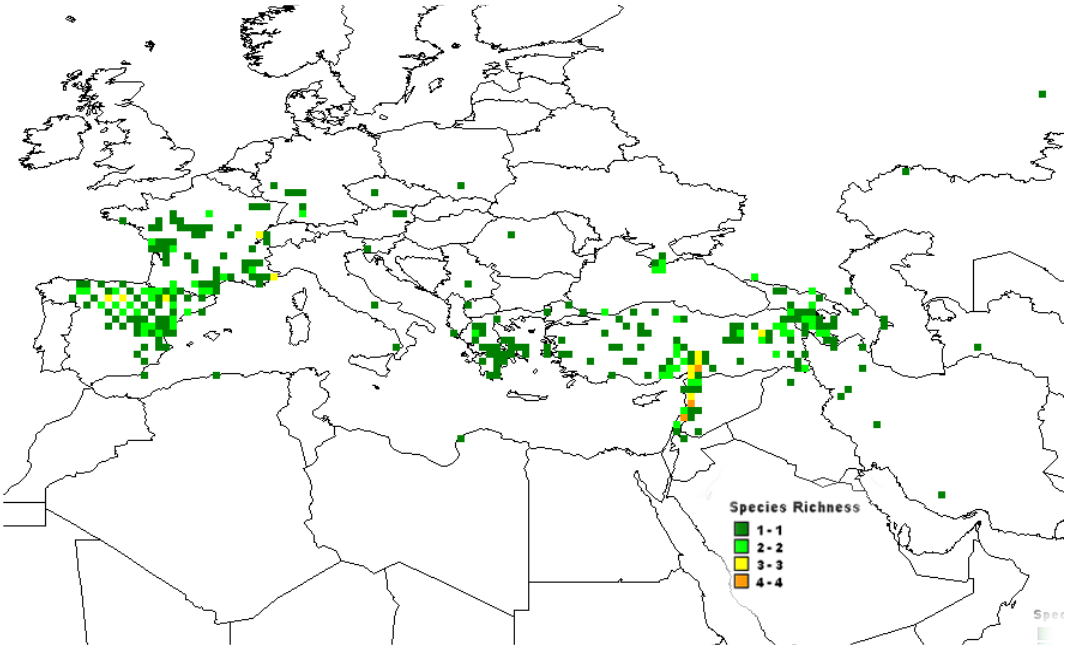

Figure 1-7 Current species richness for species of section *Lathyrostylis* in 100 x 100 km grid cells (Adopted from Shehadeh, 2011)

Most of the species are mesophytes from open habitats or forest margins, but species like *L. palustris* L. (Holarctic) and some others from the wet Chaco and South American regions have adapted to inundated marshlands. Species such as *L. hitchcockianus* Barneby & Reveal and *L. tomentosus* Lamarck belong to the xerophytes. *L. japonicus* Willdenow is a classic patch-forming littoral species with creeping, sand-catching rhizomes and semi-succulent glaucous leaves, while *L. subandinus* Phil. in the high Andes of Chile would make an extremely attractive alpine plant.

Lathyrus species are herbaceous with about 40 annuals and 120 perennials. Most annuals are relatively delicate. *L. odoratus, L. paranensis* Burkart are robust, and their feeble and non-perennation rootstocks show that they are annual species. Perennials have creeping or thickened rhizomes or tubers and may form extensive clumps or patches. There are two primary life forms in the mesophytic members of *Lathyrus*; sprawling or tall-climbing (almost tendrilous species) and erect, free-standing species (lack of or reduced tendrils). A species of sect. *Lathyrus* (p.38) that climbs with the aid of tendrils and some species of the North American members of

sect. *Orobus* (p.40) may carpet scrubby hillside or climb surrounding plants in more densely vegetated regions. More open habitats or forest area without a tall herb layer tend to harbor erect species that lack tendrils (sect. *Lathyrostylis*) (Kenicer, 2007, 2008).

1.2.5 Economic Use of *Lathyrus*

Some legumes are weeds of cereal agriculture as others are major grain crops. Thus Leguminosae include many economically significant species. Legume symbiosis plays a very important role in the terrestrial nitrogen cycle in ecosystems and agriculture. The tribe Fabeae contains peas (*Pisum* – 3 spp), lentils (*Lens* – 5, 6 spp), true vetches (*Vicia* c.150 spp), and sweet peas and chickling vetches (*Lathyrus* c.160 spp) which are considered to be major and minor crops species (Kenicer, 2007).

In the genus *Lathyrus*, there are many species such as *L. ochrus* DC, *L. tingitanus* L. (tangier pea) and *L. sativus* which are suitable fodder for livestock (Fedchenko, 1948). *L. cicera* and, *L. clymenum* are other examples of species which function as important animal fodder (Kenicer, 2007). *L. sativus* (grass pea) is the *Lathyrus* species most widely cultivated for human consumption and is a key famine food for rural populations in countries like Nepal, Ethiopia, Sudan, India, Pakistan, Kenya and Bangladesh (Kenicer, 2007; Smykal et al., 2015).

However, despite the fact that it is one of the hardiest crop species, erosion in the genetic diversity of the grass pea has been observed. It is an annual, cool season legume crop with economic and ecological significance in many places. The grass pea is favored for its ability to mature and produce a yield in times of drought when other crops have failed (Shehadeh, 2011).The grass pea is not affected by excessive rainfall (Campbell et al., 1994) and has also been used medicinally.

The *Lathyrus* species shows resistance to a serious disease (ascochyta blight) which is observed in the field pea *(P. sativum* L.) (Weimer, 1947). As explained by Kenicer (2007), Miller, (1768) stated that the seeds of *Lathyrus japonicus* (sea pea) have also been eaten in England in past centuries and Lawson, (1852); Johnson and Sowerby,

(1862) stated that edible tubers (swollen rhizomes) of *Lathyrus tuberosus* is cultivated throughout northern Europe. Also, *L. linifolius* Bässler has edible tubers which are collected in Scotland.

In addition to the usefulness of *Lathyrus* as a crop species the genus contains popular ornamental species such as *L. odoratus*. As explained by Doğan et al., (1992) Chittenden, (1951) stated that approximately 33 species are used for ornamental purposes in the genus *Lathyrus*. The most important ornamental species in commercial horticulture are *L. odoratus* (garden sweet pea) and *L. latifoliuos* (broad-leaved everlasting pea), as shown in **Figure 1-8** and **Figure 1-9**. Both belong to sect. *Lathyrus* and are known by their climbing habit. *L. grandiflorus, L. rotundifolius* and *L. undulatus* from this section are significant ornamentals. *L. aureus* and *L. vernus* (section *Orobus*) are also popular as ornamental species (Kenicer, 2008).

Figure 1-8 *Lathyrus latifoliuos* 'White Pearl'

Figure 1-9 *Lathyrus odoratus* 'Black Knight'

Lathyrus taxa are used as a model organism because of their features of widespread distribution and manageable size. The cultivars of *L. odoratus* and *L. sativus* are propagated as experimental populations (Kenicer, 2007). *Lathyrus* has been an important genus in genetic research. Chromosome evolution have been investigated in some species (Narayan, 1982; Narayan and Durrant, 1983; Seijo and Fernandez, 2003). Karyological analyses of *Lathyrus* species have been conducted by researchers in Turkey and around the world (Yamamoto et al., 1984; Seijo and Fernandez, 2003; Ayaz and Ertekin, 2008; Gunes and Cirpici, 2008; Gunes, 2011). Similarly, chemical analyses of the *Lathyrus* species have been conducted by Simola (1986).

Ecological studies make use of a *Lathyrus* species as a model organism with in-depth analyses of phenological and community behavior in *L. vernus* provided by Ehrlen (1992, 1995a, 1995b) and Ehrlen and Erikson (1995).

Some of the annual species in *Lathyrus* like, *L. aphaca* and *L. clymenum* and their close relatives, have a weedy lifestyle. They are classic ruderals which do well in disturbed areas like roadsides. *L. aphaca* and *L. ochrus* are important weedy species which function as drought- tolerant annuals (Holm et al., 1979). *L. hirsutus* (rough pea) is used for pasture, hay, winter cover, and soil improvement. *L. sylvestris* has been used for erosion control in the USA (Whyte et al., 1953). *L. pratensis* is a weedy alien that can be found everywhere.

Thus, the Leguminosae are used as food, as fodder crops or as ornamentals, and to provide oils, fiber, fuel, timber, medicines, as well as for the extraction of numerous chemicals, cultivated horticultural varieties, soil nitrifiers, dune stabilizers, or weeds, and to extract numerous chemicals (Wojciechowski, 2003). Because of the widespread diversity of *Lathyrus* and its vast usefulness in the world, it is significant to understand the relationships within the genus (Kenicer, 2007). At the same time it is necessary to conserve available cultivars and wild species.

1.2.6 Conservation Status of the *Lathyrus* species in Turkey

As stated previously (in section 1.2.4), Turkey has the richest diversity of *Lathyrus* species; however, its environment is being changed by human intervention through dam building, the construction of recreational areas, the introduction of new alien species, and overgrazing.

There is a vast ex-situ seed collection of wild and cultivated *Lathyrus* species and the most diverse species have been collected by Maxted and coworkers in conjunction with Biodiversity International and ICARDA (International Center for Agriculture and Research in Dry Area). ICARDA, the second largest collection, is a conservation organization concerned with the *Lathyrus* species in the Mediterranean region as well as other places in the world. ICARDA is focused mostly on three specific species of *Lathyrus* (*L. sativus, L. cicera*, *L. ochrus*) which are socio- economically important as human or animal feed and as a source of fodder (GCDT, 2009).

Twenty-one species, one subspecies, and two varieties of *Lathyrus* are listed in the 1998 IUCN Red Data Book (Walter and Gillott, 1998). *L. dominianus* Litvinov is extinct (Ex); four species are endangered (E); five are vulnerable (V), and eleven species are considered as rare (R). Unfortunately, reflecting the lack of a solid conservation strategy, the number of *Lathyrus* species on the Red list has been increasing for example, *L. belinensis* is critically endangered (native in Turkey) and *L. odoratus* (sweet pea) is nearly threatened (native in Italy) and are mentioned in the IUCN Red List by Maxted (2012), Branca and Donnini (2013). The conservation status of the species examined in the current study have not yet been assessed for the IUCN Red List.

There is also a valuable reference tool, the ILDIS (International Legume Database and Information Service), which has collected taxonomical and country level distribution data together with assessment of the conservation status of *Lathyrus*. However, conservation assessment of many species of *Lathyrus* is uncertain in ILDIS.

1.3 Aim of the Study

The aim of the study was to investigate the taxonomic potential of unique micromorphological characters for differentiating the taxa, particularly the species indicated with asterisks (*) in **Table 1-2** and the sectional delimitations (Kupicha, 1983) as summarized in **Table 1-1.**

For this purpose:

Firstly, the taxonomic value of 11 micromorphological leaf characters were considered for 15 species of sect. *Lathyrostylis* found in Turkey and the characters were examined by light microscope.

Secondly, the diagnostic value of 26 micromorphological leaf characters and 6 micromorphological calyx characters were investigated for 28 species (again, all

species are present in Turkey) of sect. *Lathyrostylis, Orobus, Orobon, Pratensis, Lathyrus, Clymenum.* This time the characters were examined using a scanning electron microscope (SEM).

Based on subsequent observations, similarity indices between the species were calculated and displayed by coordination and phylogenic analyses. The groups observed were evaluated comparatively with the sections of *Lathyrus* given in **Table 1-1.** Thus, the characters studied were evaluated with respect to their diagnostic value in the infrageneric classification of the genus *Lathyrus* in Turkey.

Since the species considered belong to the six sections of *Lathyrus,* it was expected that the micromorphological characters employed for the species examined would group into six clusters where each cluster belongs to one section.

2. MATERIAL AND METHODS

2.1 Material for Light Microscope

Two different kinds of microscope were employed in the study to evaluate the potential use of micromorphological characters in the classification of *Lathyrus* species and sections.

All materials of the species included in the section *Lathyrostylis* were collected from Turkey between the years 2005 and 2009 in a field survey in order to study micromorphological characters. In **Table 2-1**, the species examined with a light microscope are shown and their location information and voucher number are presented in APPENDIX A. These species were pressed and dried using standard techniques and then preserved at the Laboratory of Plant Systematics, Department of Biological Science, Middle East Technical University after crosschecks using the identification keys in the flora of Turkey (Davis, 1970) and the flora of Iraq (Townsend, 1974). In addition, species were compared with species at the Herbarium of Hacettepe University, Gazi University and Ankara University**.**

Among these species, *L. karsianus, L. tukhtensis, L. armenus, L. atropatanus, L. satdaghensis, L. elangatus, L. cilicicus, L. brachypterus,* and *L. haussknechtii* are endemic.

Table 2-1 List of species of *Lathyrus* belonging to section *Lathyrostylis* examined using a light microscope

1. *L. digitatus* (Bieb)
2. *L. karsianus* P.H. Davis
3. *L. tukhtensis* Czecz
4. *L. cyaneus* var. *cyaneus* (Stev) Koch
5. *L. armenus* (Boiss. Huet)
6. *L. boissieri* Sirj
7. *L. pallesence* (Bieb) Koch
8. *L. atropatanus* (Grossh) Sirj
9. *L. satdaghensis* P.H. Davis
10. *L. elangatus* (Bornm) Sirj
11. *L. spathulatus* Cel
12. *L. variabilis* (Boiss. & Ky.)
13. *L. cilicicus* Hayek & Siehe
14. *L. brachypterus* Cel
15. *L. haussknechtii* Cel

2.2 Light Microscope Method

Full grown leaves of species belonging to sect. *Lathyrostylis* (5-10 leaves) (**Figure 2-1**) from the middle part of a stem in each species were soaked in 5% KOH solution at room temperature for 2- 4 days. Following this, the leaf epidermis was torn off as a strip by using small blade. Epidermis on slides were stained with 1 % safranin for 2-2.5 minutes, dehydrated with 95% alcohol for about 1 minute, and then mounted by enthellan or Canadian balsam (Modified method of Simola, 1968).

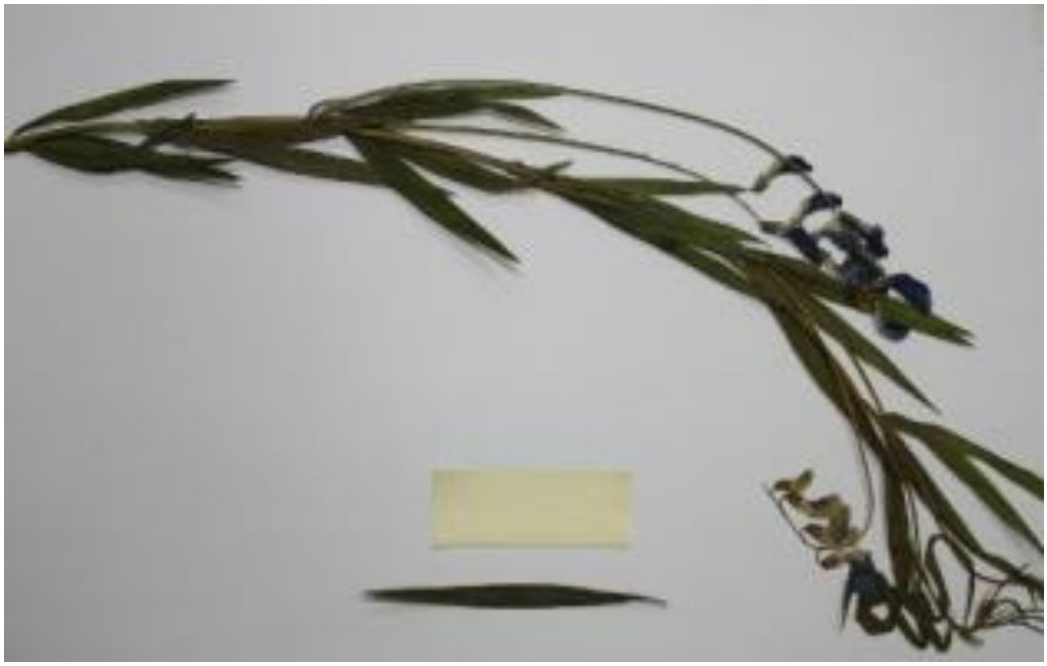

Figure 2-1 *L. cyaneus* var. *cyaneus* and single leaf

Prepared slides were taken using a light microscope attached to a Leica colour camera 1000 DM. Images were captured and digitized by means of photo express 1.0 software (X 40 Magnified) for analyses of their epidermal cell shape, pattern of cell wall, presence of stomata, presence of trichome, stomata index and stomata ratio. The leaflet area examined was 1/8 mm and was mostly sampled from the center of the mid lamina on both leaflet surfaces.

The stomata index was calculated in accordance with Salisbury's (1927) definition: Stomatal index = {S/ (E + S)} 100, where S = number of stomata per unit area and E = number of other epidermal cells in the same unit area. It indicates how many meristemoids result in the formation of stomata produced for each one hundred epidermal cells.

Stomata and epidermal cells were counted on 5–15 randomly selected areas (microscopic fields) on both sides, except in *L. variabilis* (Adaxial) and *L. pallesence* (Abaxial) which had 3 and 4 replicates due to material rarity. Stomata and epidermal cells were counted in all parts excluding main veins.

Micromorphological characters investigated herein are listed in Table 2-2 and include: epidermal cell shape of adaxial surface, epidermal cell shape of abaxial surface, cell wall pattern in adaxial surface, cell wall pattern in abaxial surface, presence of stomata in adaxial surface, presence of stomata in abaxial surface, presence of trichomes in adaxial surface, presence of trichomes in abaxial surface, stomata index of adaxial surface, stomata index of abaxial surface and stomata ratios. In total, 11 micromorphological characters were observed using a light microscope in the 15 species given in **Table 2-1** of sect. *Lathyrostylis.*

Table 2-2 Micromorphological characters investigated by LM

<table>
<tr><th>Qualitative characters</th><th>Quantitative characters</th></tr>
<tr><td>Epidermal cell shape (ada, aba)</td><td rowspan="2">Stomata Index (ada, aba)</td></tr>
<tr><td>Cell wall pattern (ada, aba)</td></tr>
<tr><td>Presence of stomata (ada, aba)</td><td rowspan="2">Stomata Ratios</td></tr>
<tr><td>Presence of trichome (ada, aba)</td></tr>
</table>

ada: adaxial surface; aba: abaxial surface

2.3 Material for Scanning Electron Microscope

Species belonging to sect. *Lathyrostylis* were obtained from the laboratory of the Plant Systematics, and the remaining species from the other sections were acquired from Ankara University (Herbarium). Each location number and voucher number is given in APPENDIX A. The entirely of these materials as shown on **Table 2-3** were crosschecked with the flora (Davis, 1970; Townsend, 1974) and compared with species at the Hacettepe and Gazi herbariums.

These species belonged to the section *Orobus* (*L. aureus, L. vernus, L. niger*), *Pratensis* (*L. laxiflorus, L. pratensis, L. czeczottianus*), *Orobon* (*L. roseus*),

Clymenum (*L. clymenum*) and section *Lathyrus* (*L. annuus, L. sativus, L. cicera, L. gorgoni, L. undulatus*).

Type species were available in some sections like *Lathyrostylis, Pratensis* and *Orobon,* but in the other sections the quality of herbarium materials was not sufficient to study. However, the endemic species of *Lathyrostylis* section as previously mentioned, *L. undulatus* and *L. czeczottianus,* are endemic in Turkey.

Table 2-3 List of species of *Lathyrus* examined using SEM which belong to six sections. Endemic species are shown with an asterisk.

1. *L. digitatus* (Bieb)
2. *L. karsianus* P.H. Davis*
3. *L. tukhtensis* Czecz*
4. *L. cyaneus* var. *cyaneus* (Stev)
5. *L. armenus* (Boiss & Huet) *
6. *L. boissieri* Sirj
7. *L. pallesence* (Bieb)
8. *L. atropatanus* (Grossh) Sirj*
9. *L. satdaghensis* P.H. Davis*
10. *L. elangatus* (Bornm)*
11. *L. spathulatus* Cel
12. *L. variabilis* (Boiss. & Ky.)
13. *L. cilicicus* Hayek & Siehe*
14. *L. brachypterus* Cel *
15. *L. haussknechtii* Cel*
16. *L. aureus* (Stev)
17. *L. vernus* L. Bernh
18. *L. laxiflorus* (Desf)
19. *L. roseus* (Stev)
20. *L. annuus* L.
21. *L. niger* L. Bernh
22. *L. clymenum* L.
23. *L. sativus* L.
24. *L. pratensis* L.
25. *L. cicera* L.
26. *L. gorgoni* Parl
27. *L. undulatus* Boiss *
28. *L. czeczottianus* Bässler *

In order to analyze micromorphological traits using SEM, leaves and calyx of *Lathyrus* species were examined, but the corolla parts of *Lathyrus* species were very shaded and micro traits thus obtained were not reliable for the analyses.

2.4 Scanning Electron Microscope Method

Leaf and calyx samples were mounted on double–sided carbon tape affixed to aluminum stubs, covered with gold or silver and photographed with a JEOL–JSM 6400 Scanning Electron Microscope in order to analyze their micro traits. Both surfaces of the leaves and the outer surface of the calyx were examined to determine if there were any visible differences which could be used as micromorphological traits. These are listed in **Table 2-4.**

The width and length of epidermal cell, stomata size (guard cells), and length of non-glandular and glandular trichomes were measured using Image tool software.

Table 2-4 Micromorphological characters investigated by SEM

<table>
<tr><th colspan="2">Leaf</th><th colspan="2">Calyx</th></tr>
<tr><th>Qualitative Characters</th><th>Quantitative Characters</th><th>Qualitative Characters</th><th>Quantitative Characters</th></tr>
<tr><td>Epidermal cell shape</td><td>Size of epidermal cells</td><td rowspan="2">Trichome Type</td><td rowspan="2">Length of non-gland trichome</td></tr>
<tr><td>Cell wall pattern</td><td>Size of stomata</td></tr>
<tr><td>Presence of stomata</td><td>Length of non – gland trichome</td><td rowspan="5">Presence of Stomata</td><td rowspan="2">Length of gland Trichome</td></tr>
<tr><td>Stomata position</td><td rowspan="4">Length of gland Trichome</td></tr>
<tr><td>Presence of trichome</td><td rowspan="3">Size of stomata</td></tr>
<tr><td>Trichome Type</td></tr>
<tr><td>Granular pattern</td></tr>
</table>

a. Qualitative Characters studied in the leaves were;

Epidermal Cell Shape (ECS), Cell Wall Pattern (CWP), Presence of Stomata (P.S), Stomata Position on epidermis (S.Pos), Presence of Trichome (P.T), Trichome Type (T.T), Presence of Granular pattern (G. pat).

These traits were considered as taxonomic characters in the leaf surface of *Lathyrus* or other plants by many researchers (Simola, 1968; Krstic et al., 2002; Ma et al., 2004; Stenglein et al., 2005; Yang and Lin, 2005; Zoric et al., 2008; Zou et al., 2008; Moon et al., 2009; Carvalho et al., 2010; Saheed and Illoh, 2010; Celep et al., 2011; Tripathi and Mondal, 2012; Srivastava et al., 2013).

b. Quantitative Characters studied in the leaves were;

Size of epidermal cell (Width of epidermal cell, Length of epidermal cell), size of stomata (Width of stomata, Length of stomata), Length of non-glandular trichome, Length of glandular trichome.

Thickness of epidermal cell (described by width and length) in various flowering plants like species in the family Portulaceae or some species of *Bauhinia* (Leguminosae) are used by Albert and Sharma (2013); Srivastava et al (2013) respectively.

Also, stomata size (described by width and length) has been measured in species like Chinese endemic *Glyptostrobus penisilis* (Ma et al., 2004), *Schisandra* (Yang and Lin, 2005), *Mentheae* species (Moon et al., 2009), *Cassia* (Tripathi and Mondal, 2012), *Tamarix* species (Abbruzzese et al., 2013), and other species.

Trichomes (non-glandular and glandular) length in different species have been analyzed in numerous studies; for example; Stenglein et al., (2005); Krajsek et al., (2006); Moon et al., (2009) and Albert and Sharma, (2013). In total (qualitative and quantitative), 26 characters (13 characters in the adaxial leaf surface and 13 characters in the abaxial leaf surface) were studied in the surfaces of the leaves.

c. Qualitative characters studied in the Calyx were;

Trichome Type (T.T) and the presence or absence of stomata (P.S). Other traits like epidermal cell shape, cell wall pattern, or stomata position were thought insignificant to consider as all species possess the same kind of epidermal cell shapes and pattern.

Trichome type in the calyx of *Lathyrus* is considered a taxonomic character by Çıldır et al (2012), and Kahraman et al (2013). The presence or absence of stomata in the calyx is an additional trait which is considered for the first time in this study. There are many morphological studies regarding the calyx but very few considering its micromorphology. Trichome type for the calyx was considered in other plants like *Nepeta sibthorpii, Salvia chrysophylla,* and *Ocimum selloi* Benth respectively by Rapisarda et al (2001); Kahraman et al (2009) and Goncalves et al (2010).

d. Quantitative Characters studied in the Calyx were;

Length of non-glandular trichome, Length of glandular trichome, Size of Stomata (width and length of stomata). Only the outer surface of the calyx were studied here as the inner surface lacks trichome and stomata. Trichome length of different parts of plant like calyx, stem, or leaf were considered as mixed data in *Epilobium* by Krajsek et al (2006). In total (qualitative and quantitative), 6 micro traits were studied in the calyx in this study.

No more micromorphological features in the leaf and calyx of *Lathyrus* species examined were readily identifable.

2.5 Statistical Method

2.5.1 Mean ± Standard Error

Mean values of stomata index (using a light microscope) were considered between 5–and 10 microscopic fields for every species except *L. variabilis* (Adaxial) and *L. pallesence* (Abaxial) which had 3 and 4 replicates due to material rarity. Standard error was calculated by using standard deviation. Means of epidermal size, stomata

size, non-glandular trichome, and glandular trichome length were measured between 15-20 individuals for every species.

2.5.2 Numerical Taxonomy

a. Ordination

In ordination analyses, similar operational taxonomic units (OTUs), here the species, appear closer to each other, and dissimilar objects are farther apart. In multivariate analyses, ordination is a method complementary to data clustering. Among the many ordination methods, principal component analyses (PCA) (Sneath and Sokal, 1973), is one of the most widely used methods, and is employed in the present study.

I. PCA

In PCA (Principal Component Analyses), the similarity of the species are displayed on a two or three dimensional space. Dimensions, each perpendicular to the others, are represented by axes called principal components. Components are composed of the measured variables where each of them is weighted differentely. Each component accounts for a certain proportion of the total variance of the multivariate data.

The amount of the variance accounted for is given by the eigenvalue, and the eigenvalues (or the amount of the total variance that is accounted) decreases from the first to the last components. Thus, the first two components or dimensions account for the highest two proportions of the total variance of the data.

On the other hand, weights of the variables on each component depict the discrimination power of the variable in discriminating the species on that particular component. Weights are presented in this study as loading scores. Only the loading scores of the first principal components are given.

Gower, Euclidean and Bray Curtis are widely used similarity coefficients and are used mostly to analyze data having multiple scales such as binary, qualitative, or

quantitative characters and can also be used with missing values (Gower, 1971; Rosemberg, 1984; St-Laurent et al., 2000).

II. Seriation

Seriation is another way to visualize the data and is a method based on the character similarities between the OTUs (between the species). The algorithm described by Brower and Kile (1988), is constructed by the seriation absence-presence (0/1) matrix of the chracters. There are two algorithms: constrained and unconstrained optimization. In constrained optimization, only the rows (species) are free to move, and in the unconstrained mode, both rows (species) and columns (characters) are free to move (Brower and Kile, 1988). The unconstrained mode is used in the present study.

b. Cluster analyses

In order to group the examined *Lathyrus* species, OTUs based on their micromorphological similarities of leaves and calyx, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) is chosen as the type of clustering analyses. In UPGMA, the OTUs exhibiting the smallest distances between them first form clusters. These clusters are then united to form bigger clusters. While undergoing unification, the distance between these two clusters depends on the mean distance between all OTUs in these two clusters; thus, OTUs with the highest mutual similarity are grouped in a hierarchical tree-like structure called a phenogram, where the horizontal axis exhibits the linkage distance, and the vertical axis exhibits the OTUs (Stuessy, 2009) in most of their illustrations.

Cophenetic correlation indicates degree of fit between the “similarity matrix” of the distances and the “cophenetic value matrix” of the UPGMA tree. They are all calculated based on UPGMA cluster analyses (Ozturk et al., 2013).

Bootstrap (simply the number of times a particular branch appears in a tree-like structure in repeated sampling of characters) is used in this study as a statistical technique to assign measures of accuracy to sample estimates.

The PAleontological STatistics (PAST) software program (3.05) is comprehensive but simple to use for numerical analyses and has been used here. PAST includes univariate, multivariate statistics, curve fitting, data plotting, and many other tools and is therefore a complete educational package.

3. RESULTS AND DISCUSSION

3.1 Light Microscope Analyses

All qualitative and quantitative micromorphological data results (epidermal cell shape, cell wall pattern, presence of stomata and trichome, stomata index, stomata ratios) using a light microscope are shown in **Table 3-1** and **Table 3-2**. Using numerical analyses and visual illustrations, species are represented by the numbers assigned to them. Photographs are shown between Figure 3-1 and Figure 3-15.

Table 3-1 Micromorphological traits (qualitative) in leaf surface among the 15 species of sect. *Lathyrostylis*

Species (numbers)	**Surface**	**Major epidermal cell shape**	**Major cell wall pattern**	**PS**	**PT**
L.digitatus (1)	Adaxial	SE	WS	POS	POS
	Abaxial	E	ST	POS	POS
L.karsianus (2)	Adaxial	SE	WS	POS	POS
	Abaxial	E	ST	POS	POS
L.tukhtensis (3)	Adaxial	SE	WS	POS	NEG
	Abaxial	E	ST	POS	POS
L.cyaneus var. *cyan* (4)	Adaxial	SE	WS	POS	POS
	Abaxial	E	ST	POS	POS
L.armenus (5)	Adaxial	SE	WS	POS	NEG
	Abaxial	E	ST	POS	NEG
L.boissieri (6)	Adaxial	IS/SE	ST	POS	POS
	Abaxial	E	ST	POS	NEG
L.pallesence (7)	Adaxial	SE	WS	POS	POS
	Abaxial	E	ST	POS	POS
L.atropatanus (8)	Adaxial	SE	WS	POS	POS
	Abaxial	E	ST	POS	POS

Table 3-1 continued

L.satdaghensis (9)	Adaxial	SE	WS	POS	POS
	Abaxial	E	ST	POS	POS
L.elongatus (10)	Adaxial	SE	WS	POS	NEG
	Abaxial	E	ST	POS	NEG
L.spathulatus (11)	Adaxial	SE	ST	POS	POS
	Abaxial	E	ST	POS	POS
L.variabilis (12)	Adaxial	SE	WS	POS	POS
	Abaxial	E	ST	POS	POS
L.cilicicus (13)	Adaxial	SE	WS	POS	POS
	Abaxial	E	ST	POS	NEG
L.brachypterus (14)	Adaxial	SE	WS	POS	POS
	Abaxial	SE	ST	POS	POS
L.haussknechtii (15)	Adaxial	E	ST	POS	POS
	Abaxial	E	ST	POS	POS

ECS; epidermal cell shape. SE; slightly elongated. IS/ SE; isodiametric slightly elongated. E; elongated.
CWP; cell wall pattern. WS; wavy or straight .ST; straight
PS; presence of stomata
PT; presence of trichome
Pos; Positive
Neg; Negative

Table 3-2 Quantitative data (Stomatal Index, Ratios) in leaf surface among 15 species of sect. *Lathyrostylis*

Species	**Sp No**	**SI (mean ± SE) adaxial**	**SI (mean ± SE) abaxial**	**S.Ratio**
L. digitatus	1	(22.98 ± 1.76)	(26.12 ± 2.01)	0.87
L. karsianus	2	(24.69 ± 2.02)	(18.37 ± 1.28)	1.34
L. tukhtensis	3	(17.51 ± 1.80)	(28.32 ± 2.17)	0.61
L. cyaneus var. *cyan*	4	(23.77 ± 1.93)	(18.80 ± 1.91)	1.26
L. armenus	5	(27.57 ± 2.46)	(23.93 ± 2.57)	1.15

Table 3-2 continued

L. boissieri	6	(20.22 ± 1.83)	(22.62 ± 0.89)	0.89
L. pallesence	7	(23.90 ± 1.79)	(17.69 ± 3.96)	1.35
L. atropatanus	8	(18.04 ± 2.80)	(18.30 ± 1.54)	0.98
L. satdaghensis	9	(25.18 ± 2.01)	(17.04 ± 1.53)	1.47
L. elangatus	10	(23.56 ± 2.21)	(27.45 ± 2.79)	0.85
L. spathulatus	11	(30.07 ± 2.12)	(21.99 ± 2.02)	1.36
L. variabilis	12	(27.93 ± 3.02)	(24.83 ± 1.07)	1.12
L. cilicicus	13	(21.73 ± 2.18)	(16.02 ± 3.07)	1.35
L. brachypterus	14	(19.88 ± 2.63)	(24.59 ± 2.11)	0.8
L. haussknechtii	15	(25.69 ± 2.09)	(19.69 ± 1.57)	1.3
SI = Stomatal Index, SE = Standard Error, S.Ratio = Stomatal Ratio				

3.1.1 Micromorphological properties of the Adaxial Surface

3.1.1.1 Epidermal Cell Shape

Epidermal cell shapes and their wall patterns of *Lathyrus* species were first described by Bässler (1966). In the present study, there are three different cell shapes on the adaxial surface among 15 species of *Lathyrus* as given in **Table 3-1**. *L. digitatus, L. karsianus, L. tukhtensis, L. cyaneus* var. *cyaneus, L. armenus L. pallesence, L. atropatanus, L. satdaghensis, L. elangatus, L. spathulatus L. variabilis, L. cilicicus,* and *L. brachypterus* have slightly elongated epidermal cell shape. *L. boissieri* has an isodiametric slightly elongated cell shape. *L. haussknechtii* has an elongated cell shape. Photographs are provided in **Figure 3-1** and **Figure 3-15**. Epidermal cells are different in size among species, and their width and length in non-stomatal areas have been calculated using SEM.

3.1.1.2 Cell Wall Pattern

There are two types of cell wall patterns herein. Some demonstrate a wavy or straight pattern (in some parts, the wall is wavy and in other parts, it is straight) and others

have a straight wall pattern in their adaxial surface as given in **Table 3-1**. *L. boissieri, L. spathulatus, L. haussknechtii* have a straight wall pattern given in **Figure 3-6**, **Figure 3-11** and **Figure 3-15** respectively. Others are wavy or straight with variable degrees of wall undulation among species. For example; *L. atropatanus* and *L. variabilis* have the least waviness and *L. pallesenc*e has the most waviness in their wall pattern. However, these species show a predominantly wavy or straight wall pattern.

3.1.1.3 Stomata

All examined species have stomata in their adaxial surface as given in **Table 3-1.** In all species the stomata index of the adaxial surface was calculated using the Salisbury Formula, and means are shown in **Table 3-2**. They ranged from 17.51 in *L. tukhtensi*s to 30.07 in *L. spathulatus.* The standard error is also shown in **Table 3-2**. Stomatal size was measured by SEM in order to detail analyses in the next part. In some cells stomata have no subsidiary cells, and in some cells they include one or two subsidiary cells.

3.1.1.4 Trichomes

The diagnostic roles of trichomes have been accepted for many years as waste repositories and as primary environmental stimuli (Payne, 1978). All species in sect. *Lathyrostylis* have trichomes in their adaxial leaf surface except *L. tukhtensis, L. armenus* and *L. elangatus* as shown in **Table 3-1**. Trichome types are not clear using a light microscope. Types and length of trichomes are elucidated in the next part.

3.1.2 Micromorphological properties of the Abaxial Surface

3.1.2.1 Epidermal Cell Shape

There is no significant difference between epidermal cell shapes among species in abaxial surfaces as all have elongated forms as demonstrated in **Table 3-1**. Only *L. brachypterus* has slightly elongated cell shapes in their epidermis as shown in **Figure**

3-14. This species has the same cell type epidermal shapes in both surfaces. Their size in width and length was calculated as follows.

3.1.2.2 Cell Wall Pattern

All species in their abaxial surfaces are straight in their wall pattern as shown in **Table 3-1**. It seems that this kind of wall pattern is specific to the abaxial surface in the species examined. Although some species have quiet a straight wall pattern, others have few or very few undulations in their wall, which implies the wall pattern is straight. Photographs are as shown between **Figure 3-1** and **Figure 3-15**.

3.1.2.3 Stomata

Like adaxial surfaces in all species in their abaxial surface have stomata as shown in **Table 3-1**. In all species, the stomata index of the abaxial leaf surface was calculated using the Salisbury Formula, and means are found in **Table 3-2**. They ranged from 16.02 in *L. cilicicus* to 28.32 in *L. tukhtensis.*

In some cells stomata have no subsidiary cells, and in some cells stomata include one or two subsidiary cells. Their size is different between species, and these were calculated as follows.

3.1.2.4 Trichomes

Except *L. armenus, L. boissieri, L. elangatus* and *L. cilicicus* other species have trichomes in their abaxial leaf surface as seen in **Table 3-1**. Their type and length have been elucidated as follows.

The micromorphological study of the *Lathyrus* species of sect. *Lathyrostylis* using a light microscope shows adaxial surfaces which have more variable microtraits than the abaxial surfaces. In the abaxial surface, epidermal cell shape and cell wall pattern do not indicate any significant difference between species. Adaxial surfaces mostly have a slightly elongated epidermal cell shape whereas abaxial surfaces have only one type of epidermal cell shape demonstrating elongation. Some species like *L.*

haussknechtii with elongated epidermal cell shape and *L. brachypterus* with a slightly elongated epidermal cell shape have the same kind of epidermal cell shape on both surfaces. Among the species, *L. boissieri* has a different kind of epidermal cell shape, isodiametric and slightly elongated in its adaxial surface. The cell wall patterns in the adaxial surface are mostly wavy or straight except *L. boissieri, L. brachypterus,* and *L. haussknechtii* with only a straight wall pattern. All cell wall patterns in abaxial are straight. It seems undulation of cell walls belongs to adaxial surfaces between 15 examined species in the *Lathyrostylis* section. Many different suggestions have been made about the factors causing undulation of epidermal cell walls. Drought and too much light have both been cited as preventing undulation of epidermal cell walls (Simola, 1968).

These species in sect. *Lathyrostylis* are amphistomatous, although their stomata distribution as xerophytic species are more numerous on the abaxial surface. The stomatal index indicates significant differences between the fifteen species. They ranged from 17.5 in *L. tukhtensis* to 30. 07 in *L. spathulatus* in the adaxial surface and from 16.02 in *L. cilicicus* to 28.32 in *L. tukhtensis* in the abaxial surface. Therefore, *L. tukhtensis* has the lowest stomata number in adaxial surface whereas in the abaxial surface this species has the highest stomata number. Stomata ratios are calculated by division of the stomata index of adaxial to abaxial as is exhibited in **Table 3-2.** They ranged from 0.61 in *L. tukhtensis* to 1.47 in *L. satdaghensis.*

L. armenus and *L. elangatus* do not have trichomes in their leaf surface. These species are glabrous, which is also confirmed by SEM analyses in the next chapter. *L. tukhtensis* has trichomes in just the abaxial surface, and *L. boissieri* and *L. cilicicus* have trichomes in only the adaxial surface. Glandular and non-glandular trichome types are not quite clear here using a light microscope, so SEM has been used to identify different trichome types and other micromorphological characters in the vegetative and reproductive organs of the *Lathyrus* species.

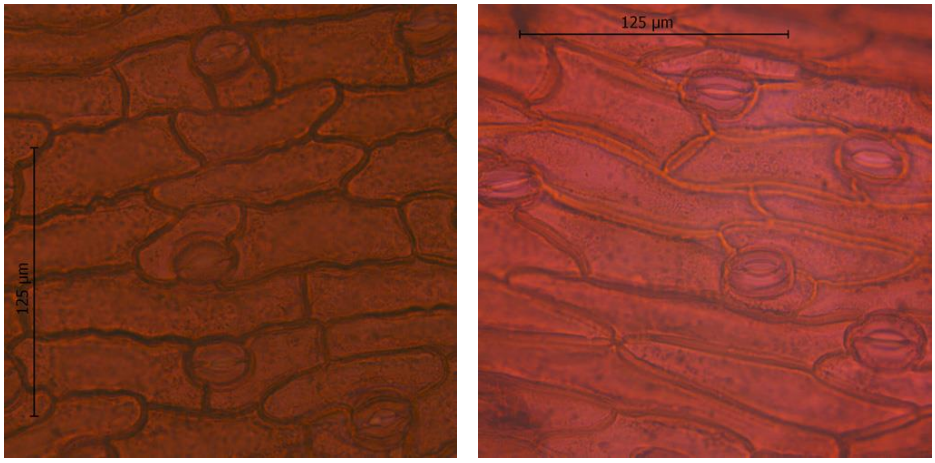

Figure 3-1. Left, *L. digitatus* adaxial surface; right, *L. digitatus* abaxial surface

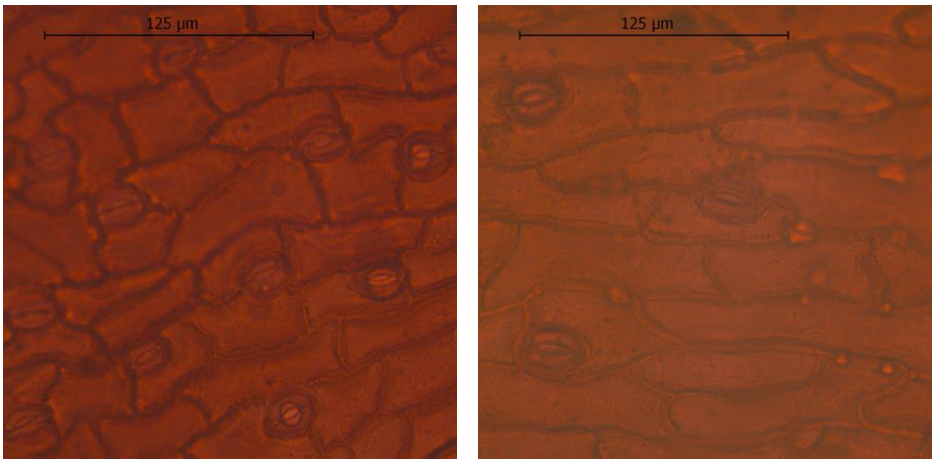

Figure 3-2. Left , *L. karsianus* adaxial surface, *L. karsianus* abaxial surface

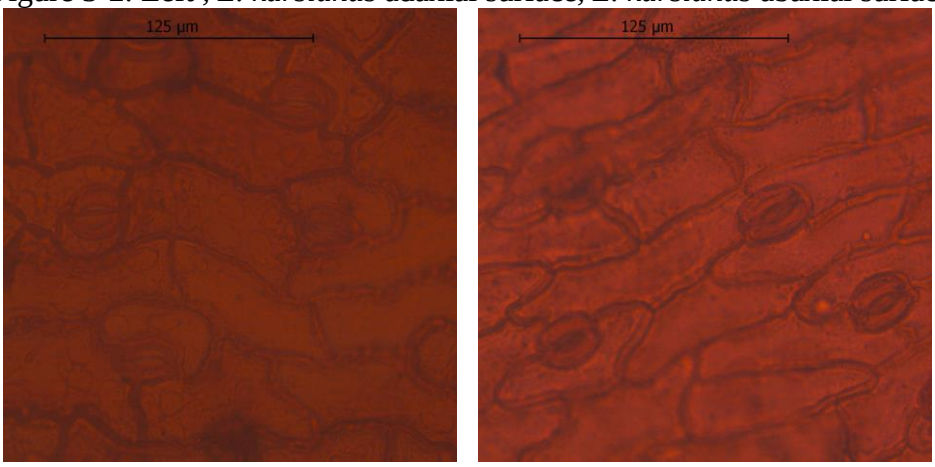

Figure 3-3. Left, *L. tukhtensis* adaxial surface and right, *L. tukhtensis* abaxial surface

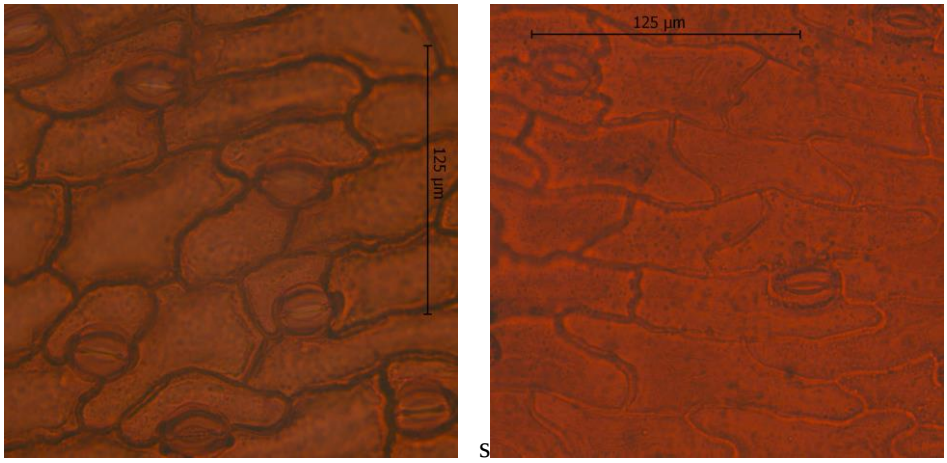

Figure 3-4 . Left, *L. cyaneus* var. *cyan* adaxial surface and right, *L. cyaneus* var *cyan* abaxial surface.

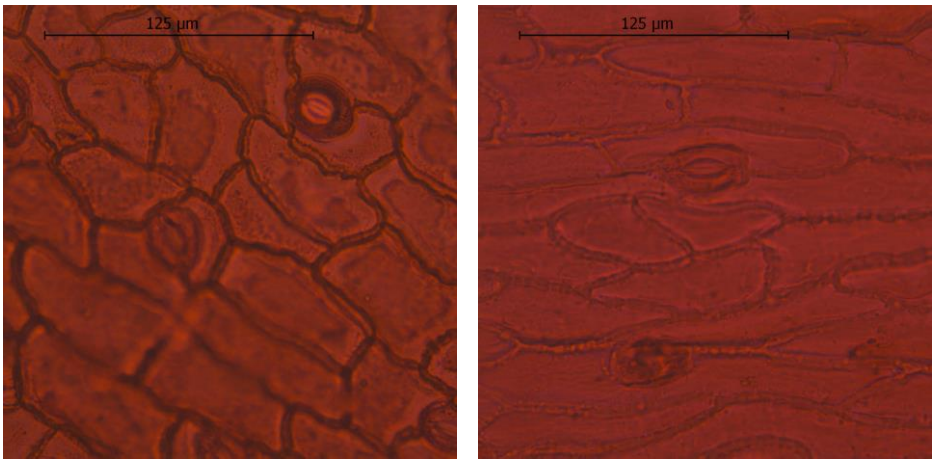

Figure 3-5. Left, *L. armenus* adaxial surface and right, *L. armenus* abaxial surface

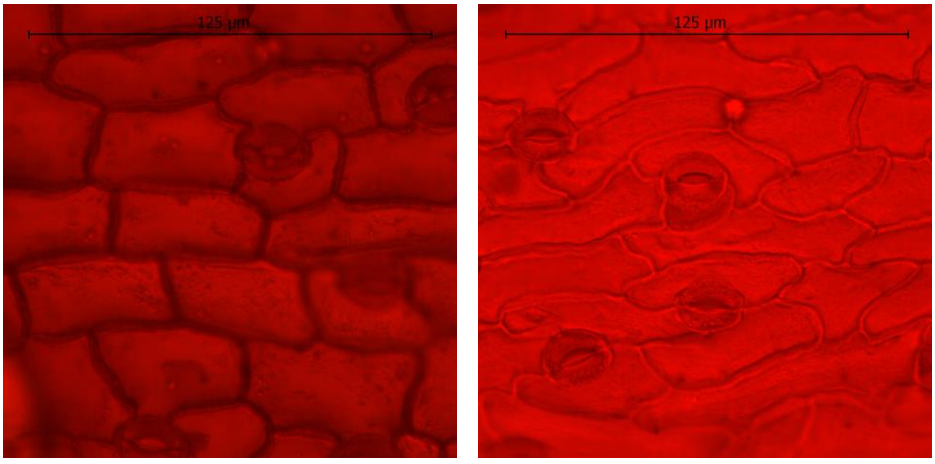

Figure 3-6 . Left, *L. boissieri* adaxial surface and right, *L. boissieri* abaxial surface

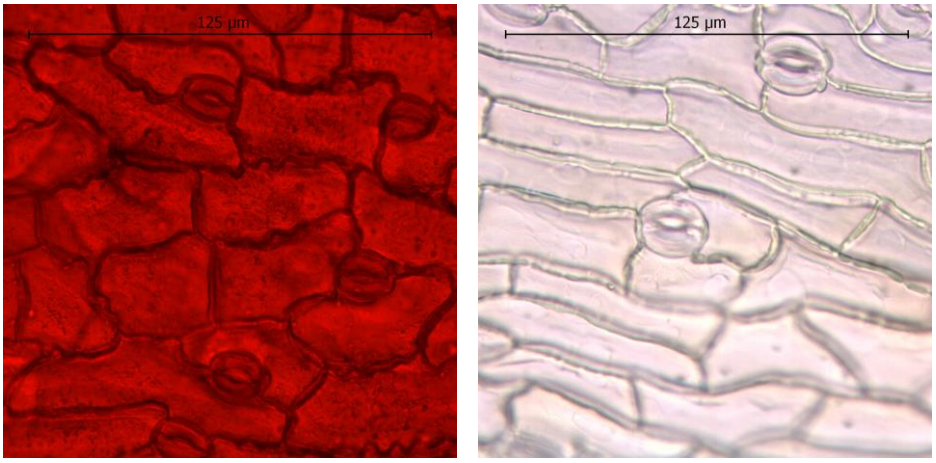

Figure 3-7. Left, *L. pallesence* adaxial surface and right, *L. pallesence* abaxial surface

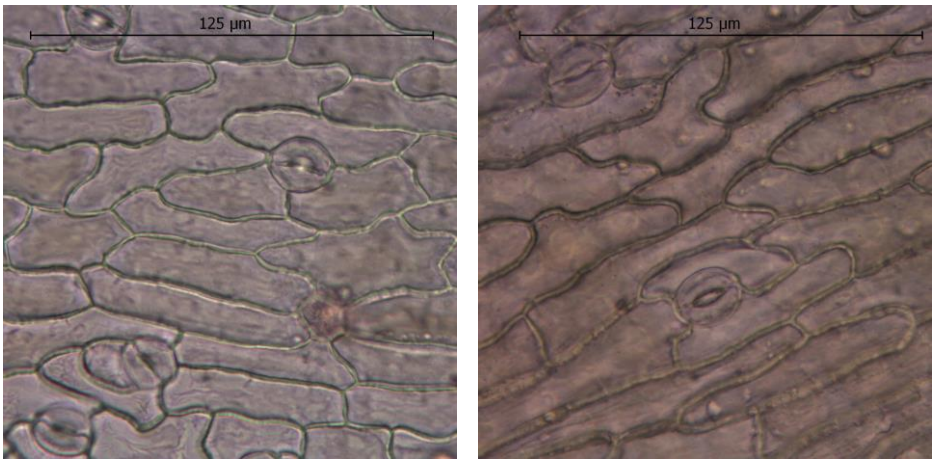

Figure 3-8. Left, *L. atropatanus* adaxial surface and right, *L. atropatanus* abaxial surface

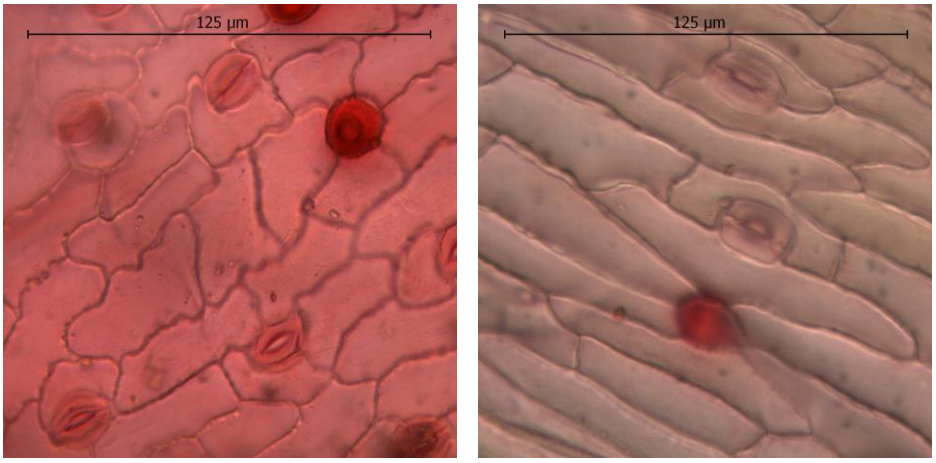

Figure 3-9 Left, *L. satdagensis* adaxial surface and right, *L. satdagensis* abaxial surface

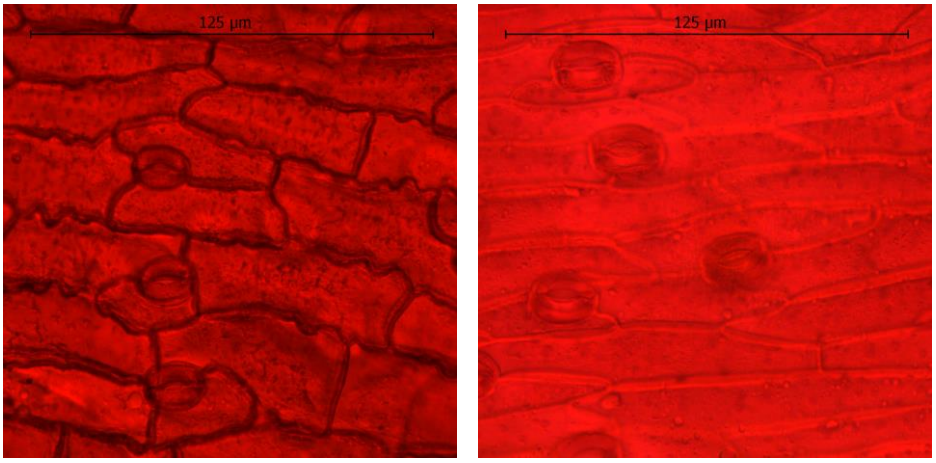

Figure 3-10 Left, *L. elangatus* adaxial surface and right, *L. elangatus* abaxial surface

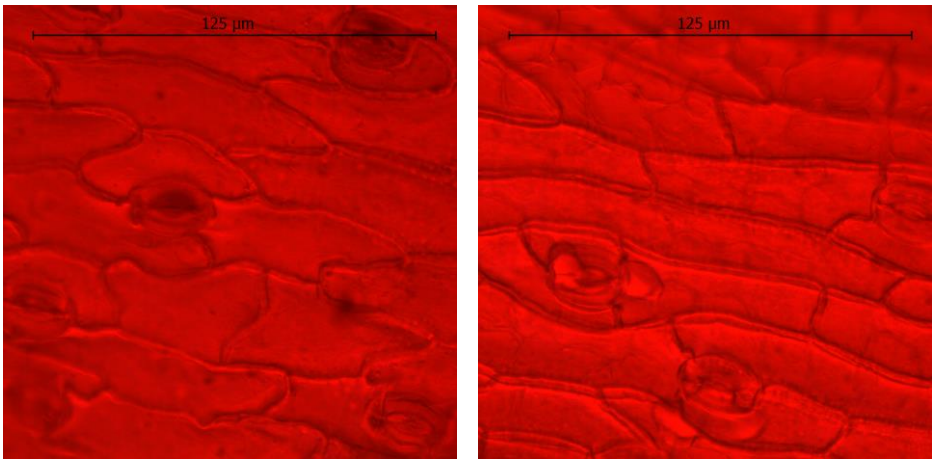

Figure 3-11. Left, *L. spathulatus* adaxial surface and right, *L. spathulatus* abaxial surface

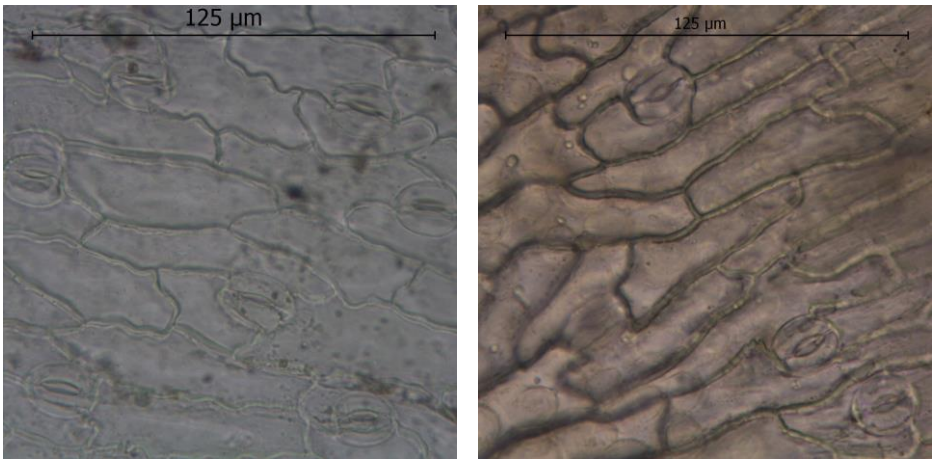

Figure 3-12. Left, *L. variabilis* adaxial surface and right, *L. variabilis* abaxial surface

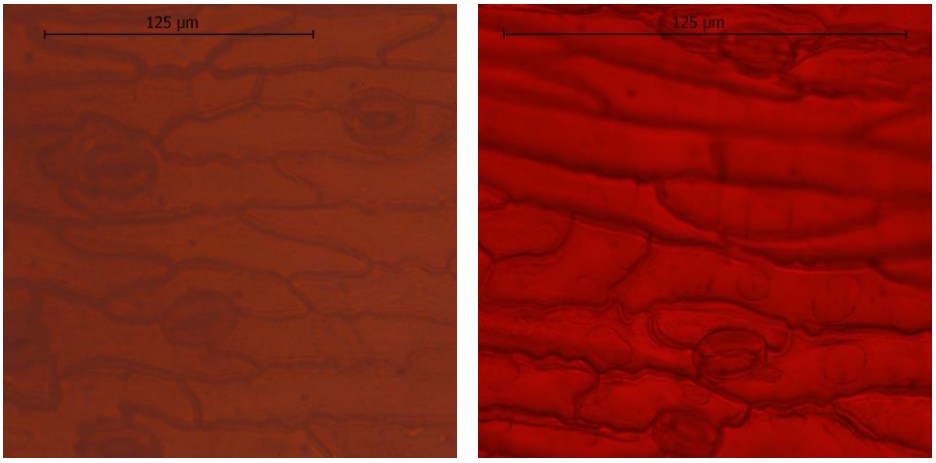

Figure 3-13. Left, *L. cilicicus* adaxial surface and right, *L. cilicicus* abaxial surface

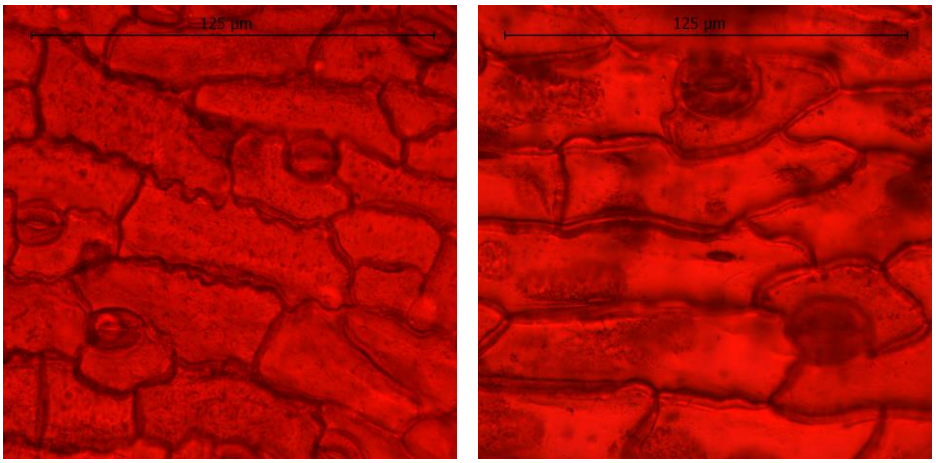

Figure 3-14. Left, *L. brachypterus* adaxial surface and right, *L. brachypterus* abaxial

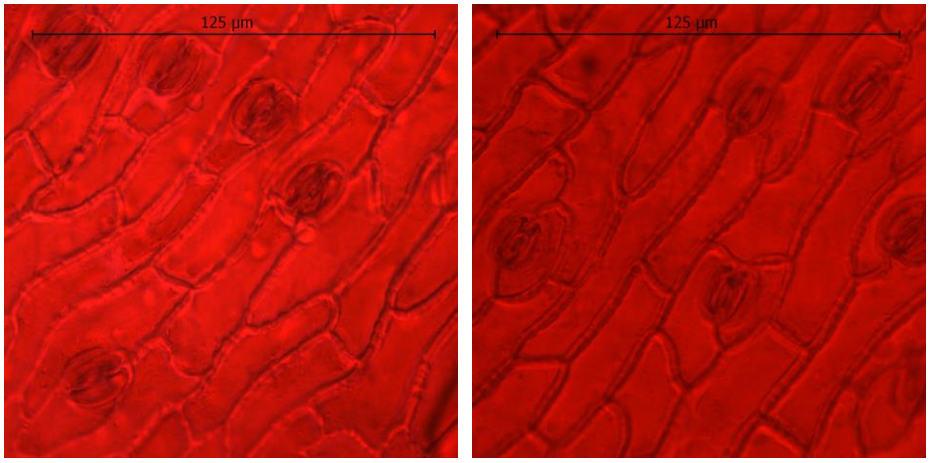

Figure 3-15. Left, *L. haussknechtii* adaxial surface and right, *L. haussknechtii* abaxial

3.1.3 Ordination analyses and Phenetic Cluster results for Micromorphological traits

For the Principal Component Analyses and UPGMA phenogram tree construction, quantitative and qualitative microtraits were analyzed separately. The PAST 3.05 software program was used to evaluate the data.

3.1.3.1 Quantitative data Results

As quantitative data stomata index and ratios of both leaf surfaces, **Table 3-2**, are analyzed with the PAST 3.05 software and in two-dimensional scatter plot is shown in **Figure 3-16**, which is constructed in the PCA based on first two axes (X axis is PC 1 and Y axis is PC 2). In the scatter plot, numbers represent species of sect. *Lathyrostylis* examined using a light microscope. Loading plot (value is coefficients) is shown in **Figure 3-17**. A, B and C represent respectively the stomata index of adaxial surface, stomata index of abaxial surface, and stomata ratios.

It is possible to distinguish two groups in the PCA. *L. digitatus* (1), *L. elangatus* (10), *L. boissieri* (6), *L. brachypterus* (14) and *L. tukhtensis* (3) are in one group (I), and the second group (II) consistes of *L. variabilis* (12), *L. armenus* (5), *L. spathulatus* (11), *L. haussknechtii* (15), *L. pallesence* (7), *L. cyaneus* var. *cyaneus* (4), *L. karsianus* (2) *L. satdaghensis* (9), *L. cilicicus* (13) and *L. atropatanus* (8).

L. digitatus (1), *L. elangatus* (10), and *L. boissieri* (6), *L. brachypterus* (14), respectively are in close proximity due to their quantitative microtraits. Their stomata ratios are very close in value. *L. tukhtensis* (3) is distanced from the others based on this scatter plot. It has the least amount of stomata ratios. Also, *L. pallesence* (7), *L. cyaneus* var. *cyaneus* (4), *L. karsianus* (2) and *L. satdaghensis* (9) show a close relationship in scatter plot. *L. cilicicus* (13) and *L. haussknechtii* (15) are close to these species. Also, *L. armenus* (5), *L. variabilis* (12) and *L. spathulatus* (11) exhibit close proximity on the scatter plot. According to the loading plots of data as given in **Figure 3-17**, the stomata index of abaxial surface (B) hold an important place in comparison to others.

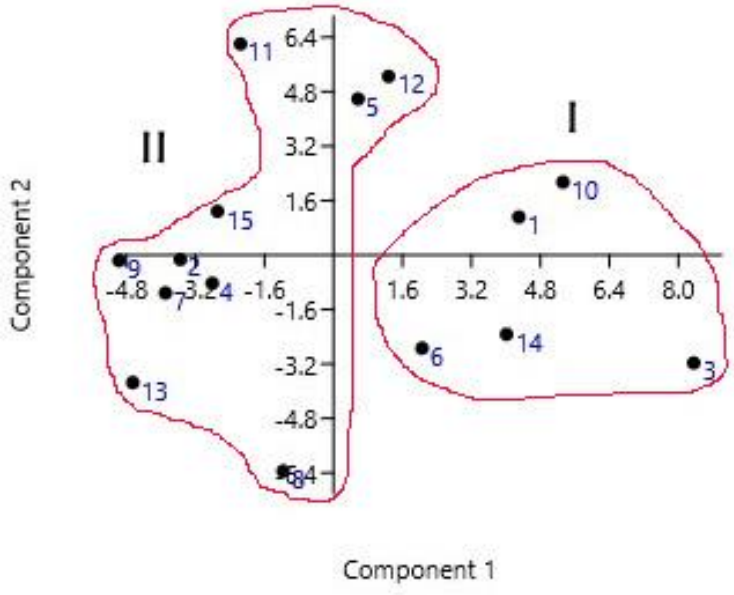

Figure 3-16 A Two dimensional scatter plot in the PCA for Quantitative data

Group I covers: *L.digitatus* (1), *L.tukhtensis* (3), *L.boissieri* (6), *L.elangatus* (10), *and L.brachypterus* (14).

Group II covers: *L.armenus* (5), *L.variabilis* (12), *L.spathulatus* (11), *L. haussknechtii* (15), *L.karsianus* (2), *L.satdaghensis* (9), *L.pallesence* (7), *L. cyaneus* var. *cyaneus* (4), *L.cilicicus* (13), *L.atropatanus* (8)

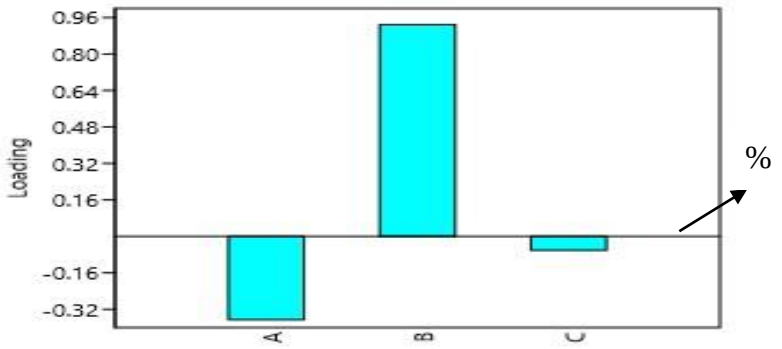

Figure 3-17 Loading plot of Quantitative data

A: is stomata index of adaxial, B: is stomata index of abaxial, and C is stomata ratios

Percent of the total variation that is accounted by the first axis (57.41%) is shown by the arrow

The phenogram tree constructed in the UPGMA cluster analyses using Euclidean‘s similarity coefficient with three quantitative characters is given in **Figure 3-18** (Cophen corr is 0.7896). **Figure 3-19** indicates the phenogram using Gower’s similarity index with 0.7939 Cophen corr. Cophenetic correlation, indicating a good fit of the phenogram to the distance matrix (Rohlf, 1992).

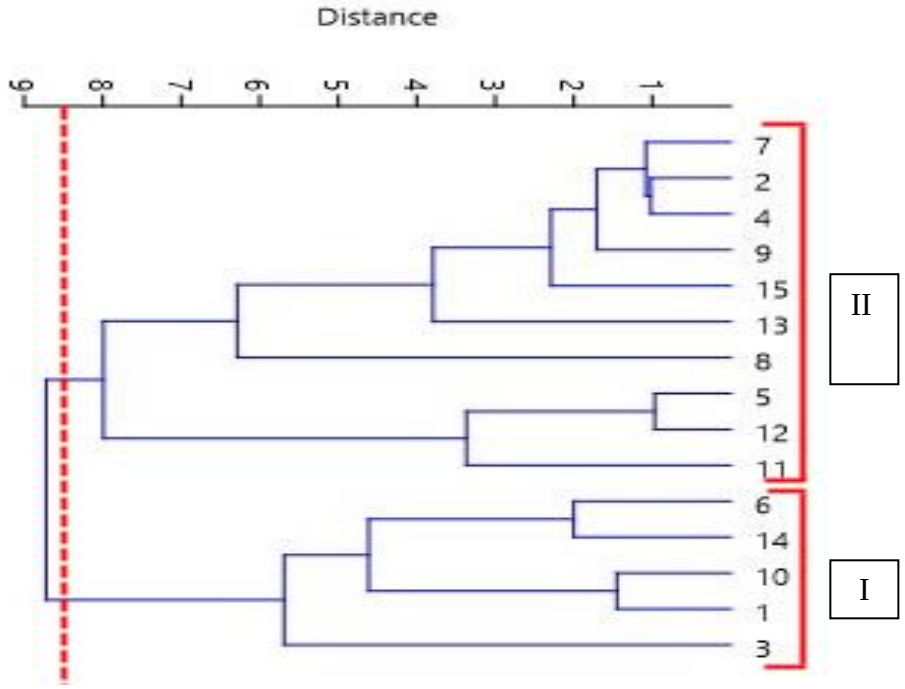

Figure 3-18 UPGMA based on Euclidean similarity index with quantitative data

Group I covers: *L.digitatus* (1), *L.tukhtensis* (3), *L.boissieri* (6), *L.elangatus* (10), *and L.brachypterus* (14).

Group II covers*: L.spathulatus* (11), *L.variabilis* (12), *L.armenus* (5), *L.atropatanus* (8), *L.cilicicus* (13), *L.haussknechtii* (15), *L.satdaghensis* (9), *L.cyaneus* var. *cyaneus* (4), *L.karsianus* (2), *L.pallesence* (7).

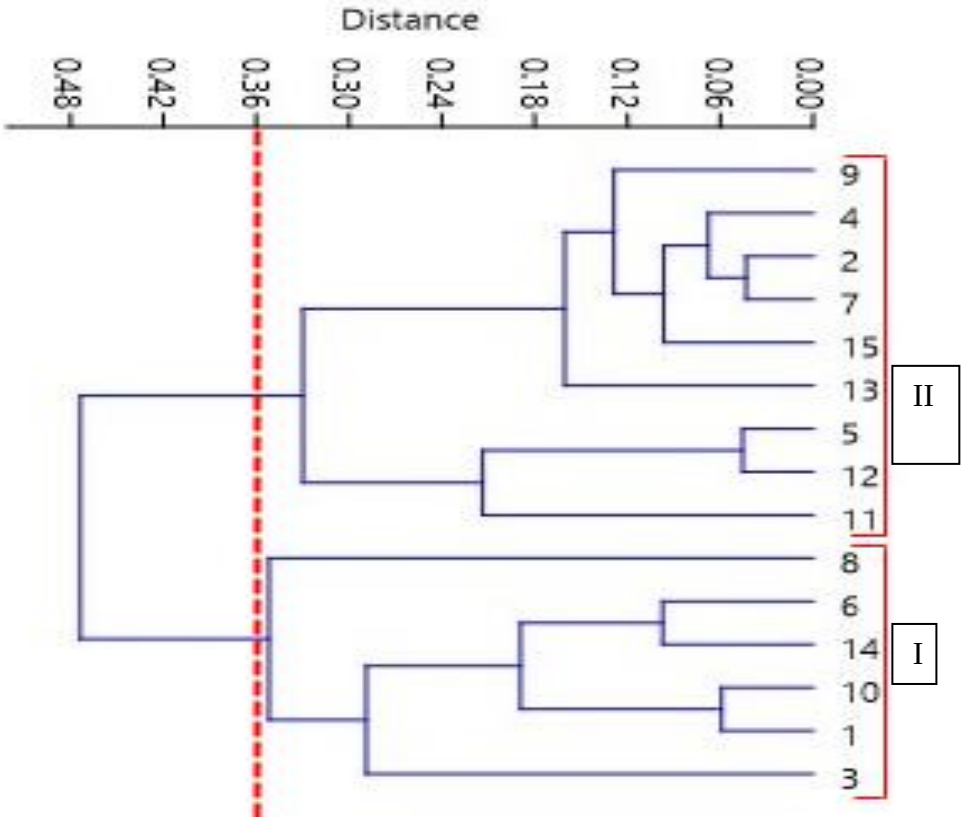

Figure 3-19 UPGMA based on Gower similarity index with quantitative data

Group I covers*: L.digitatus* (1), *L.tukhtensis* (3), *L.boissieri* (6), *L.elangatus* (10), *L. brachypterus* (14), and *L. atropatanus* (8).

Group II covers*: L.spathulatus* (11), *L.variabilis* (12), *L.armenus* (5), *L.cilicicus* (13), *L. haussknechtii* (15), *L.satdaghensis* (9), *L.cyaneus* var. *cyaneus* (4), *L.karsianus* (2), *L.pallesence* (7).

The phenon line at 8.5 (for Euclidean similarity index) and 0.36 (for Gower similarity index) creates two groups or phenon in the UPGMA trees and results in both phenograms being almost the same in the PCA. *L. boissieri* (6), *L. brachypterus* (14), *L. elangatus* (10), *L. digitatus* (1), *L. tukhtensis* (3) are in one phenon, and the rest of the species appear in the second phenon. Only *L. atropatanus* (8) exhibits a different relationship with other species as its placement is changed in both phenograms. In a phenogram with Euclidean index, *L. atrapatanus* (8) is in relation to *L. pallesence* (7), L. *karsianus* (2), *L. cyaneus* var. *cyaneus* (4), *L. satdaghensis* (9), *L. haussknechtii* (15), and *L. cilicicus* (13). But in a phenogram with Gower

similarity index, this species has a relationship with *L. boissieri* (6), *L. brachypterus* (14), *L. elangatus* (10), *L. digitatus* (1), and *L. tukhtensis* (3).

In both phenograms, *L. digitatus* (1), *L. elangatus* (10) with 0.06 distance (Gower similarity index), 1.5 distance (Euclidean similarity index) exhibit a closer relationship when compared to other species. Similarly, *L. armenus* (5), *L. variabilis* (12) with 0.04 distance (Gower similarity index), 1 distance (Euclidean similarity index) exhibits a closer relationship when compared to other species. In all species stomata ratios are very close. *L. karsianus* (2), *L. cyaneus* var. *cyaneus* (4) and *L. pallesence* (7) are closely related on both trees. However, observed quantitative characters are very few to confirm obtained tree in this part.

3.1.3.2 Qualitative data Results

After obtaining numeric forms for qualitative micromorphological characters as given in **Table 3-3**, the PAST 3.05 software program was used to evaluate data (qualitative micro characters that do not show any differentiation among species were eliminated from analyses and others which show different features were coded and scored as 0, 1 and 2 for clustering and ordination analyses as given in Table 3-3).

Table 3-3 Numeric forms of micromorphological traits (qualitative) in the 15 species of *Lathyrus*

Species (numbers)	Ada ecs	Ada cwp	Ada PT	Aba PT
***L.digitatus* (1)**	0	0	1	1
***L.karsianus* (2)**	0	0	1	1
***L.tukhtensis* (3)**	0	0	0	1
***L.cyan* var. *cya* (4)**	0	0	1	1
***L.armenus* (5)**	0	0	0	0
***L.boissieri* (6)**	2	1	1	0
***L.pallesence* (7)**	0	0	1	1
***L.atropatanus* (8)**	0	0	1	1
***L.satdaghens* (9)**	0	0	1	1
***L.elangatus* (10)**	0	0	0	0

Table 3.3, continued

***L.spathulatus*(11)**	0	1	1	1
***L.variabilis* (12)**	0	0	1	1
***L.cilicicus* (13)**	0	0	1	0
***L.brachypterus*(14)**	0	0	1	1
L.hausknechti* (15*)	1	1	1	1

Epidermal cell shape on Adaxial (Ada ecs), Cell wall pattern on Adaxial (Ada cwp), Presence of trichome on Adaxial (Ada PT), Presence of trichome on Abaxial (Aba PT)

Epidermal cell shapes and their wall pattern of adaxial surface, presence of trichome on both leaf surface as qualitative data are analyzed with the PAST 3.05 software, and in **Figure 3-20**, a two-dimensional scatter plot is constructed in the PCA based on the two first axes (X axis is PC 1, and Y axis is PC 2). Loading plot (value is coefficients) is shown in **Figure 3-21**. A, B, C and D demonstrate epidermal cell shape of adaxial surface, cell wall pattern of adaxial surface, presence of trichome in adaxial surface, and presence of trichome in abaxial surface respectively.

There are two groups in the PCA. *L. boissieri* (6) in one group (I) (separated from others), and the remainder of the species are in the second group. In the second group (II), *L. armenus* (5), *L. elangatus* (10) overlap as *L. pallesence* (7), *L. cyaneus* var. *cyaneus* (4), *L. karsianus* (2), *L. digitatus* (1), *L. atropatanus* (8), *L. satdaghensis* (9), *L. variabilis* (12) and *L. brachypterus* (14) show a very close relationship in the scatter plot. According to loading plots of data, **Figure 3-21**, epidermal cell shape (A) and cell wall pattern of adaxial leaf surface (B) play an important part when compared to others.

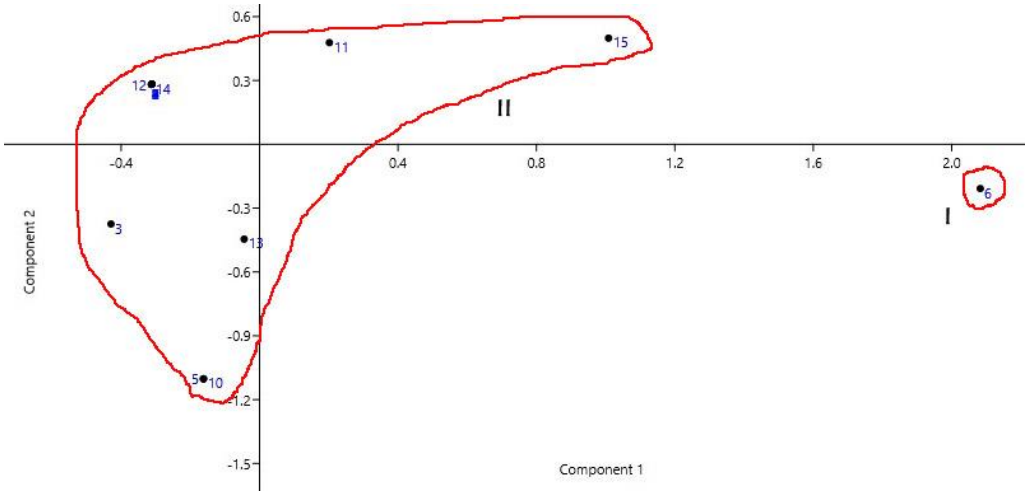

Figure 3-20 A Two dimensional scatter plot in the PCA for qualitative data

Group I covers*: L. boissieri* (6)

Group II covers: *L.digitatus* (1),*L.karsianus* (2),*L.tukhtensis* (3),*L.cyaneus* var.*cyaneus* (4), *L.armenus* (5), *L.pallesence* (7),*L.atropatanus* (8),*L.satdagensis* (9),*L.elangatus* (10), *L.spathulatus* (11), *L.variabilis* (12), *L.cilicicus* (13*), L.brachypterus* (14), *L.haussknechtii* (15)

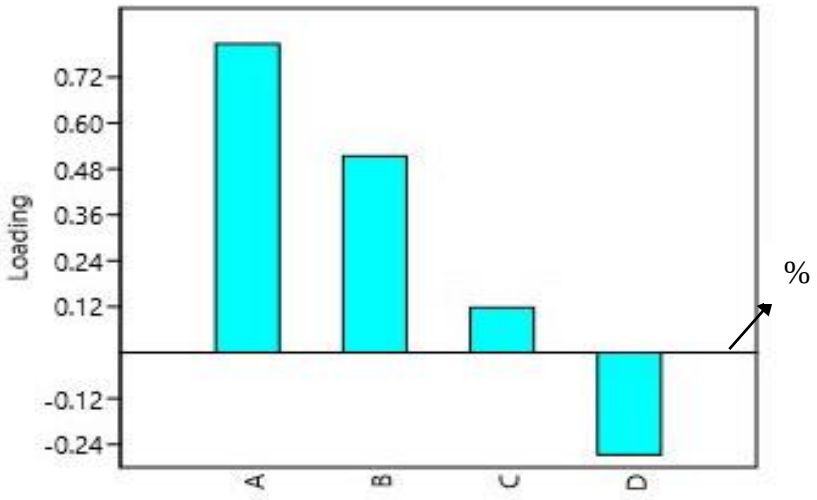

Figure 3-21 Loading plot of qualitative data

A: is epidermal cell shape of adaxial, B: is cell wall pattern of adaxial, C: is presence of trichome in adaxial and D: is presence of trichome of abaxial.

Percent of the total variation that is accounted by the first axis (%52.84) is shown by the arrow.

The phenogram tree constructed in the UPGMA cluster analyses employing Euclidean‘s similarity coefficient with four qualitative characters as given in **Figure**

3-22 (Cophen corr is 0.9633). **Figure 3-23** indicates a phenogram employing Gower's similarity index with 0.8926 Cophen corr. According to Rohlf, 1992, Cophenetic correlation, indicating a good fit of the phenogram to the distance matrix.

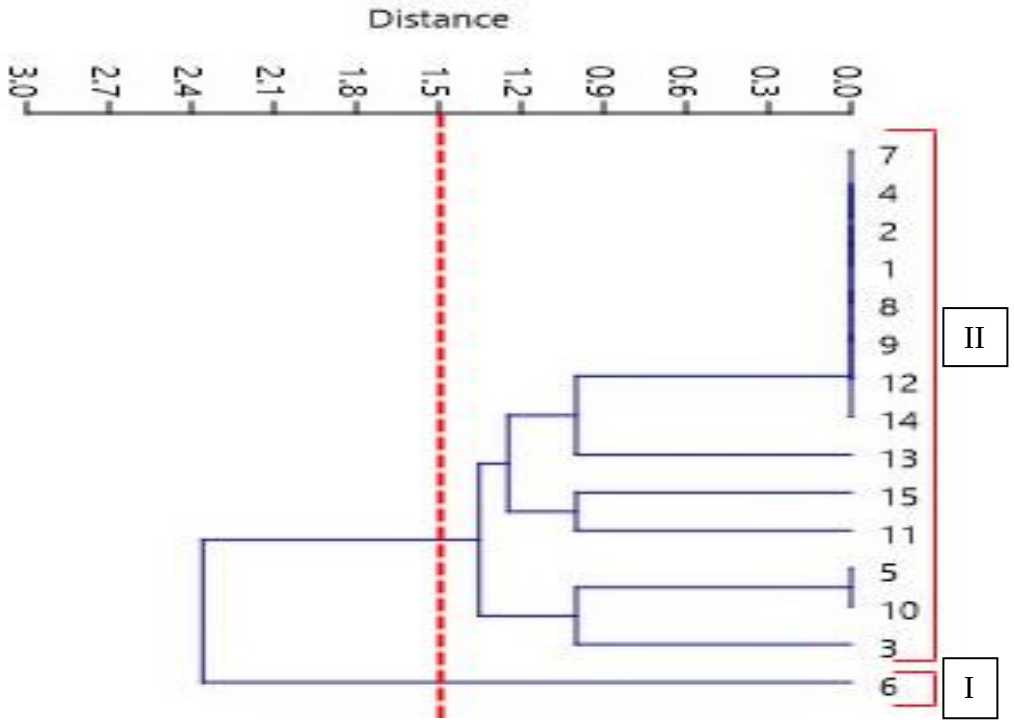

Figure 3-22 UPGMA based on Euclidean Similarity index with qualitative data

Group I covers*: L.boissieri (6)*

Group II covers*: L.digitatus* (1), *L.karsianus* (2), *L.tukhtensis* (3), *L.cyaneus* var. *cyaneus* (4) , *L.armenus* (5), *L.pallesence* (7), *L.atropatanus* (8), *L.satdagensis* (9), *L.elangatus* (10), *L. spathulatus* (11), *L.variabilis* (12), *L.cilicicus* (13), *L.brachypterus* (14), *L.haussknechtii* (15)

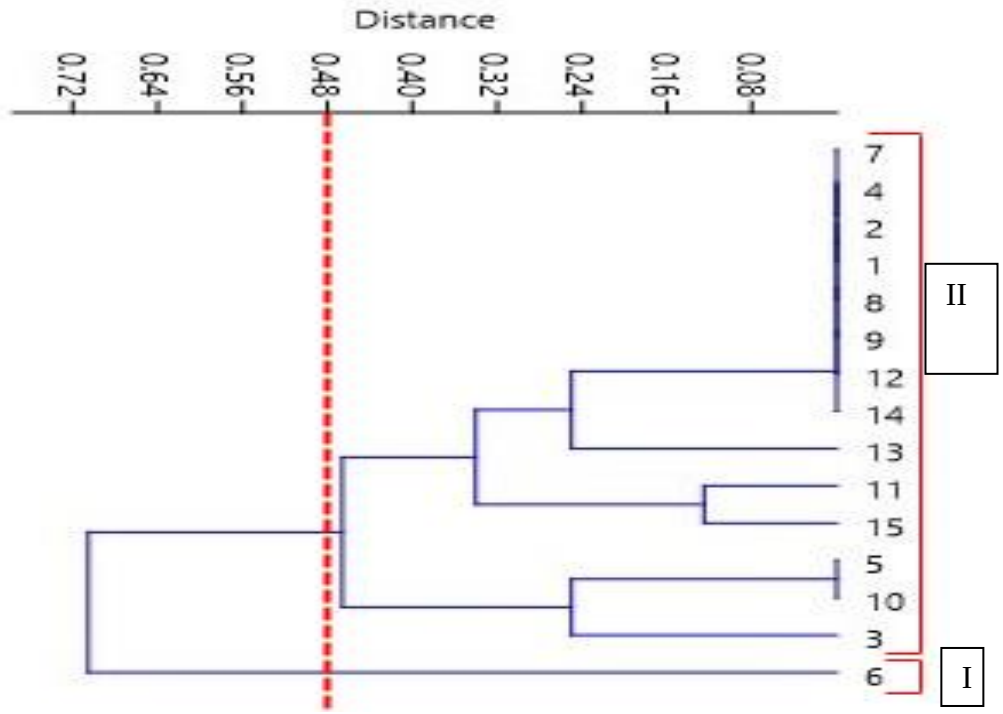

Figure 3-23 UPGMA based on Gower similarity index with qualitative data
Group I covers: *L.boissieri* (6)

Group II covers*: L.digitatus* (1), *L.karsianus* (2), *L.tukhtensis* (3), *L.cyaneus* var. *cyaneus* (4), *L.armenus* (5), *L.pallesence* (7), *L.atropatanus* (8), *L.satdagensis* (9), *L.elangatus* (10), *L. spathulatus* (11), *L.variabilis* (12), *L.cilicicus* (13*), L.brachypterus* (14), *L.haussknechtii* (15)

The phenon line at 1.5 (for Euclidean similarity index) and 0.48 (for Gower similarity index) creates two groups or phenon in the UPGMA trees, and results in both phenograms being almost identical in the PCA. *L. boissieri* (6) is in one phenon, and the others are in the second phenon. *L. boissieri* (6) is separated from other species in both phenograms. Its cell shape is more specific among species with isodiametric and slightly elongated cell shapes and a straight wall pattern in the adaxial surface. This species has trichome only in the adaxial surface. In both phenograms, species *L. pallesence* (7), *L. cyaneus* var. *cyaneus* (4), *L. karsianus* (2), *L. digitatus* (1), *L. atropatanus* (8), *L. satdaghensis* (9), *L. variabilis* (12), and *L. brachypterus* (14) are very close (with 0 distance) based on qualitative micromorphological traits. All of these species have a slightly elongated cell shape with a wavy or straight cell wall pattern in their adaxial leaf surface. Also, these

species all have trichome in both surfaces. In both phenograms, *L. armenus* (5) and *L. elangatus* (10) exhibit a very close relationship (with 0 distance). Both of these have a slightly elongated cell shape with a wavy or straight cell wall pattern in their adaxial leaf surface, and both lack trichomes (glabrous), which is also confirmed by SEM analyses.

Results of qualitative and quantitative data in the PCA (characters are few in the present study, so only two axes were used) and UPGMA are only parallel for some species, and important quantitative data among microtraits is the stomata index of abaxial surface, as important qualitative data is epidermal cell shape and wall pattern of adaxial leaf surface due to light microscope studies of *Lathyrus* species.

In the present study *L. digitatus* (1) and *L. elangatus* (10) show close proximity due to their quantitative data as examined by light microscope (stomata index, ratios), and these species have about 0.50 similarity index based on numeric taxonomy study of morphological characters in the genus *Lathyrus* provided by Doğan et al (1992). Also, *L. armenus* (5) and *L. variabilis* (12) show a close relationship due to their quantitative data (stomata index, ratios), and these species belong to the first karyology group (12m + 2sm) based on karyotype analyses of section *Lathyrostylis*, provided by Gunes (2011). *L. armenus* (5) and *L. variabilis* (12) have the same style type (Cse–style type) based on micromorphological studies of *Lathyrus* (Oskoueiyan, 2011). *L. brachypterus* (14) and *L. boissieri* (6) exhibit close similarities due to their quantitative data (stomata index, ratios), and these species have the same style type (sle- style type), according to Oskoueiyan, 2011.

L. digitatus (1) and *L. cyaneus* var. *cyaneus* (4) are very closely related (overlapped in phenograms) according to their qualitative data, and *L. digitatus* (1) and *L. cyaneus* are supported by 90 percent bootstrap in cladogram base on morphological data of *Lathyrus* species, which is provided by Leht (2009).

The group composed of *L. digitatus* (1), *L. spathulatus* (11) and *L. filiformis, L. pallesence* (7) have been supported by 100 percent bootstrap in molecular phylogeny

(Maximum Likelihood tree), which was conducted by Schaefer et al (2012). Their total relationships have been supported by 80 percent bootstrap in the phylogeny tree, and *L. digitatus* (1) and *L. pallesence* (7) are very close (overlapped in phenograms) according to their qualitative data in the present study.

L. cyaneus var. *cyaneus* (4), *L. karsianus* (2), *L. pallesence* (7) and *L. satdaghensis* (9) are closely related due to their qualitative (overlapped in phenogram) and quantitative data. *L. digitatus* (1), *L. cyaneus* and *L. karsianus* (2), *L. satdaghensis* (9) have about 0.75 similarity index based on numerical taxonomic study of morphological characters in the genus *Lathyrus* provided by Doğan et al (1992) repectively.

As mentioned before, *L. atropatanus* (8) is very similar to *L. nivalis*, which is also already placed in the *Lathyrostylis* section. *L. atropatanus* is newly recorded as a different species, according to Gunes and Cirpici, (2011) and its placement in the phenogram (quantitative data) is altered with a different similarity index. The relationship of *L. atropatanus* (8) to the rest of the species needs further study before any firm systematic decision can be made.

Although micromorphological traits of the leaf surfaces of these species in the *Lathyrostylis* section are investigated for the first time in the present study, this indicates novel data regarding the relationships of these species. However, it is very early to say which grouping is more natural as very few micromorphological characters were studied. Therefore, the taxonomic value of these characters would be more clrealy elucidated by a scanning electron microscope with the consideration of more micromorphological characters and by adding other species.

3.2 Scanning Electron Microscope Analyses

The surface of leaves (as a vegetative organ) and calyx (as reproductive organ) were analyzed in much greater detail by using a scanning electron microscope to provide highly accurate information.

3.2.1 Leaf surface

It seems that all species have wax deposition on their leaf surfaces with a light or heavy density. Epidermal cells have been photographed from non-margin and non-stomatal parts among the *Lathyrus* species examined. All qualitative and quantitative micromorphological character results of both leaf surface (epidermal cell shape, cell size, cell wall pattern, presence of stomata, stomata size, position of stomata, presence of trichome, trichome length and types and presence of granular pattern) using a scanning electron microscope are outlined in **Table 3-4**, **Table 3-5** and **Table 3-6**. Numerical analyses and their visual illustrations of species are represented by numbers assigned to them. Photographs are shown between **Figure 3-24** and **Figure 3-51.**

Table 3-4 Micromorphological traits (qualitative data) in leaf surface among the 28 species examined. (Every species observed between 10 – 15 samples)*

sp/ no	L. sur	ECS	CWP	PS	S.Pos	P.T	T.T	Granular Pattern
1	Adaxial	SE	WS	Present	sunken	Present	III	Absent
	Abaxial	E	ST	Present	sunken	Present	III , II	Absent
2	Adaxial	SE	WS	Present	sunken	Present	III	Absent
	Abaxial	E	ST	Present	sunken	Present	III	Absent
3	Adaxial	SE	WS	Present	sunken	Absent	–	Absent
	Abaxial	E	ST	Present	sunken	Present	III , II	Absent
4	Adaxial	SE	WS	Present	sunken	Present	III	Absent
	Abaxial	E	ST	Present	sunken	Present	III , II	Absent
5	Adaxial	SE	WS	Present	sunken	Absent	–	Absent
	Abaxial	E	ST	Present	sunken	Absent	–	Absent
6	Adaxial	IS/SE	ST	Present	sunken	Present	III	Absent
	Abaxial	E	ST	Present	sunken	Absent	–	Absent
7	Adaxial	SE	WS	Present	sunken	Present	III	Absent
	Abaxial	E	ST	Present	sunken	Present	III , II	Absent
8	Adaxial	SE	WS	Present	sunken	Present	III	Absent
	Abaxial	E	ST	Present	sunken	Present	III	Absent
9	Adaxial	SE	WS	Present	sunken	Present	III	Absent
	Abaxial	E	ST	Present	sunken	Present	III	Absent

Table 3- 4 continued

10	Adaxial	SE	WS	Present	sunken	absent	–	Absent
	Abaxial	E	ST	Present	sunken	absent	–	Present
11	Adaxial	SE	ST	Present	sunken	present	III	Absent
	Abaxial	E	ST	Present	sunken	present	III , II	Absent
12	Adaxial	SE	WS	Present	sunken	present	III	Absent
	Abaxial	E	ST	Present	sunken	present	III	Absent
13	Adaxial	SE	WS	Present	sunken	present	III	Absent
	Abaxial	E	ST	Present	sunken	absent	–	Absent
14	Adaxial	SE	WS	Present	sunken	present	III	Absent
	Abaxial	SE	ST	Present	sunken	present	III	Absent
15	Adaxial	E	ST	Present	sunken	present	III, II	Present
	Abaxial	E	ST	Present	sunken	present	II	Absent
16	Adaxial	IS	DS	Absent	–	present	III , II	Present
	Abaxial	IS	DS	Present	Even	present	III , II	Present
17	Adaxial	IS	DS	Present	Even	present	III , II	Absent
	Abaxial	IS	DS	Absent	–	present	III , II	Present
18	Adaxial	SE	DS	Present	sunken	present	III	Absent
	Abaxial	SE	WS	Present	sunken	absent	–	Absent
19	Adaxial	IS/SE	DS	Absent	–	absent	–	Absent
	Abaxial	IS/SE	DS	Present	Even	present	II	Absent
20	Adaxial	E	DS	Present	sunken	absent	–	Absent
	Abaxial	E	WS	Present	sunken	present	II	Absent
21	Adaxial	IS	DS	Absent	–	absent	–	Absent
	Abaxial	IS	DS	Present	Even	present	III	Absent
22	Adaxial	E	WS	Present	sunken	absent	–	Absent
	Abaxial	E	DS	Present	sunken	absent	–	Absent
23	Adaxial	E	ST	Present	sunken	present	III, II	Absent
	Abaxial	E	ST	Present	sunken	present	III, II	Absent
24	Adaxial	SE	WS	Present	sunken	present	III	Absent
	Abaxial	SE	WS	Present	sunken	present	III, II	Absent
25	Adaxial	E	DS	Present	sunken	present	III,II	Absent
	Abaxial	E	ST	Present	sunken	present	III,II	Absent
26	Adaxial	E	ST	Present	sunken	present	II	Absent
	Abaxial	E	ST	Present	sunken	present	III , II	Absent
27	Adaxial	SE	DS	Present	sunken	absent	–	Absent
	Abaxial	SE	WS	present	sunken	present	II	Absent

Table 3-4 continued

28	Adaxial	SE	DS	Present	sunken	Present	III	Absent
	Abaxial	SE	WS	Present	sunken	Present	III	Absent

ECS; epidermal cell shape, SE; slightly elongated, IS/SE; isodiametric/slightly elongated, IS; isodiametric, E; elongated.

CWP; cell wall pattern, WS; wavy or straight, ST; straight, DS; deeply sinuous

P.S; presence of stomata.

S. Pos; stomata position on epidermis.

P.T; presence of trichome.

T.T; trichome type.

Granular Pattern (presence of granule).

L.digitatus (1),*L.karsianus* (2), *L.tukhtensis* (3), *L.cyaneus* var. *cyaneus* (4), *L.armenus* (5), *L.boissieri* (6),*L.pallesence* (7),*L.atropatanus* (8),*L.satdagensis* (9),*L.elangatus* (10),*L.spathulatus* (11), *L.variabilis* (12), *L.cilicicus* (13), *L.brachypterus* (14), *L.haussknechtii* (15), *L.aureus* (16), *L.vernus* (17), *L.laxiflorus* (18), *L.roseus* (19),*L.annuus* (20), *L.niger* (21), *L.clymenum* (22), *L.sativus* (23), *L.pratensis* (24), *L.cicera* (25), *L.gorgoni* (26), *L.undulatus* (27) , *L.czeczottianus* (28)

Table 3-5 Mean values of Quantitative Adaxial Leaf surface characters (missing data are shown with -1)

Species (numbers)	**Epidermal cell width**	**Epidermal cell length**	**Stomatal width**	**Stomatal length**	**Non gland – length**	**Gland length**
***L.digitatus* (1)**	129.03	18.74	11.87	2.37	152.19	−1
***L.karsianus* (2)**	79.78	23.33	10.41	2.77	52.23	−1
***L.tukhtensis* (3)**	78.75	28.28	12.46	4.6	−1	−1
***L.cyan* var. *cya* (4)**	81.81	20.59	11.83	2.52	68.77	−1
***L.armenus* (5)**	99.21	18.87	17	4.51	−1	−1
***L.boissieri* (6)**	44	18.69	7.52	2.58	102.19	−1
***L.pallesence* (7)**	65.1	16.61	11.51	3.44	106.62	−1
***L.atropatanus*(8)**	63.1	22.47	10.14	4.33	179	−1
***L.satdaghens*(9)**	58.27	20.7	11.16	3	237.46	−1
***L.elangatus*(10)**	78.77	23.93	14.42	5.51	−1	−1
***L.spathulatus*(11)**	69.56	17.93	10.2	2.97	67.08	−1
***L.variabilis*(12)**	71.98	24.24	10.71	3.32	45.72	−1
***L.cilicicus*(13)**	84.25	14.25	15.82	4.1	352.88	−1
***L.brachypterus*(14)**	87.42	26.77	13.07	3.6	122.22	−1
***L.hausknechtii(*15)**	92.04	14.99	12.34	3.81	176.81	84.59
***L.aureus*(16)**	42.06	15.55	−1	−1	283.45	56.77

Table 3-5 continued

L.vernus(17)	52.57	23.57	11.16	2.43	146.25	81.14
L.laxiflorus(18)	95.92	29.97	12.64	3.89	289.97	−1
L.roseus(19)	72.56	36.47	−1	−1	−1	−1
L.annuus(20)	95.21	23.02	10.96	3.91	−1	−1
L.niger(21)	74.31	33.98	−1	−1	−1	−1
L.clymenum(22)	102.69	24.92	14.03	2.25	−1	−1
L.sativus(23)	156.84	23.27	13.85	3.33	80.24	70.213
L.pratensis(24)	67.65	20.12	13.03	3.87	245.86	−1
L.cicera(25)	111.97	23.38	10.57	2.91	204.42	53.83
L.gorgoni(26)	169.8	16.6	9.83	1.81	−1	56.18
L.undulatus(27)	77.38	28.49	11.11	1.76	−1	−1
L.czeczottianus(28)	114.59	22.43	12.11	3.32	514.07	−1

Table 3-6 Mean values of Quantitative Abaxial Leaf surface characters (missing data are shown with -1)

Species (numbers)	Epidermal cell width	Epidermal cell length	Stomatal width	Stomatal length	Non gland – length	Gland length
L.digitatus(1)	95.08	14.56	12.21	3.38	91.39	71.13
L.karsianus(2)	95.81	16.06	12.69	3.37	77.34	–
L.tukhtensis(3)	139.71	23.02	16.2	3.99	185.89	64.68
L.cyan var. *cya*(4)	101.93	19.14	14.73	3.96	97.92	45.05
L.armenus(5)	70.72	23.52	16.61	4.67	–	–
L.boissieri(6)	55.37	12.28	9.78	1.95	–	–
L.pallesence(7)	118.48	15.75	15.36	3.17	111.26	55.73
L.atropatanus(8)	79.35	16.69	11.7	2.78	174.58	–
L.satdaghens(9)	120.46	15.92	15.24	2.94	204.29	–
L.elangatus(10)	114.22	15.44	16.67	4.47	–	–
L.spathulatus(11)	97.89	15.92	13.22	2.02	185.99	73.22
L.variabilis(12)	97.38	21.06	12.51	2.89	111.13	–
L.cilicicus(13)	84.43	12.29	13.31	3.45	–	–
L.brachypteru(14)	88.65	22.66	12.48	3.03	193.15	–
L.hausknechti(15)	75.11	22.73	13	3.04	–	70.77
L.aureus(16)	75.93	22.36	16.59	4.96	560.44	60.53
L.vernus(17)	59.08	20.27	–	–	149.82	50.3
L.laxiflorus(18)	109.83	33.51	13.17	4.24	–	–

Table 3- 6 continued

***L.roseus*(19)**	91.91	42.55	16.07	2.94	–	108.76
***L.annuus*(20)**	128.42	20.35	14.5	3.37	–	55.63
***L.niger*(21)**	72.22	21.75	13.49	2.49	350.96	–
***L.clymenum*(22)**	125.42	25.92	13.27	3.66	–	–
***L.sativus*(23)**	108.13	17.04	11.85	2.9	–	58.78
***L.pratensis*(24)**	81.04	18.87	12.77	3.74	437.92	48.92
***L.cicera* (25)**	144.09	18.44	12.77	2.65	417.17	50.13
***L.gorgoni*(26)**	167.35	20.64	11.77	3.07	431.82	67.67
***L.undulatus*(27)**	144.95	28.22	15.96	2.97	–	70.21
***L.czeczottian*(28)**	73.15	22.25	8.95	2.64	457.66	–

3.2.1.1 Micromorphological properties of the Adaxial surface

3.2.1.1.1 Epidermal Cells

There are four different kinds of epidermal cell shapes in the adaxial surface as shown in **Table 3-4**. The most common epidermal cell shape is slightly elongated as seen in *L. digitatus* and *L. laxiflorus.* Some species have elongated as demonstrated in *L. annuus, L. sativus* and others have an isodiametric slightly elongated shape like *L. boissieri* or *L. roseus.* A few species have an isodiametric cell shape like *L. vernus* in their adaxial leaf surface (photographs are given between Figure 3-24 and Figure 3-51). There are three different kinds of cell wall patterns in the adaxial leaf surface as given in **Table 3-4**. The most common cell wall pattern is a wavy or straight cell wall pattern. Some species have a deeply sinuous wall pattern, and other species have a straight wall pattern. All species of sect. *Lathyrostylis* exhibit a slightly elongated epidermal cell shape with wavy or straight cell wall pattern in their adaxial leaf surface except *L. boissieri* with its isodiametric slightly elongated and straight wall pattern. Also, *L. spathulatus* has a slightly elongated epidermal cell shape with straight wall pattern. *L. haussknechtii* demonstrates an elongated epidermal cell shape with a straight wall pattern in its adaxial leaf surface.

All species of sect. *Orobus* (*L. aureus, L. vernus* and *L. niger*) have an isodiametric epidermal cell shape with a deeply sinuous cell wall pattern in their adaxial leaf surface. *L. laxiflorus* and *L. czeczottianus* both belong to sect. *Pratensis* and have a slightly elongated epidermal cell shape with a deeply sinuous cell wall pattern in their adaxial leaf surface. *L. roseus* exhibits different cell shapes from the rest of species. It demonstrates an isodiametric slightly elongated cell shape with a deeply sinuous cell wall pattern in its adaxial leaf surface. This species belong to sect. *Orobon.* All species of sect. *Lathyrus* (*L. annuus, L. sativus, L. cicera, L. gorgoni*) exhibit an elongated epidermal cell shape except *L. undulatus* with slightly elongated cell shapes. Their cell wall patterns are the mostly deeply sinuous. *L. clymenum* (sect. *clymenum*) has an elongated epidermal cell shape with wavy or straight wall pattern in its adaxial leaf surface.

In conclusion, a slightly elongated epidermal cell shape with a wavy or straight wall pattern is most common in adaxial leaf surface of the *Lathyrus* species examined.

Epidermal cell width ranges between 169.8 µm in *L. gorgoni* and 42.06 µm in L. *aureus* and epidermal cell length ranges from 36.47 µm in *L. roseus* to 14.25 µm in *L. cilicicus* as shown in **Table 3-5**.

3.2.1.1.2 Stomata

Except for *L. aureus, L. niger (*sect. *Orobus)* and *L. roseus (*sect. *Orobon),* all other species have stomata in their adaxial leaf surface as shown in **Table 3-4**.

Stomatal width ranges between 7.52 µm in *L. boissieri* to 7 µm in *L. armenus.* Stomatal length ranges between 1.76 µm in *L. undulatus* to 5.51 µm in *L. elongatus* as shown in **Table 3-5**.

Stomata position in all 28 *Lathyrus* species is sunken except *L. vernus* which is at the level of epidermis (even) or a little raised as shown in **Table 3-4**. This species belongs to sect. *Orobus.*

3.2.1.1.3 Trichomes

Except for *L. tukhtensis, L. armenus, L. elangatus* (sect. *Lathyrostylis*), *L. roseus* (*sect. Orobon), L. niger (*sect. *Orobus), L. clymenum (*sect. *Clymenum), L. annuus* and *L. undulatus* (sect. *Lathyrus*), other species have trichomes on their adaxial leaf surface. Trichomes are found in two types in adaxial leaf surface: glandular and non–glandular (photographs are given between **Figure 3-52** and **Figure 3-57**). The presence of a non- glandular trichome is a common condition in adaxial leaf surface among the species examined. Some species such as, *L. haussknechtii, L. aureus, L. vernus, L. sativus* and *L. cicera* have both types of trichomes. Non- glandular trichomes are a typical bicellular type accompanied by an elongated terminal (tapering gradually towards the apex). Glandular hairs are of capitate type, which is unicellular containing stalk, neck cell, and cutinized secretory head. The length of the stalk is less than, more than or equal to the length of the head. Non glandular trichomes have been found to contribute to heightened resistance to pests (Levin, 1973), but glandular trichomes may act through secretion of secondary metabolites as defensive, repellent, or toxic compounds.

In addition, the density of trichomes in the adaxial leaf surface varies among the species examined. For example, *L. atropatanus, L. czeczottianus, L. satdaghensis* and *L. aureus* are densely covered in trichomes. Trichome density is the result of genetic differences according to Stenglein et al., 2005, and other studies but it changes with age so was not studied in the present study. For this reason fresh material will provide more accurate and appropriate results.

L. czeczottianus with a length of 514.07 µm has the longest and *L. variabilis* with a length of 45.72 µm the shortest non-glandular trichome in the adaxial leaf surface. *L. haussknechtii* with a length of 84.59 µm has the longest and *L. cicera* with a length of 53. 83 µm has the shortest glandular trichome in the adaxial leaf surface. Glandular trichomes are generally shorter than non -glandular trichomes.

3.2.1.1.4 Granular pattern

Among the species examined, only *L. haussknechtii* (**Figure 3-60***),* and *L. aureus (***Figure 3-58**) have a granular pattern on their adaxial leaf surfaces. Some species such as, *L. cyaneus* var. *cyaneus, L. boissieri, L. pallesence, L. variabilis, L. cilicicus* (sect. *Lathyrostylis*) and *L. undulatus* (sect. *Lathyrus*) have granules which appears only at their adaxial leaf margins. As was explained by Moraes et al (2011), Vieria and Gomes, (1995) stated that the presence of a granular pattern had been observed in the species of the Rubiaceae family (*Psychotria*) to separate the species. Also, its type is genetically determined, but its density and distribution on the epidermis surfaces may be influenced by environment (Boyer, 1982).

3.2.1.2 Micromorphological properties of the Abaxial surfaces

3.2.1.2.1 Epidermal Cells

There are four different kinds of epidermal cell shapes in the abaxial leaf surface as shown in **Table 3-4**. The most common epidermal cell shape is elongated as seen in *L. tukhtensis*, and *L. sativus.* Some species have slightly elongated cell shapes as in *L. brachypterus* or *L. undulatus.* Others like *L. aureus* or *L. vernus* demonstrate an isodiametric epidermal cell shape. Only one species (*L. roseus*) has an isodiametric slightly elongated epidermal cell shape in its abaxial leaf surface. There are three different kinds of cell wall patterns in the abaxial leaf surface as shown in **Table 3-4**. The most common cell wall pattern is straight. Some species have a wavy or straight wall pattern, while others have a deeply sinuous cell wall pattern. All species of sect. *Lathyrostylis* have an elongated epidermal cell shape with a straight wall pattern except *L. brachypterus* which has a slightly elongated cell shape and straight wall pattern. Species of sect. *Orobus* (*L. aureus, L. vernus* and *L. niger*) have an isodiametric epidermal cell shape and deeply sinuous wall pattern. *L. laxiflorus, L. pratensis* and *L. czeczottianus* have a slightly elongated epidermal cell shape with wavy or straight wall pattern in their abaxial leaf surfaces. These belong to sect.

*Pratensis. L. roseus (*sect. *Orobon)* have an isodiametric epidermal cell shape with a slightly elongated wall pattern in its abaxial leaf surface. All species of sect. *Lathyrus* have a mostly elongated epidermal cell shape with straight wall pattern except *L. undulatus* with its slightly elongated cell shape and wavy or straight wall pattern and *L. annuus* with an elongated epidermal cell shape and wavy or straight wall pattern. *L. clymenum (*sect. *clymenum)* has an elongated epidermal cell shape and deeply sinuous wall pattern in its abaxial leaf surface.

Therefore, an elongated epidermal cell shape with a straight wall pattern is most common in the abaxial leaf surfaces of the *Lathyrus* species examined. Photographs are provided between **Figure 3-24** and **Figure 3-51.**

Epidermal cell width ranges from 167.35 µm in *L. gorgoni* to 55.37 µm in *L. boissieri* and epidermal cell length ranges between 42.55 µm in *L. roseus* and 12.28 µm, 12.29 µm in *L. boissieri* and *L. cilicicus* as shown in **Table 3-6**. Therefore, *L. gorgoni* has the longest width of epidermal cells on both leaf surface among species examined, and *L. roseus* has the longest length on both leaf surfaces.

3.2.1.2.2 Stomata

All species exhibit stomata in their abaxial leaf surface as given in **Table 3-4**. Stomatal width ranges between 8.95 µm in *L. czeczottianus* to 16.67 µm in *L. elongatus*. Stomatal length ranges between 1.95 µm in *L. boissieri* to 4.96 µm in *L. aureus* as shown in **Table 3-6**.

Stomata position in all *Lathyrus* species is sunken except in *L. aureus, L.niger* (sect. *Orobus)* and *L. roseus* (sect. Orobon), which is at the level of the epidermis (even) or a bit raised as shown in **Table 3-4**.

3.2.1.2.3 Trichome

Except for *L. armenus, L. boissieri, L. elangatus, L. cilicicus* (*sect. Lathyrostylis), L. laxiflorus* (*sect. pratensis),* and *L. clymenum* (sect. clymenum), other species have trichomes on their abaxial leaf surface. Trichomes consist of two types in abaxial leaf

surface: glandular and non-glandular. The presence of both types of trichome is a common condition in abaxial leaf surface among the species examined. Some species have only non-glandular trichomes, such as *L. karsianus, L. atropotanus, L. satdaghensis, L. variabilis, L. brachypterus, L. niger* and *L. czeczottianus*. Also, the density of trichomes in the abaxial leaf surface among the species examined can vary. For example, *L. brachypterus, L. roseus* and *L. sativus* have very few trichomes.

L. aureus with a length of 560.44 µm has the longest non-glandular trichome in the abaxial leaf surface, and *L. karsianus* with a length of 77.34 µm has the shortest. *L. roseus* with a length of 108.76 µm has the longest and *L. cyaneus* var. *cyaneus* with a length of 45.05 µm has the shortest glandular trichome in the abaxial leaf surface. Similarly, adaxial surface glandular trichomes are generally shorter than non-glandular trichomes.

3.2.1.2.4 Granular pattern

Among examined species, only *L. elongatus* (**Figure 3-59**), *L. aureus*, and *L. vernus* exhibit a granular pattern on their abaxial leaf surface. Also, some species, such as *L. digitatus, L. tukhtensis, L. cyaneus* var. *cyaneus, L. pallesence, L. variabilis*, and *L. gorgoni* have granules only in their abaxial leaf margin.

Micromorphological study of the *Lathyrus* species of *Lathyrostylis, Orobus, Orobon, Clymenum, Lathyrus* and *Pratensis* sections using SEM exhibit adaxial leaf surface have more variable microtraits than abaxial leaf surface. Adaxial surface mostly exhibit a slightly elongated epidermal cell shape whereas abaxial surface demonstrates an elongated cell shape. Isodiametric epidermal cell shape and deeply sinuous wall pattern is specific to sect. *Orobus*. Adaxial leaf surface of *L. boissieri* (sect. *Lathyrostylis*) and both surfaces of *L. roseus* (sect. *Orobon*) have the same epidermal cell shape as isodiametric slightly elongated. Most of species have sunken stomata except species of sect. *Orobus* and *L. roseus.* Also, in the abaxial surface of

L. elongatus as well as in the adaxial surface of *L. haussknechtii*, and both surfaces of *L. aureus* and *L. vernus* have a granular pattern in their epidermal surfaces. The remainder of species lack granules.

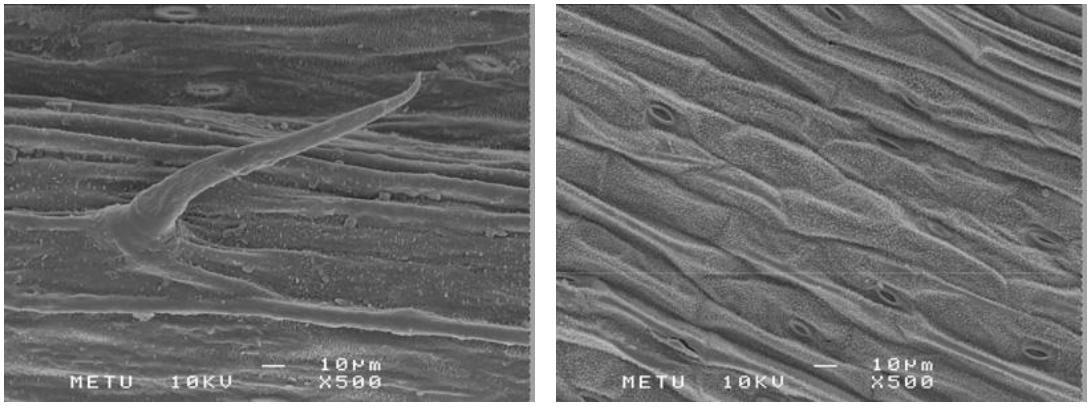

Figure 3-24 Leaf surface of *L. digitatus* showing slightly elongated cell shape with wavy and straight wall in adaxial (left) and elongated cell shape with straight wall in abaxial (right).

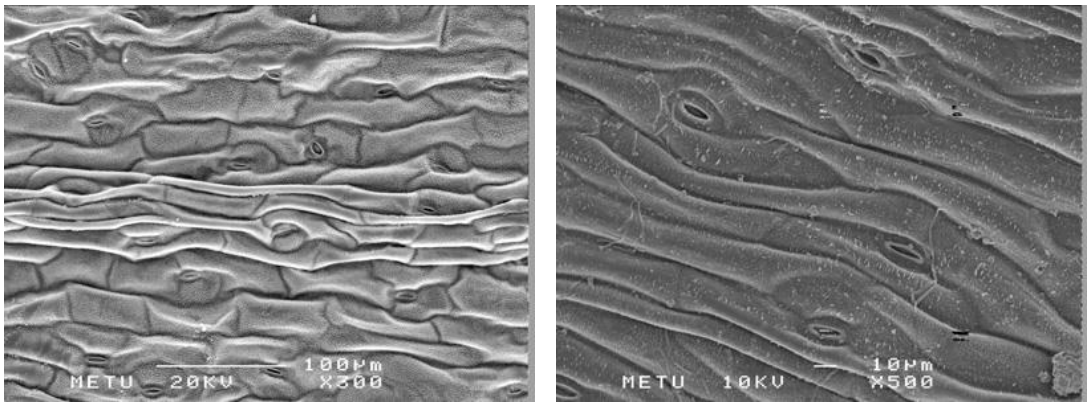

Figure 3-25 Leaf surface of *L. karsianus* showing slightly elongated cell shape with wavy and straight wall in adaxial (left) and elongated cell shape with straight wall in abaxial (right).

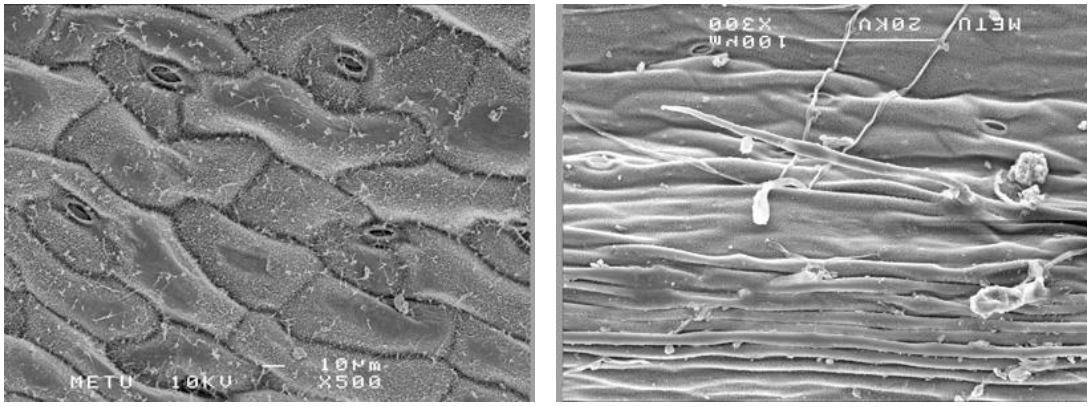

Figure 3-26 Leaf surface of *L. tukhtensis* showing slightly elongated cell shape with wavy and straight wall in adaxial (left) and elongated cell shape with straight wall in abaxial (right).

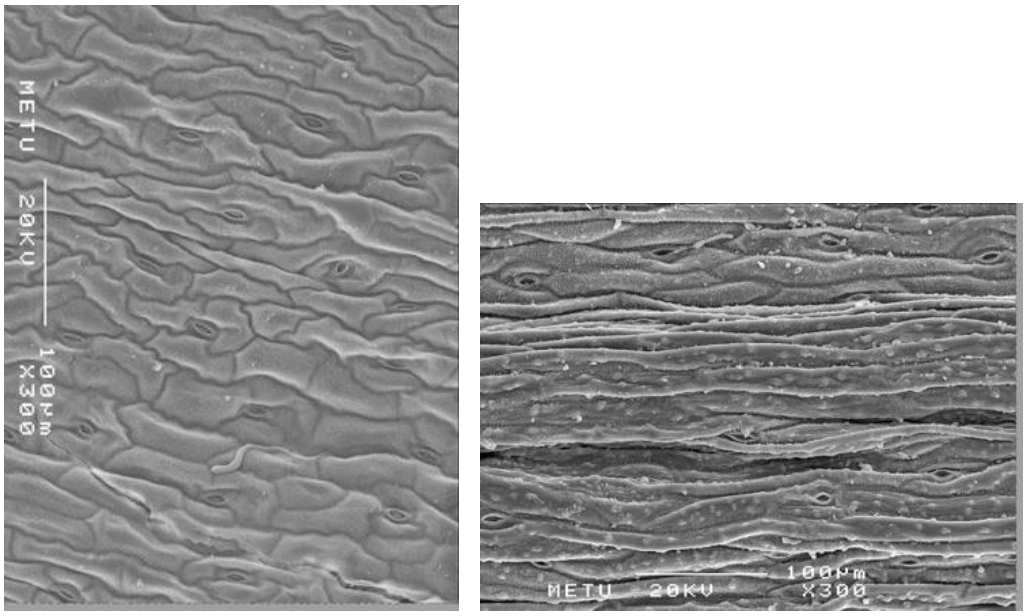

Figure 3-27 Leaf surface of *L. cyaneus* var. *cyaneus* showing slightly elongated cell shape with wavy and straight wall in adaxial (left) and elongated cell shape with straight wall in abaxial (right).

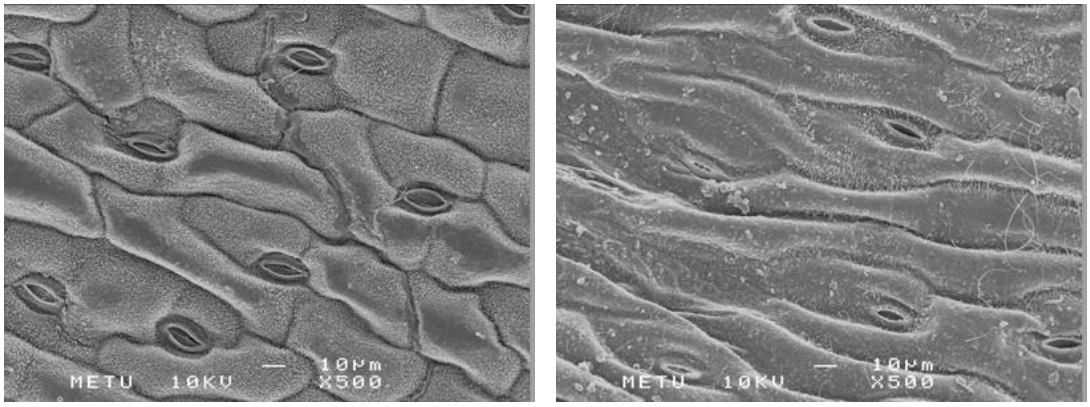

Figure 3-28 Leaf surface of *L. armenus* showing slightly elongated cell shape with wavy and straight wall in adaxial (left) and elongated cell shape with straight wall in abaxial (right).

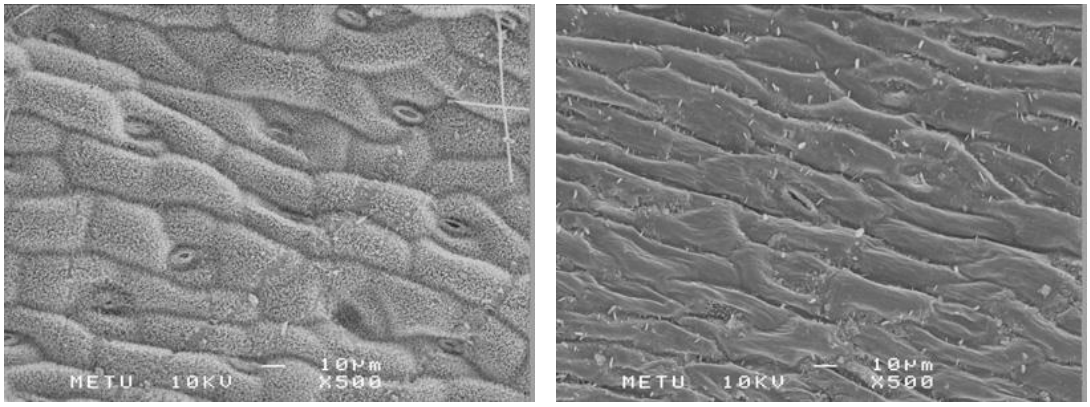

Figure 3-29 Leaf surface of *L. boissieri* showing isodiametric slightly elongated cell shape with straight wall in adaxial (left) and elongated cell shape with straight wall in abaxial (right).

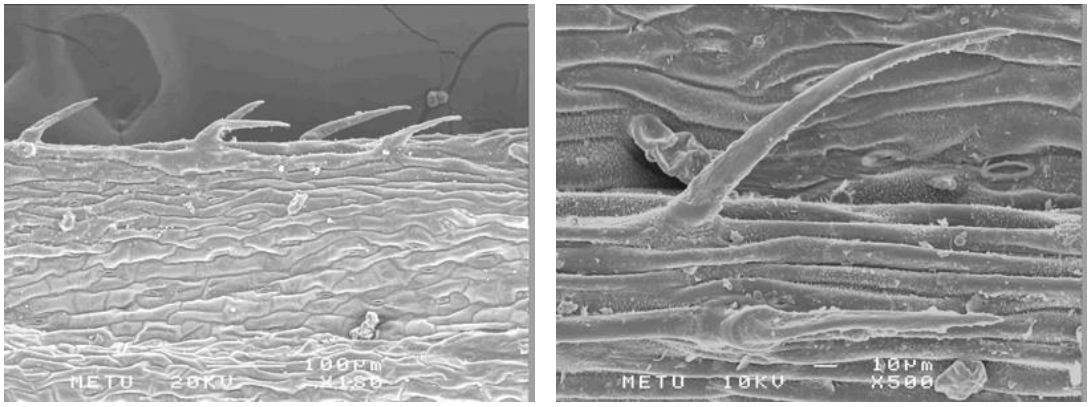

Figure 3-30 Leaf surface of *L. pallesence* showing slightly elongated cell shape with wavy and straight wall in adaxial (left) and elongated cell shape with straight wall in abaxial (right).

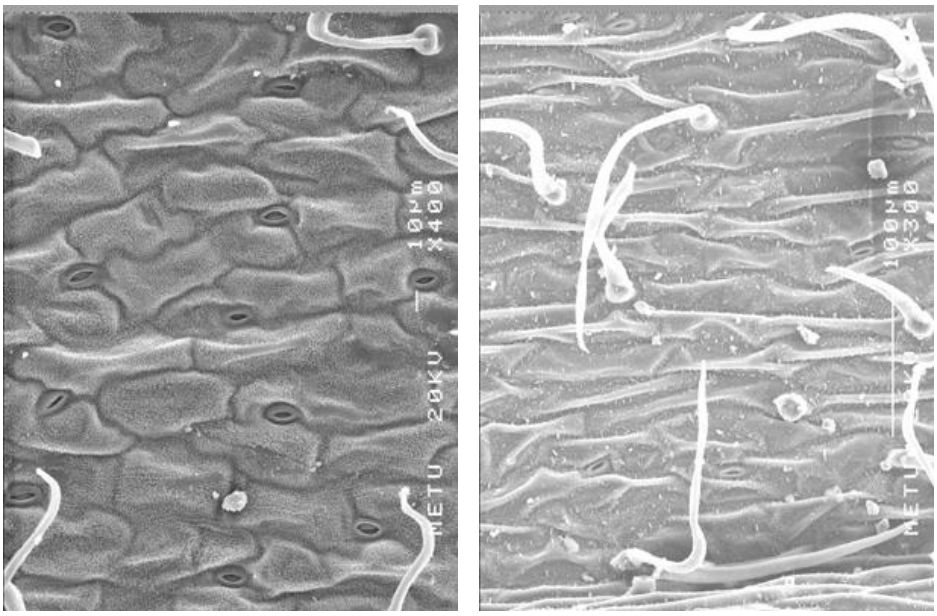

Figure 3-31 Leaf surface of *L. atropatanus* showing slightly elongated cell shape with wavy and straight wall in adaxial (left) and elongated cell shape with straight wall in abaxial (right).

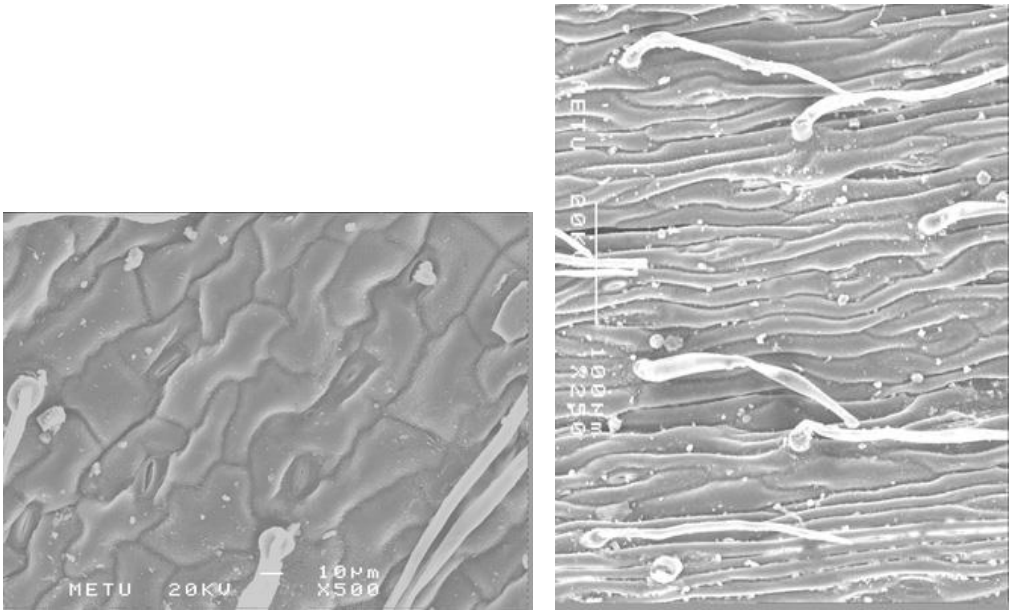

Figure 3-32 Leaf surface of *L. satdaghensis* showing slightly elongated cell shape with wavy and straight wall in adaxial (left) and elongated cell shape with straight wall in abaxial (right).

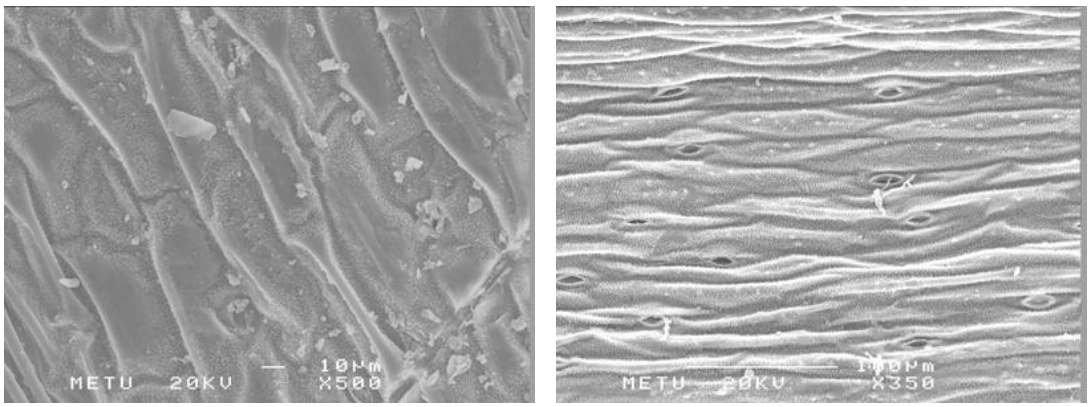

Figure 3-33 Leaf surface of *L. elangatus* showing slightly elongated cell shape with wavy and straight wall in adaxial (left) and elongated cell shape with straight wall in abaxial (right).

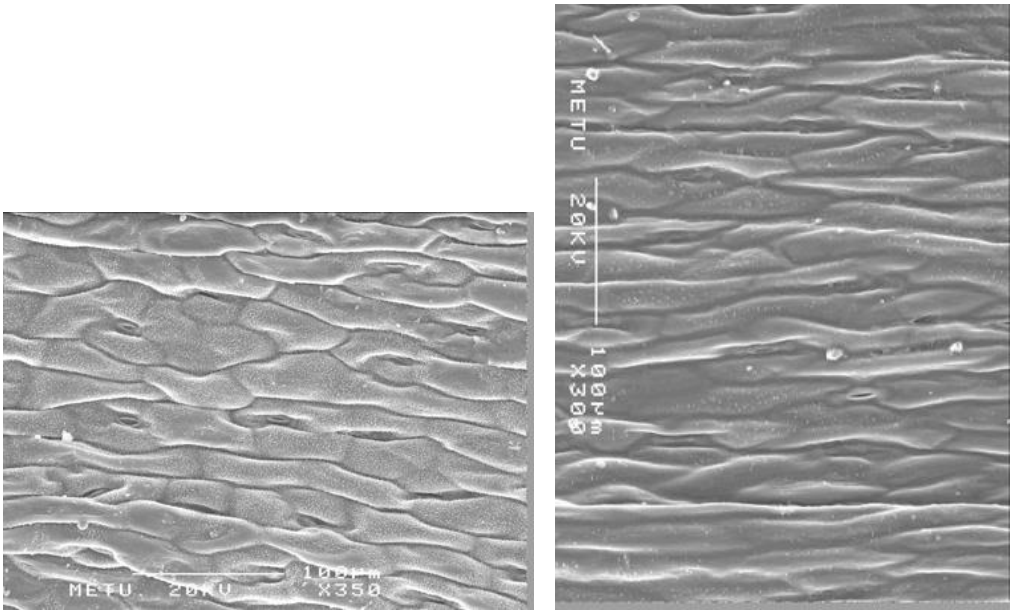

Figure 3-34 Leaf surface of *L. spathulatus* showing slightly elongated cell shape with straight wall in adaxial (left) and elongated cell shape with straight wall in abaxial (right).

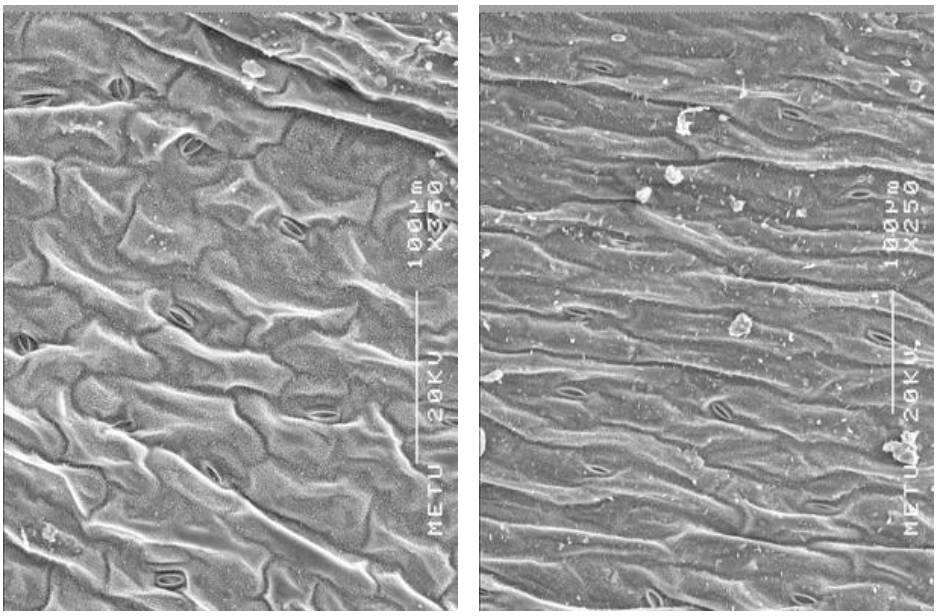

Figure 3-35 Leaf surface of *L. variabilis* showing slightly elongated cell shape with wavy and straight wall in adaxial (left) and elongated cell shape with straight wall in abaxial (right).

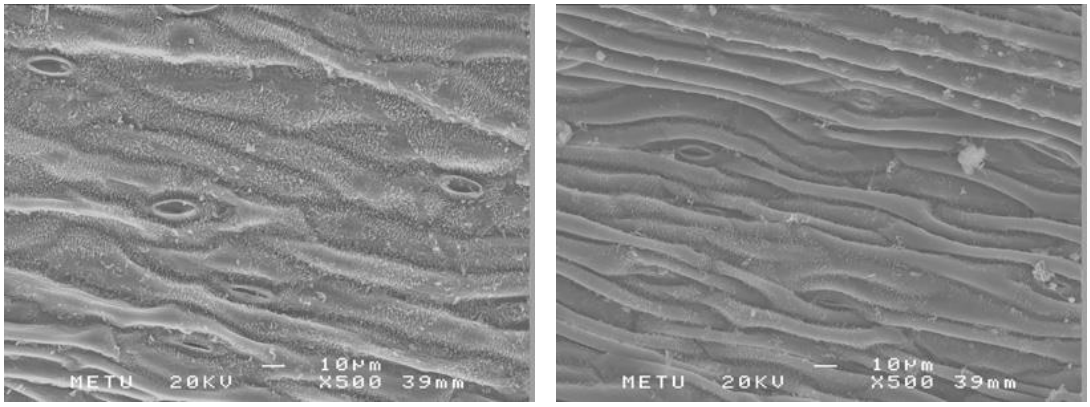

Figure 3-36 Leaf surface of *L. cilicicus* showing slightly elongated cell shape with wavy and straight wall in adaxial (left) and elongated cell shape with straight wall in abaxial (right).

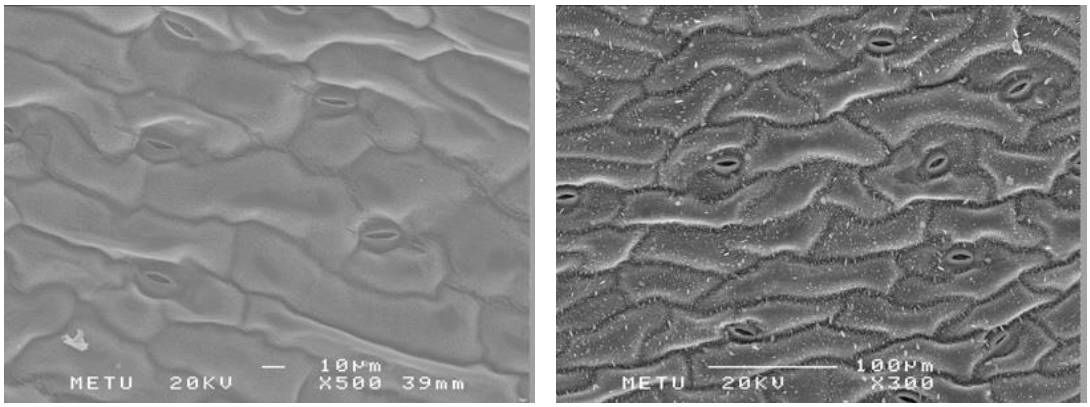

Figure 3-37 Leaf surface of *L. brachyptherus* showing slightly elongated cell shape with wavy and straight wall in adaxial (left) and slightly elongated cell shape with straight wall in abaxial (right).

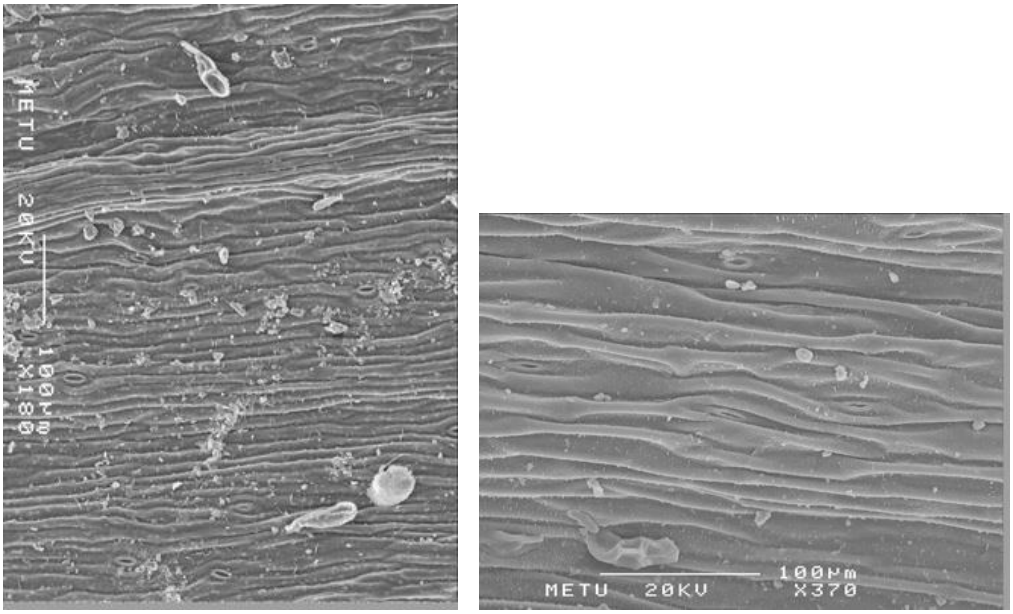

Figure 3-38 Leaf surface of *L. haussknechtii* showing elongated cell shape with straight wall in adaxial (left) and abaxial (right).

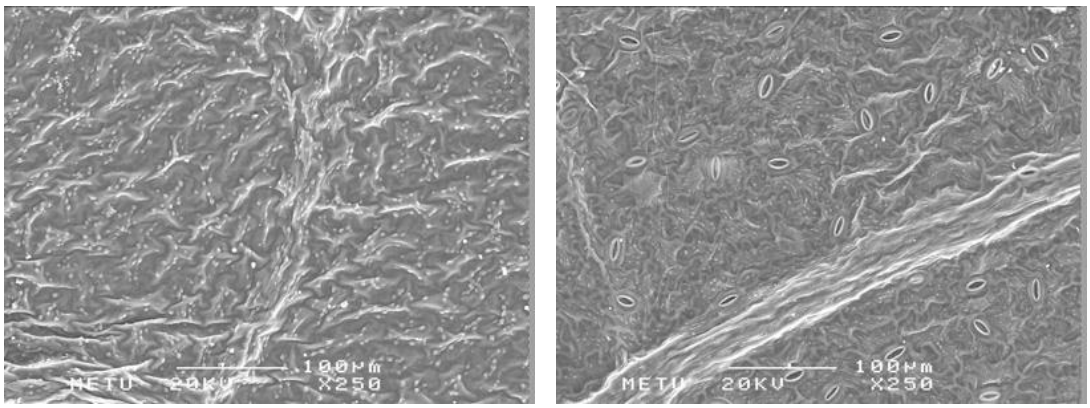

Figure 3-39 Leaf surface of *L. aureus* showing isodiametric cell shape with deeply sinuous wall in adaxial (left) and abaxial (right).

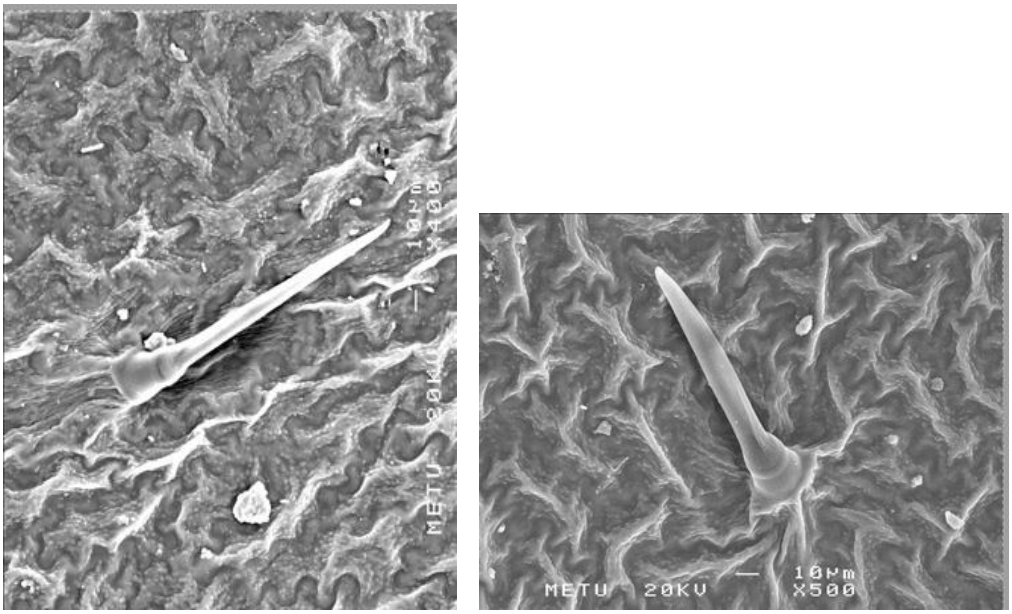

Figure 3-40 Leaf surface of *L. vernus* showing isodiametric cell shape with deeply sinuous wall in adaxial (left) and abaxial (right).

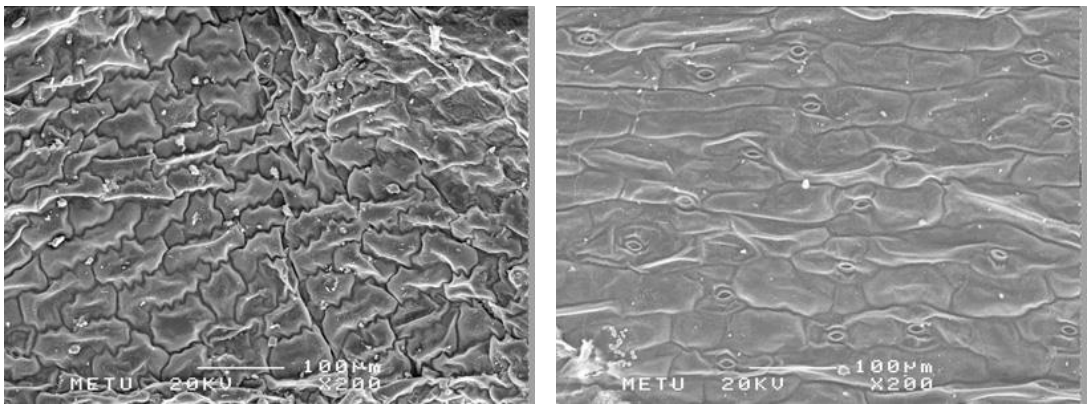

Figure 3-41 Leaf surface of *L. laxiflorus* showing slightly elongated cell shape with deeply sinuous wall in adaxial (left) and wavy and straight wall in abaxial (right).

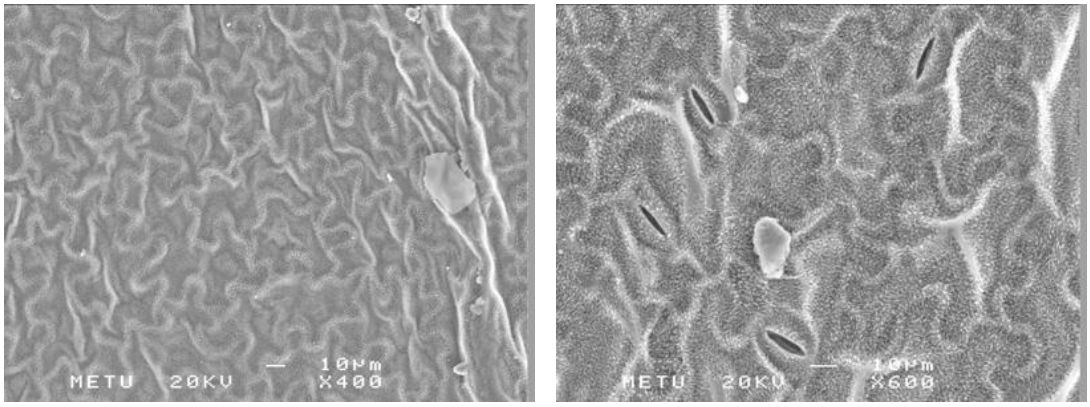

Figure 3-42 Leaf surface of *L. roseus* showing isodiametric slightly elongated cell shape with deeply sinuous wall in adaxial (left) and abaxial (right).

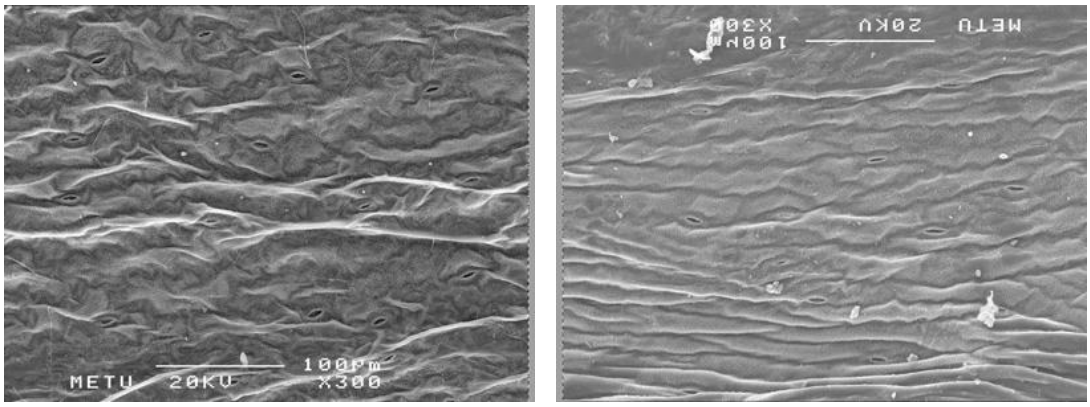

Figure 3-43 Leaf surface of *L. annuus* showing elongated cell shape with deeply sinuous wall in adaxial (left) and wavy ad straight wall in abaxial (right).

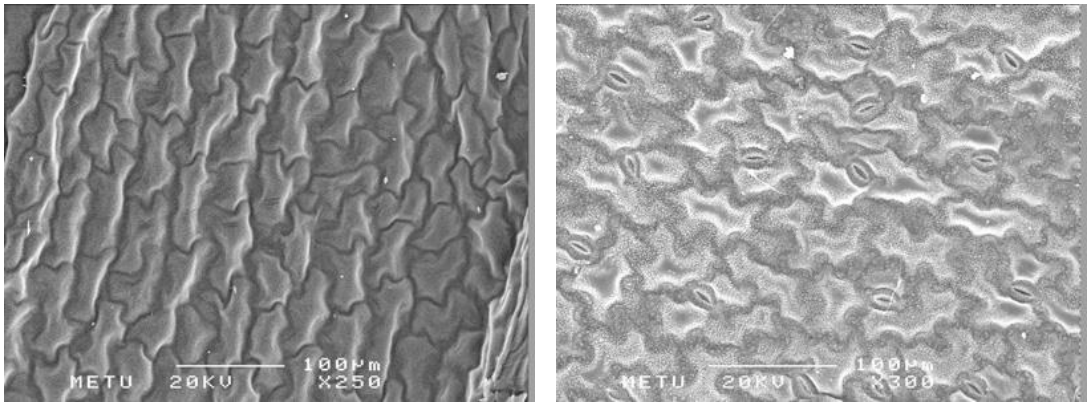

Figure 3-44 Leaf surface of *L. niger* showing isodiametric cell shape with deeply sinuous wall in adaxial (left) and abaxial (right).

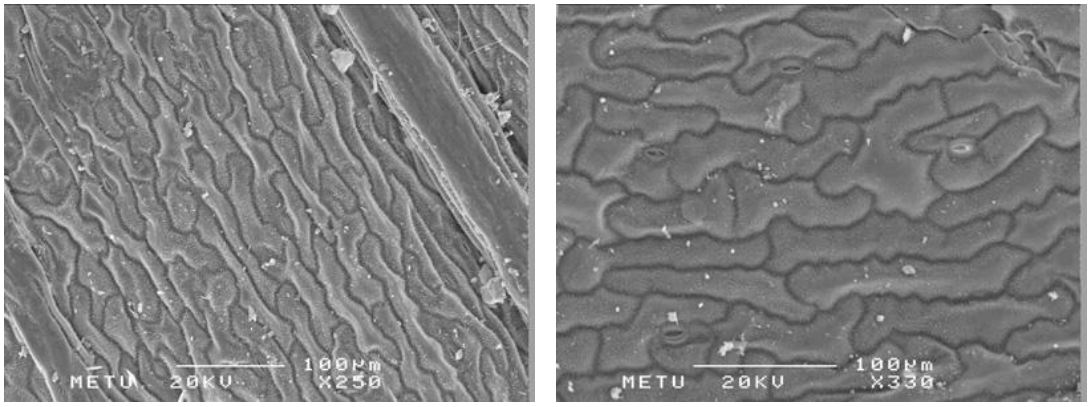

Figure 3-45 Leaf surface of *L. clymenum* showing elongated cell shape with wavy and straight wall in adaxial (left) and deeply sinuous in abaxial (right).

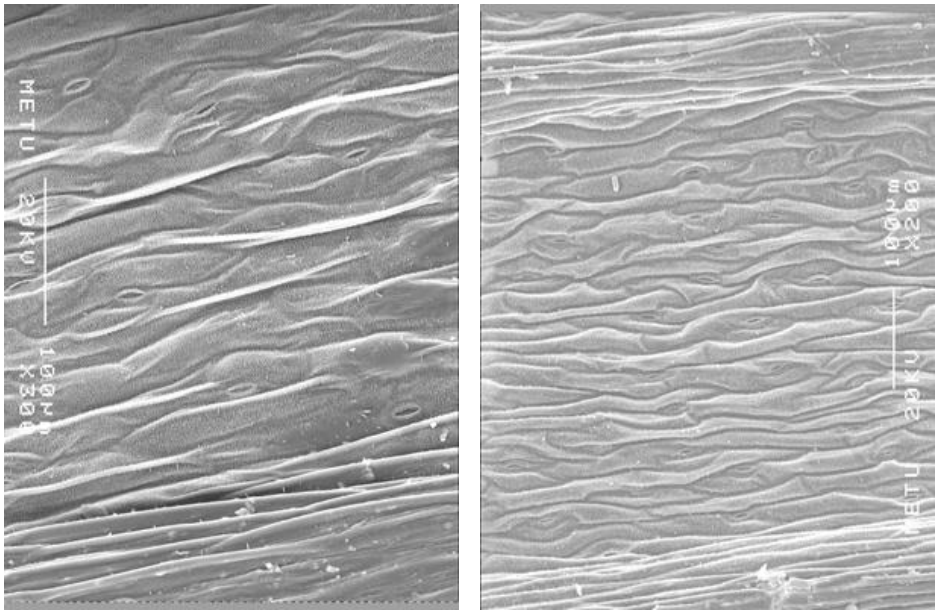

Figure 3-46 Leaf surface of *L. sativus* showing elongated cell shape with straight wall in adaxial (left) and abaxial (right).

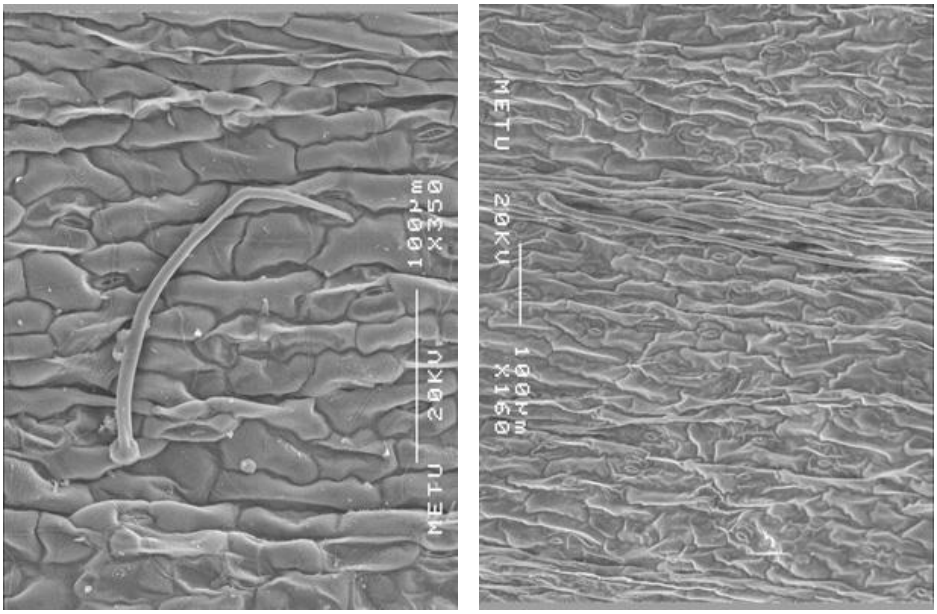

Figure 3-47 Leaf surface of *L. pratensis* showing slightly elongated cell shape with wavy and straight wall in adaxial (left) and abaxial (right).

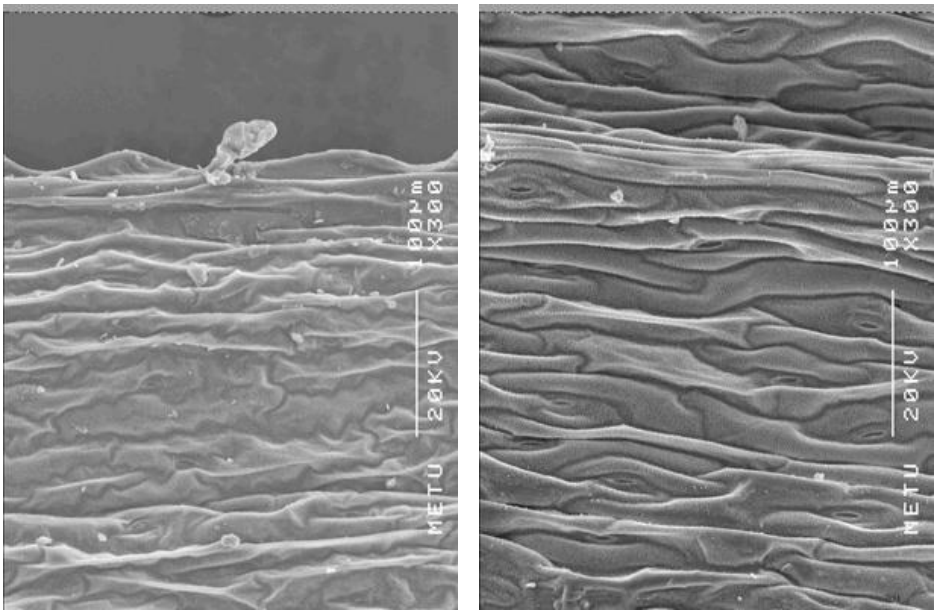

Figure 3-48 Leaf surface of *L. cicera* showing elongated cell shape with deeply sinuous wall in adaxial (left) and straight wall in abaxial (right).

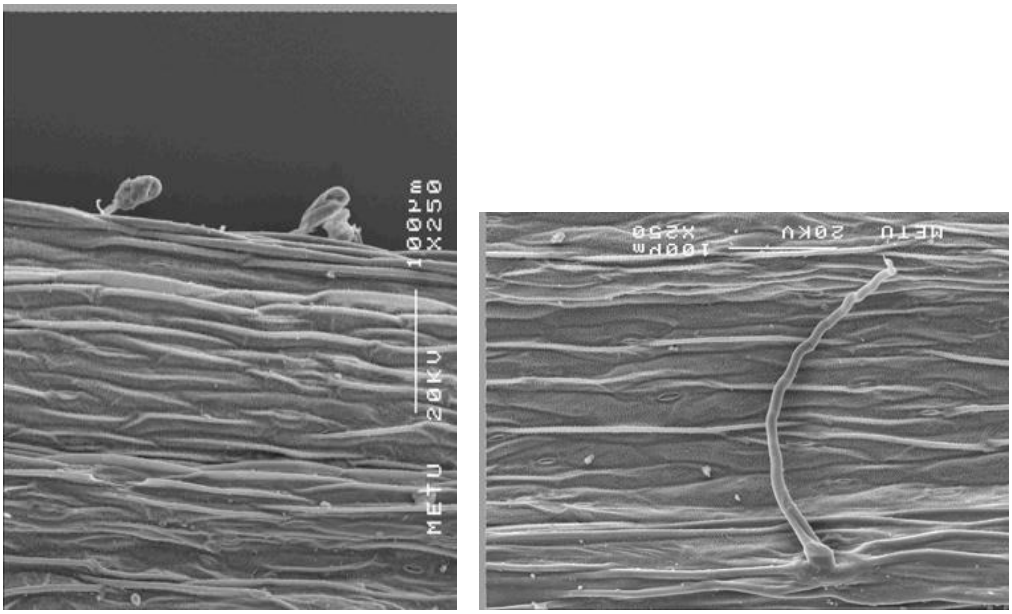

Figure 3-49 Leaf surface of *L. gorgoni* showing elongated cell shape with straight wall in adaxial (left) and abaxial (right).

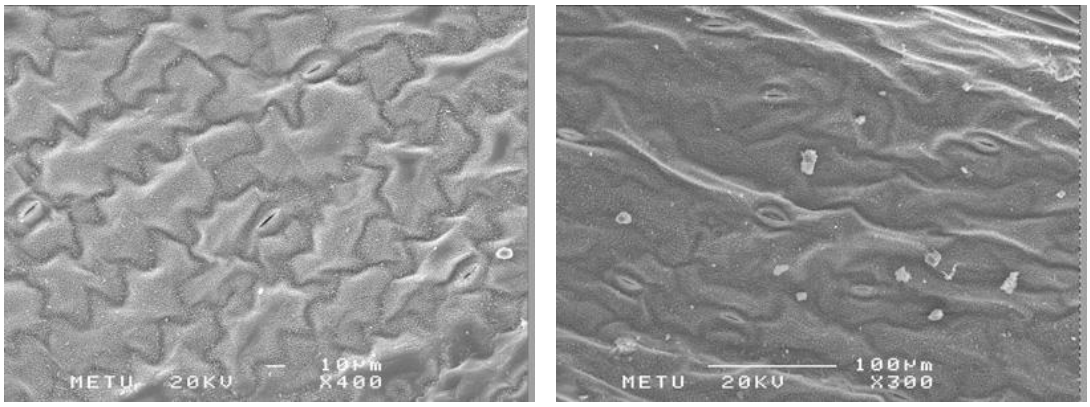

Figure 3-50 Leaf surface of *L. undulatus* showing slightly elongated cell shape with deeply sinuous wall in adaxial (left) and wavy and straight wall in abaxial (right).

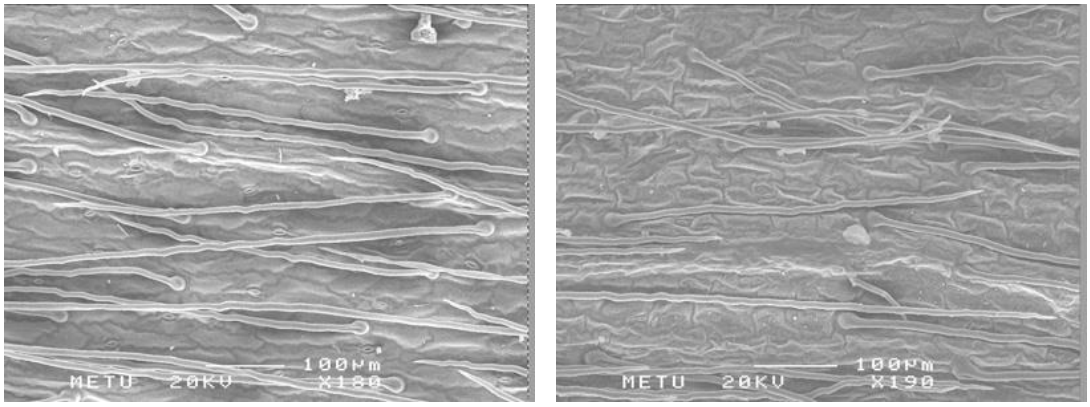

Figure 3-51 Leaf surface of *L. czeczottianus* showing slightly elongated cell shape with deeply sinuous wall in adaxial (left) and wavy and straight wall in abaxial (right).

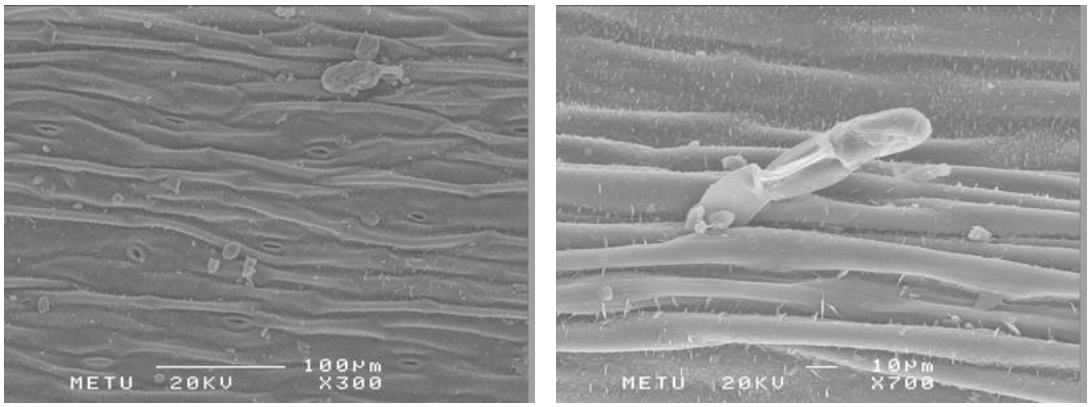

Figure 3-52 Leaf surface of *L. haussknechtii* showing glandular trichome (left) in adaxial surface and (right) in abaxial surface.

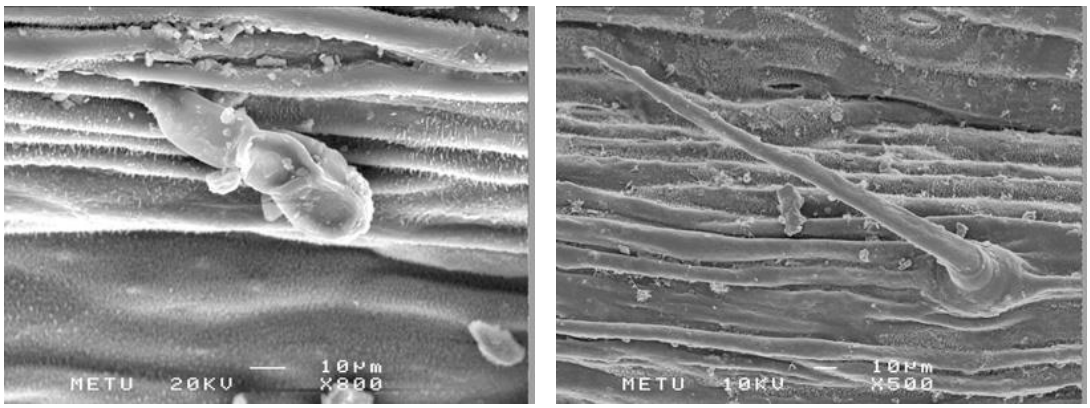

Figure 3-53 Leaf surface of *L. tukhtensis* showing glandular trichome (left) in abaxial surface and typical non glandular trichome (right) in abaxial surface.

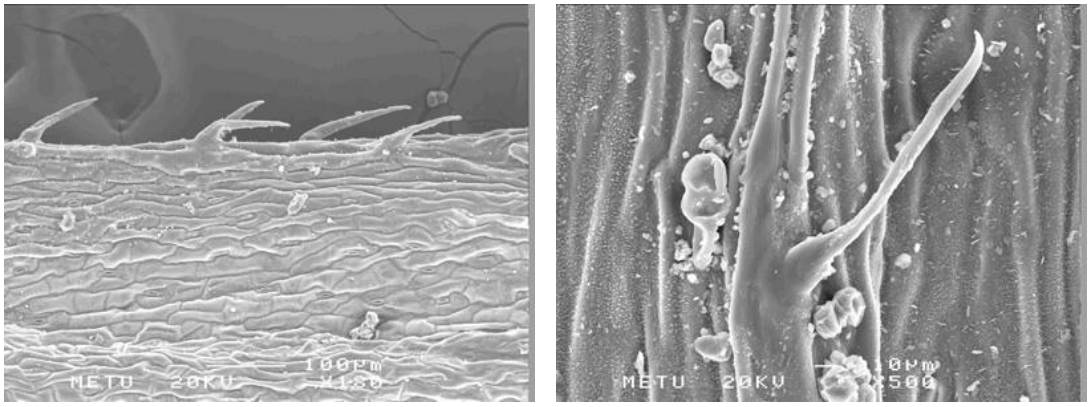

Figure 3-54 Leaf surface of *L. pallesence* showing non glandular trichome (left) in adaxial surface and typical non glandular and glandular trichome (right) in abaxial surface.

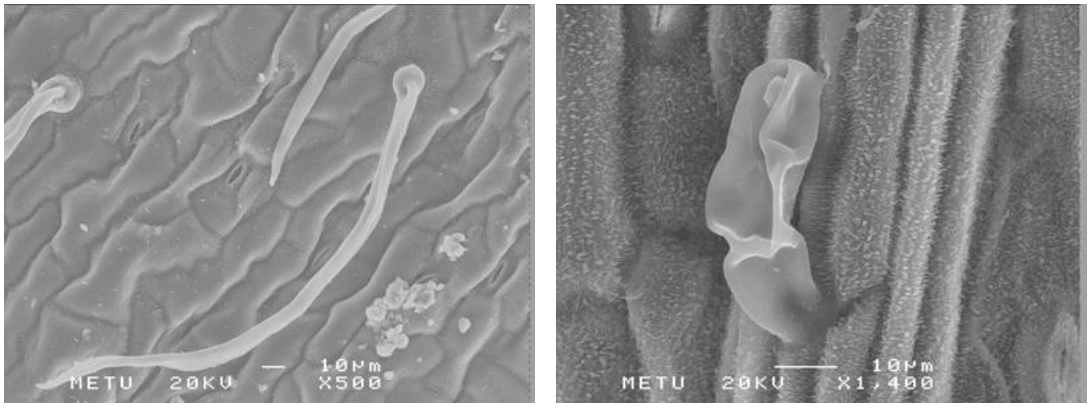

Figure 3-55 Leaf surface of *L. satdaghensis* showing non glandular trichome (left) in adaxial surface and glandular trichome (right) in abaxial surface.

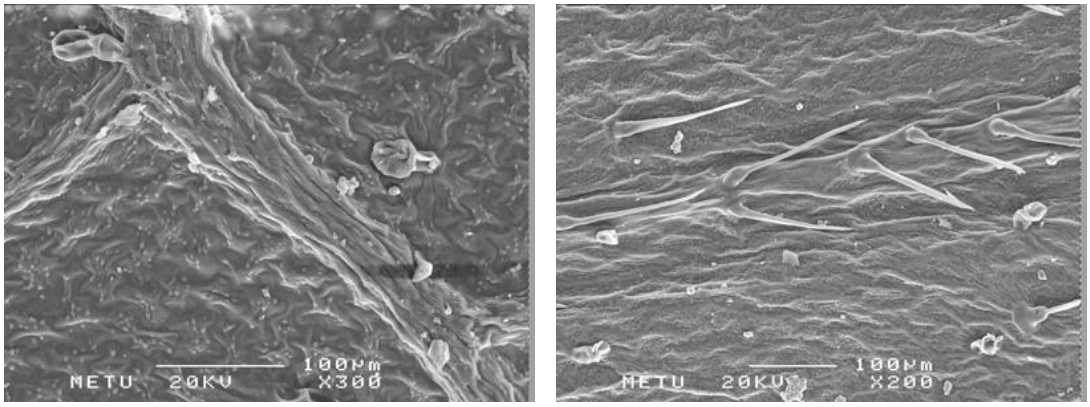

Figure 3-56 Leaf surface of *L. aureus* showing glandular trichome (left) in adaxial surface and non-glandular trichome and glandular trichome (right) in abaxial surface of *L. vernus.*

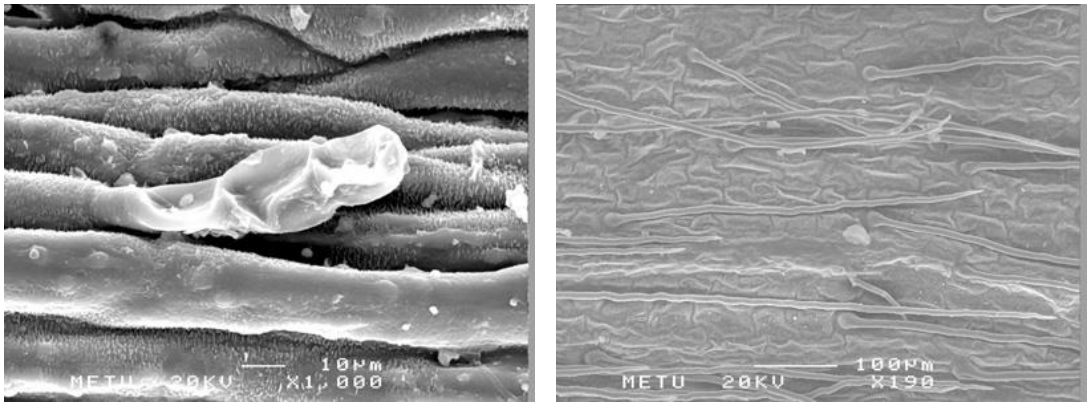

Figure 3-57 Leaf surface of *L. digitatus* showing glandular trichome (left) in abaxial surface and typical non glandular trichome (right) in abaxial surface of *L. czeczottianus*.

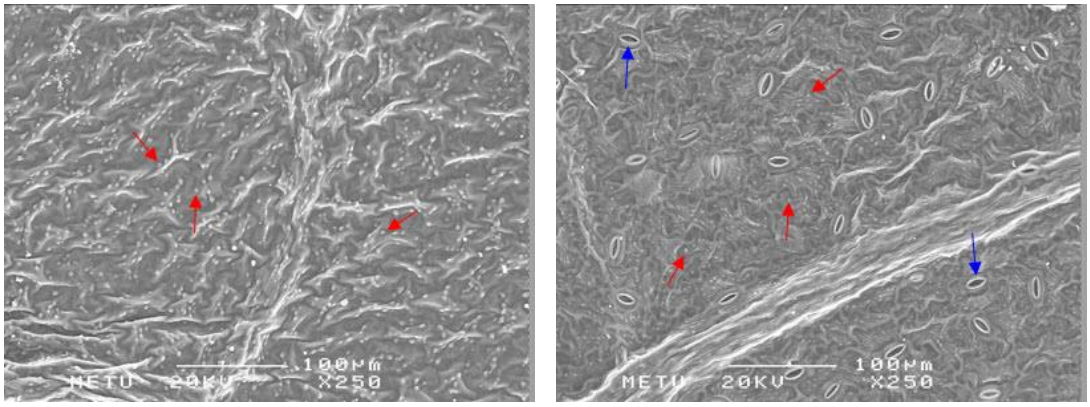

Figure 3-58. Leaf surface of *L. aureus* showing granular pattern in adaxial (left) and abaxial (right)
Red arrows show granular pattern and blue arrows show stomata

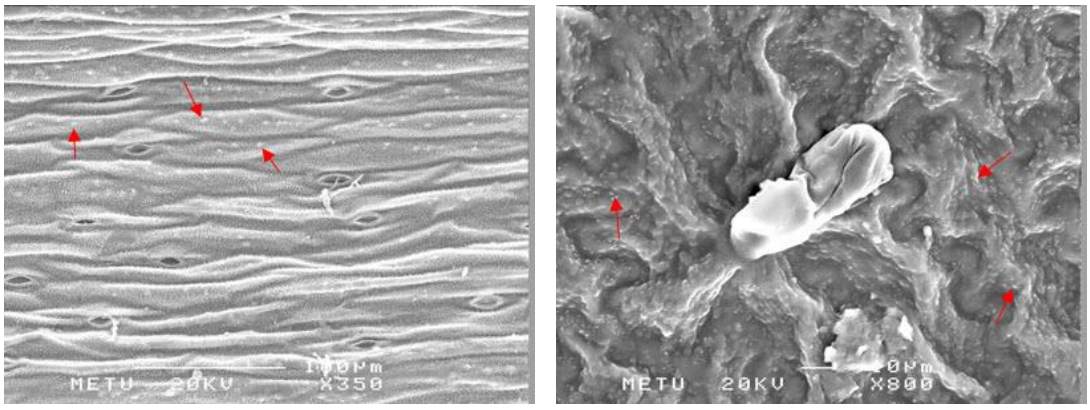

Figure 3-59. Abaxial surface of *L. elangatus* showing granular pattern (left) and *L. vernus* (right)
Red arrows show granular pattern.

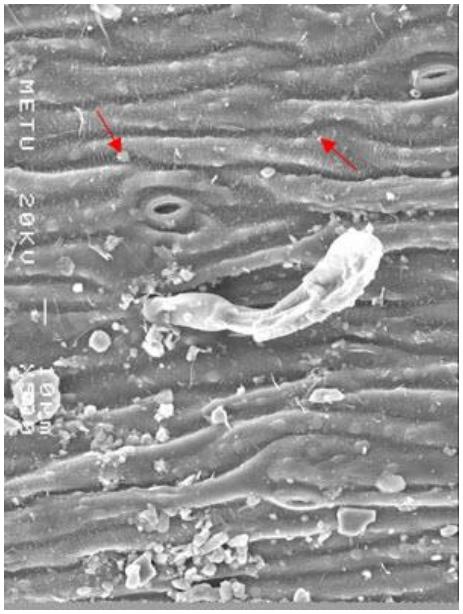

Figure 3-60. Adaxial leaf surface of *L. haussknechtii* showing granular pattern
Red arrows show granular pattern.

3.2.1.3 Ordination analyses and Phenetic Cluster results for Micromorphological traits

Observed (qualitative) characters of both leaf surfaces have been coded as 0, 1 or 2 and scored into numerical value as data matrix in order to obtain PCA, Seriation (part of the ordination analyses) and UPGMA (as clustering analyses) in the PAST 3.05 software program as shown in **Table 3-7**. PCoA results are very similar to the PCA result; therefore, PCOA results are not given.

Table 3-7 Data Matrix of micromorphological traits (qualitative) of leaf among the 28 species of *Lathyrus.*

sp/no	Ecs /ada	Ecs /aba	Cwp /ada	Cwp /aba	Ps /ada	Ps /aba	S.Pos /ada	S.Pos /aba	P.T /ada	P.T/ aba	T.T /ada	T.T/ aba	G.pat /ada	G.pat /aba
1	0	1	0	1	1	1	1	1	1	1	0	1	0	0
2	0	1	0	1	1	1	1	1	1	1	0	0	0	0

Table3-7 continued

3	0	1	0	1	1	1	1	1	0	1	–	1	0	0
4	0	1	0	1	1	1	1	1	1	1	0	1	0	0
5	0	1	0	1	1	1	1	1	0	0	–	–	0	0
6	2	1	1	1	1	1	1	1	1	0	0	–	0	0
7	0	1	0	1	1	1	1	1	1	1	0	1	0	0
8	0	1	0	1	1	1	1	1	1	1	0	0	0	0
9	0	1	0	1	1	1	1	1	1	1	0	0	0	0
10	0	1	0	1	1	1	1	1	0	0	–	–	0	1
11	0	1	1	1	1	1	1	1	1	1	0	1	0	0
12	0	1	0	1	1	1	1	1	1	1	0	0	0	0
13	0	1	0	1	1	1	1	1	1	0	0	–	0	0
14	0	0	0	1	1	1	1	1	1	1	0	0	0	0
15	1	1	1	1	1	1	1	1	1	1	1	2	1	0
16	3	3	2	2	0	1	–	0	1	1	1	1	1	1
17	3	3	2	2	1	0	0	–	1	1	1	1	0	1
18	0	0	2	0	1	1	1	1	1	0	0	–	0	0
19	2	2	2	2	0	1	–	0	0	1	–	2	0	0
20	1	1	2	0	1	1	1	1	0	1	–	2	0	0
21	3	3	2	2	0	1	–	0	0	1	–	0	0	0
22	1	1	0	2	1	1	1	1	0	0	–	–	0	0
23	1	1	1	1	1	1	1	1	1	1	1	1	0	0
24	0	0	0	0	1	1	1	1	1	1	0	1	0	0
25	1	1	2	1	1	1	1	1	1	1	1	1	0	0
26	1	1	1	1	1	1	1	1	1	1	2	1	0	0
27	0	0	2	0	1	1	1	1	0	1	–	2	0	0
28	0	0	2	0	1	1	1	1	1	1	0	0	0	0

Epidermal cell shape of adaxial surface ; Ecs / ada , Epidermal cell shape of abaxial surface ; Ecs/ aba , Cell wall pattern of adaxial surface ; Cwp/ ada , Cell wall pattern of abaxial surface ; Cwp/ aba , Presence of stomata of adaxial surface ; Ps / ada , Presence of stomata of abaxial surface ; Ps / aba , Stomata Position of adaxial surface ;S. Pos / ada , Stomata Position of abaxial surface ; S. Pos / aba , Presence of Trichome of adaxial surface ; PT/ ada , Presence of Trichome of abaxial surface ; PT/ aba , Trichome Type of adaxial surface ; TT/ ada , Trichome Type of abaxial surface ; TT/ aba , Granular Pattern of adaxial surface ; G. Pat / ada ; Granular Pattern of abaxial surface ; G.Pat / aba .

L.digitatus (1), *L.karsianus* (2), *L.tukhtensis* (3), *L.cyaneus* var.*cyaneus* (4), *L.armenus* (5), *L.boissieri* (6), *L.pallesence* (7), *L.atropatanus* (8), *L.satdagensis* (9), *L.elangatus* (10), *L.spathulatus* (11), *L.variabilis* (12), *L.cilicicus* (13*), L.brachypterus* (14), *L.haussknechtii* (15), *L.aureus* (16), *L.vernus* (17), *L.laxiflorus* (18), *L.roseus* (19), *L.annuus* (20 *), L.niger* (21), *L. clymenum* (22), *L.sativus* (23), *L.pratensis* (24), *L.cicera* (25), *L.gorgoni* (26), *L.undulatus* (27), *L.czeczottianus* (28)

Eigenvalues and their corresponding % of total variance accounted in the PCA (with variance – covariance matrix) is given in Table 3-8.

Table 3-8 Eigenvalues and % variance of each component for qualitative data in PCA.

Principal Component (PC)	Eigenvalue	% variance
1	**2.7645**	**47.252**
2	**1.20709**	**20.632**
3	**0.767963**	**13.126**
4	**0.572794**	**9.7905**
5	**0.137958**	**2.358**
6	**0.123411**	**2.1094**
7	**0.0853109**	**1.4582**
8	**0.0635747**	**1.0866**
9	**0.0532567**	**0.91029**
10	**0.0321774**	**0.54999**
11	**0.0270152**	**0.46176**
12	**0.0154814**	**0.26462**
13	**0.0105884**	**0.18098**
14	**0.0380262**	**0.64996**

The first of four variance have the highest percentage (% 47.25, % 20.63, % 13. 12 and % 9.79) of total variance.

Scatter plot (X axis is PC1, Y axis is PC2) in the PCA is shown in **Figure 3-61**. The loading plot is shown in **Figure 3-62** . In the loading plot, A is epidermal cell shape of adaxial, B is epidermal cell shape of abaxial, C is cell wall pattern of adaxial, D is cell wall pattern of abaxial, E is presence of stomata of adaxial, F is presence of stomata of abaxial, G is stomata position of adaxial, H is stomata position of abaxial, I is presence of trichome of adaxial, J is presence of trichome of abaxial, K is trichome type of adaxial, L is trichome type of abaxial, M is granular pattern of adaxial, and N is granular pattern of abaxial.

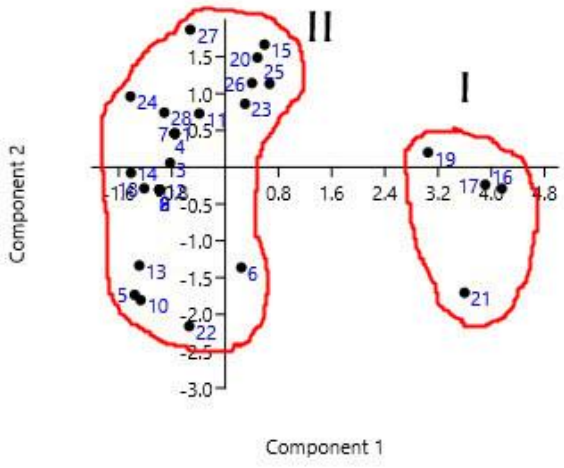

Figure 3-61. A two dimensional scatter plot constructed in the PCA.

Group I covers *: L. aureus* (16), *L. vernus* (17), *L. roseus* (19), *L. niger* (21) Group II covers*: L.digitatus* (1), *L.karsianus* (2), *L.tukhtensis* (3), *L.cyaneus* var. *cyaneus* (4) , *L.armenus* (5), *L.boissieri* (6), *L.pallesence* (7), *L.atropatanus* (8), *L.satdagensis* (9), *L.elangatus* (10),*L.spathulatus* (11),*L.variabilis* (12),*L.cilicicus* (13*), L.brachypterus* (14),*L.haussknechtii* (15), *L.laxiflorus* (18), *L.annuus* (20*), L.clymenum* (22), *L.sativus* (23), *L.pratensis* (24), *L.cicera* (25), *L.gorgoni* (26), *L.undulatus* (27) , *L.czeczottianus* (28)

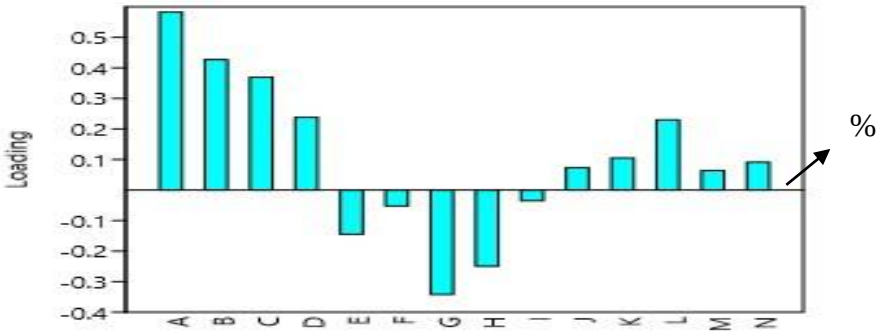

Figure 3-62 Loading plot of qualitative data constructed in the PCA.

A :is epidermal cell shape of adaxial, B: is epidermal cell of abaxial, C: is cell wall pattern of adaxial, D: is cell wall pattern of abaxial, E: is presence of stomata of adaxial , F: is presence of stomata of abaxial , G: is stomoata position of adaxial, H: is stomata position of abaxial , I: is presence of trichome of adaxial , J: is presence of trichome of abaxial , K: is trichome type of adaxial, L: is trichome type of abaxial, M: is granular pattern of

adaxial, N: is granular pattern of abaxial . Percent of the total variation that is accounted by the first axis (%47.25) is shown by the arrow.

There are two groups in the PCA. *L. aureus* (16), *L. vernus* (17), *L. roseus* (19) and *L. niger* (21) are in one group (I) and the remainder of species appear in the second group (II). In the first group *L. aureus* (16), *L. vernus* (17), and *L. niger* (21) belong to section *Orobus,* and *L. roseus* (19) belongs to section *Orobon.*

In the second group, *L. cicera* (25), *L. haussknechtii* (15), *L. annuus* (20), *L. undulatus* (27), *L. gorgoni* (26), and *L. sativus* (23) demonstrate a closer relationship to each other than to the others. All of these belong to section *Lathyrus* except *L. haussknechtii* (15), which belongs to the *Lathyrostylis* section. Also, in the second group *L. armenus* (5) and *L. elongatus* (10) are closely related. *L. cyaneus* var. *cyaneus* (4), *L. digitatus* (1), *L. pallesence* (7), and *L. satdaghensis* (9), *L. karsianus* (2), *L. atropotanus* (8) and *L. variabilis* (12) respectively belong to section *Lathyrostylis* and are overlapped. All of these species belong to section *Lathyrostylis.*

According to the loading plot of data, epidermal cell shape in the adaxial surface (A), epidermal cell shape in the abaxial surface (B), cell wall pattern in the adaxial surface (C), cell wall pattern in the abaxial surface (D), stomata position of the adaxial surface (G), stomata position of the abaxial surface (H) and trichome type in the abaxial surface (L) play the most important role in comparison to others. Loadings are only given to the highest total variation of the first axis.

The phenogram tree constructed in the UPGMA cluster analyses employing Euclidean (Cophen corr, 0. 93) (**Figure 3-63**). At the same time, bootstrap value was obtained for 1000 replicates to evaluate branch support and estimate phylogenetic relations. The phenon line at 3.6 constructs two groups or phenon in the UPGMA tree and results in phenograms which are almost identical in the PCA. *L. aureus* (16), *L. vernus* (17), *L. roseus* (19), and *L. niger* (21*)* are in one phenon, and the remainder of the species are in the second phenon.

The seriation result of qualitative data in the leaf surface is given in **Figure 3-64.** This result also corresponds with UPGMA trees in describing relationships and

closeness among the 28 *Lathyrus* species examined in this study. Species are shown as rows, and characters are shown as columns. The figure illustrates the similar (or dissimilar) qualitative leaf characters (14) between the chosen species.

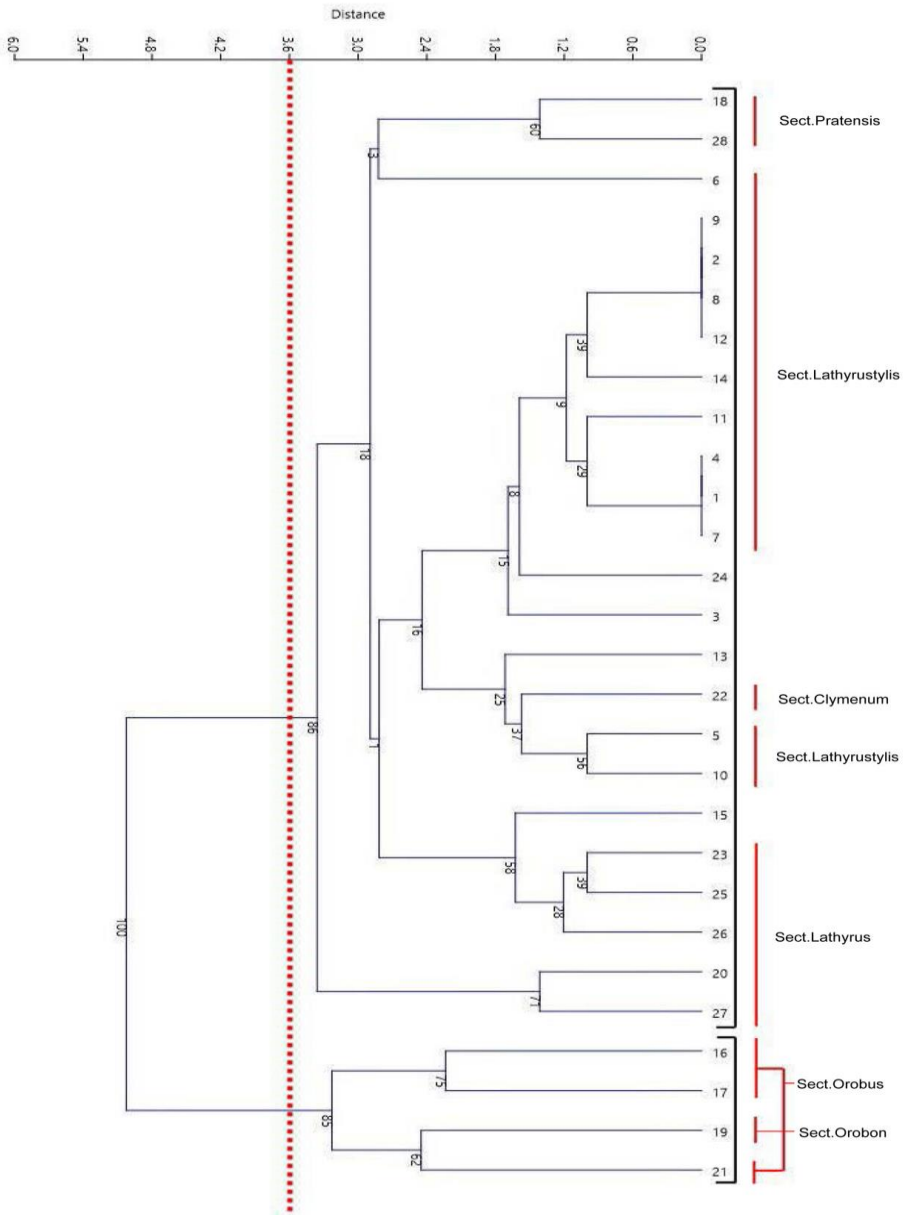

Figure 3-63 UPGMA based on qualitative data with Euclidean similarity index.
Red dash line represents phenon line (at 3.6) and solid vertical red lines represent sections.

L.digitatus (1), *L.karsianus* (2), *L.tukhtensis* (3), *L.cyaneus* var. *cyaneus* (4), *L.armenus* (5), *L.boissieri* (6), *L.pallesence* (7), *L.atropatanus* (8), *L.satdagensis* (9), *L.elangatus* (10), *L.spathulatus* (11), *L.variabilis* (12), *L.cilicicus* (13), *L.brachypterus* (14), *L.haussknechtii* (15), *L.aureus* (16), *L.vernus* (17), *L.laxiflorus* (18),

L.roseus (19), *L.annuus* (20), *L.niger* (21), *L.clymenum* (22), *L.sativus* (23), *L.pratensis* (24), *L.cicera* (25), *L.gorgoni* (26), *L.undulatus* (27) , *L.czeczottianus* (28)

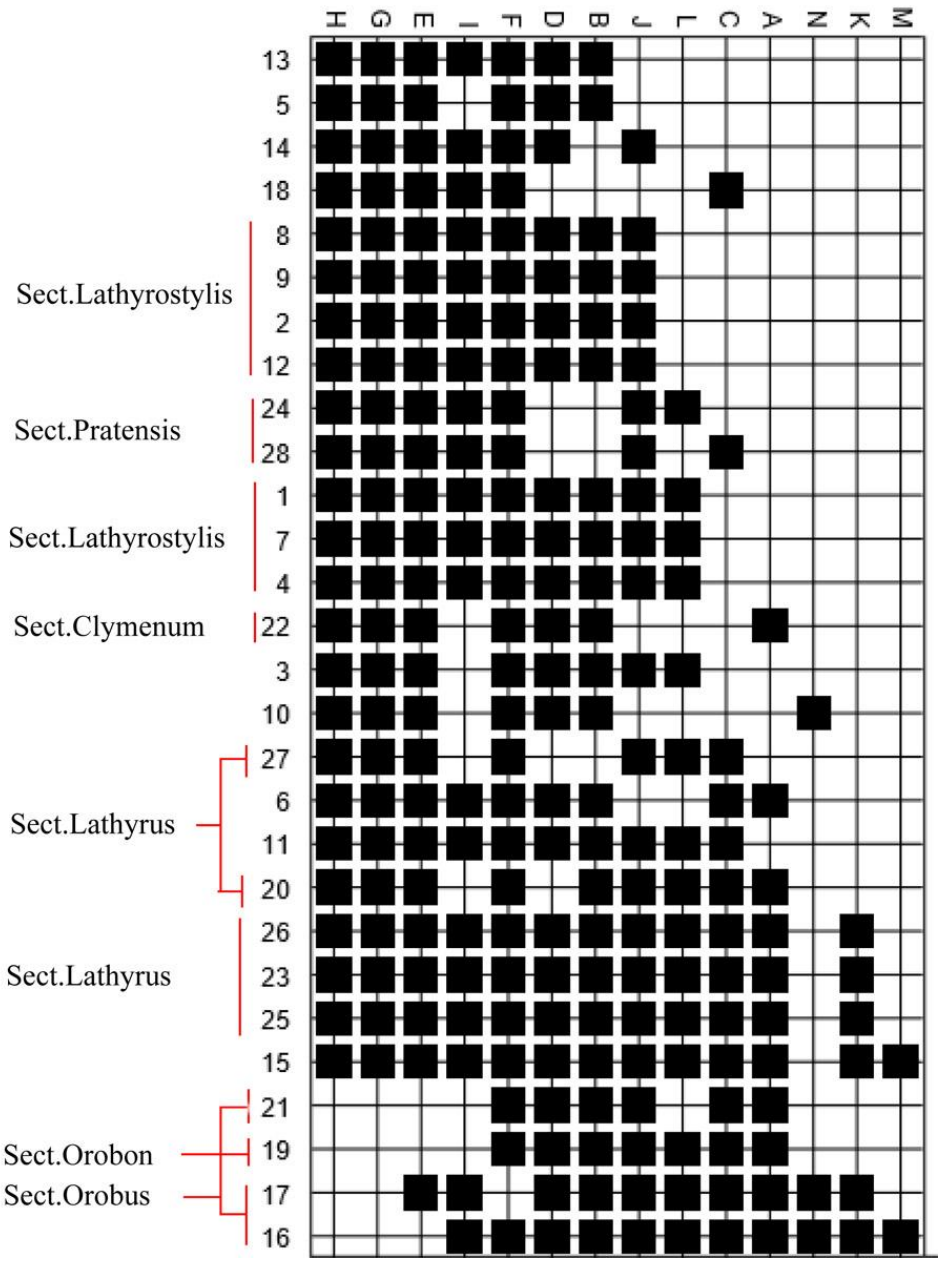

Figure 3-64 Seriation results of qualitative data of leaf surface.

Solid vertical red line represent species that belong to section. *L.digitatus* (1), *L.karsianus* (2), *L.tukhtensis* (3), *L.cyaneus* var. *cyaneus* (4) , *L.armenus* (5), *L.boissieri* (6), *L.pallesence* (7), *L.atropatanus* (8), *L.satdagensis* (9),

L.elangatus (10), *L.spathulatus* (11), *L.variabilis* (12), *L.cilicicus* (13*), L. brachypterus* (14), *L.haussknechtii* (15), *L.aureus* (16), *L.vernus* (17), *L.laxiflorus* (18), *L.roseus* (19), *L.annuus* (20*), L.niger* (21), *L.clymenum* (22), *L.sativus* (23), *L.pratensis* (24), *L.cicera* (25), *L.gorgoni* (26), *L.undulatus* (27) , *L.czeczottianus* (28).

A: is epidermal cell shape of adaxial, B: is epidermal cell of abaxial, C: is cell wall pattern of adaxial, D: is cell wall pattern of abaxial, E: is presence of stomata of adaxial, F: is presence of stomata of abaxial, G: is stomoata position of adaxial, H: is stomata position of abaxial, I: is presence of trichome of adaxial, J: is presence of trichome of abaxial, K: is trichome type of adaxial, L: is trichome type of abaxial, M: is granular pattern of adaxial, N: is granular pattern of abaxial .

In the phenon (**Figure 3-63**) which includes *L. aureus* (16), *L. vernus* (17), *L. roseus* (19), and *L. niger* (21) in the distance tree with Euclidean similarity index (their total bootstrap being 85%), all belong to section *Orobus* except *L. roseus* (19), which belongs to section *Orobon. L. aureus* (16), *L. vernus* (17) and *L. niger* (21) have exactly the same epidermal shape and cell wall pattern on both leaf surface. Also, *L. aureus* (16) and *L. vernus* (17) have the same trichome type in their both leaf surfaces, and both have a granular pattern on their surface except the adaxial surface of *L. vernus* (17). *L. roseus* (19) has a cell wall pattern (deeply sinuous) like *L. aureus* (16), *L. vernus* (17) and *L. niger* (21) but its shape is isodiametric slightly elongated.

Bartholtt, (1981) believed that the sinuous appearance of anticlinal walls of epidermal cells is taxonomically valuable. According to Bässler, (1966) and Simola, (1968) the leaves of section *Orobus* are hypostomatic (have stomata only on their abaxial surface) which is in agreement with this study except *L. vernus* (17), which has stomata on its adaxial surface. At the same time, Bassler, (1966) stated *L. vernus* (17) and *L. roseus* (19) have isodiametric cells with strongly wavy cell (deeply sinuous), which agrees with the present study.

In the phenogram obtained, species of *Orobus* section are separated from other *Lathyrus* species including *Lathyrostylis,* and according to Abou-El-Enain et al., (2007) seed data of 34 *Lathyrus* species indicated *Lathyrostylis* and *Orobus* as definitely separate sections. Other studies like Kenicer et al., (2005) and Badr et al., (2002) support this interpretation. However, sect. *Orobus* is not that distinct from other *Lathyrus* species, for instance, from sect. *Pratensis* in Schaefer‘s (2012) study.

The maximum likelihood phylogeny of the Fabaceae, which is based on the combined chloroplast and ITS data set, as conducted by Schaefer et al (2012) indicated that *L. aureus* (16) and *L. vernus* (17) belong under section of *Orobus* but in different nodes. They considered 119 *Lathyrus* species in their research with seven outgroups. *L. aureus* (16) and *L. vernus* (17) have the same form of evolution (Perennial), and their stylar pubescence evolution is abaxially hairy with dorsiventrally compression in stylar shape, according to their study.

Qualitative and quantitative characters of seed gross morphology of *L. aureus* (16) and *L. vernus* (17) are very similar including seed shape, size and testa appearance. They are found under the same subgroup (a3) in the UPGMA phenogram based on seed character in the study of Abou-El-Enain et al., (2007). *L. aureus* (16) and *L. vernus* (17) exhibit Sle-type in their style micromorphology, according to the study of Oskoueiyan et al., (2011) which has been provided for some Turkish and Iranian Fabeae tribe species.

L. roseus (19) and *L. niger* (21) have been supported with 62 % bootstrap value in UPGMA using Euclidean similarity index as given in **Figure 3-63**. Qualitative data of *L. roseus* (19) and *L. niger* (21) in the describing of cell wall pattern, presence and position of stomata, and the presence of trichome are exactly same in both leaf surface. Also, both species lack granule in their adaxial and abaxial surface.

The maximum likelihood phylogeny of the Fabeae conducted by Schaefer et al (2012) placed *L. roseus* (19) and *L. niger* (21) in the *Orobon* and *Orobus* sections respectively. Their life form evolution is perennial, and evolution of stylar pubescence is abaxially hairy. Although the biogeographic history of the Fabeae indicated *L. niger* (21) is related to central and Western Europe, *L. roseus* (19) is from the Mediterranean, according to their study.

In her morphologically based study, Leht, (2009) demonstrated that *L. roseus* (19) and *L. vernus* (17) are supported with a 68 % bootstrap value for which *L. roseus* (19) is included in Orobon section and *L. vernus* (17) is included in Orobus section.

L. aureus (16) and *L. laevigatua* are supported with the same bootstrap value under the *Orobus* section.

L. niger (21) and *L. roseus* subsp. *roseus* have a subprolate pollen shape with 1.135 and 1.30 values respectively, according to Gunes, (2011). Although, Kupicha, (1983) has been mentioned, pollen traits have minor significance as taxonomic characters.

L. laxiflorus (18) and *L. czeczottianus* (28) exhibit 60 % bootstrap support in the UPGMA tree with Euclidean similarity index with 1.4 distance as given in **Figure 3-63**. Both belong to section *Pratensis.* Qualitative data in the meaning of epidermal cell shape, cell wall pattern, presence of stomata and its position are identical in *L. laxiflorus* (18) and *L. czeczottianus* (28). Both have a slightly elongated epidermal cell shape in both leaf surfaces with a deeply sinuous wall pattern in the adaxial surface and wavy or straight wall pattern in the abaxial leaf surface. Also, none of these species have glandular trichome in their leaf surface. *L. czeczottianus* (28) is isodiametric or slightly elongated with wavy or straight wall pattern, according to Bässler, (1966). Çıldır, (2011) mentioned epidermal cells in *L. laxiflorus* subsp. *laxiflorus* is isodiametric with strongly wavy walls, and *L. czeczottianus* (28) is isodiametric with wavy or straight wall which is in disagreement with the present study. *L. pratensis* (24) is expected show a close relationship with *L. laxiflorus* (18) and *L. czeczottianus* (28) as all three species belongs to *Pratensis* section but *L. pratensis* (24) exhibits quite a weak bootstrap and is close to *Lathyrostylis* section in the UPGMA tree with Euclidean similarity index.

In a molecular-based study as conducted by Schaefer et al (2012), *L. czeczottianus* (28) and *L. laxiflorus* (18) show 91% bootstrap support, and in other phylogenic tree of the same study like biogeographic history their relationship has been supported even by 98 % bootstrap. Their life form is perennial, and evolution of stylar pubescence is abaxially hairy with dorsiventrally compressed in both species in same research. However, the evolution of chromosome is different (*L. czeczottianus* is unknown and *L. laxiflorus* is 2n=14). *L. pratensis* (24) is in sect. *pratensis* in their study and is related to *L. czeczottianus* (28) and *L. laxiflorus* (18) with unmeasured

bootstrap support. According to Oskoueiyan et al., (2011) both have Sle-type style micro morphological traits. *L. laxiflorus* subsp. *laxiflorus* and *L. czeczottianus* (28) have a subprolate pollen shape with 1.3245 and 1.3809 values respectively, indicating closely related micromorphological traits based on pollen characters due to Gunes and Aytug, (2010). *L. laxiflorus* (18) and *L. czeczottianus* (28) exhibit about 0.85 similarity level in UPGMA phenogram in the numerical taxonomy study of Turkish *Lathyrus*, which was conducted by Doğan et al (1992).

L. s*atdaghensis* (9), *L. karsianus* (2), *L. atropatanus* (8), and *L. variabilis* (12) are supported by 100 % bootstrap (overlapped) due to UPGMA tree using Euclidean similarity index as given in **Figure 3-63**. All belong to section *Lathyrostylis*. This result corresponds to the UPGMA tree obtained in **Figure 3-22** of qualitative data (light microscope method). The qualitative data of both surface in leaves in the meaning of epidermal cell shape, cell wall pattern, presence of stomata, stomata situation on the epidermis, presence or absence of trichome, trichome types and granular pattern are exactly same. These species are not considered in the research of Schaefer et al., (2012).

According to Doğan et al., (1992), *L. satdaghensis* (9) *a*nd *L. karsianus* (2) show a 0.75 similarity level and *L. nivalis* (which is very similar to *L. atropatanus*) and *L. variabilis* (12) shows about a 0.55 similarity level in the UPGMA phenogram of Turkish *Lathyrus* species.

L. cyaneus var. *cyaneus* (4), *L. digitatus* (1) and *L. pallesence* (7) exhibit 100 % bootstrap (overlapped) due to UPGMA tree using Euclidean similarity index, **Figure 3-63**. This result corresponds to the UPGMA tree obtained in **Figure 3-22** of qualitative data (light microscope method). Epidermal cell shape, cell wall pattern, presence of stomata and position, presence of trichome and trichome type, and granular pattern are identical. *L. spathulatus* (11) exhibits a quiet weak bootstrap (29%) with these species.

Schaefer et al., (2012) studied few species of section *Lathyrostylis* including (*L. digitatus* (1), *L. spathulatus* (11), *L. filiformis, L. pallesence* (7), *L. tukhtensis* (3), L. *bauhinii*, and *L. sphaericus*). *L. digitatus* (1), *L. spathulatus* (11) and *L. filiformis, L. pallesence* (7) respectively and have been supported with 100 % bootstrap.

L. cyaneus and *L. digitatus* (1) demonstrate 90% bootstrap support in the morphological study of Leht, (2009) and 57% bootstrap support with *L. pallesence* (7) relative to her research. She considered 47 *Lathyrus* species. Also, *L. cyaneus* and *L. digitatus* (1) show about 0.80 similarity level in Doğan et al., 1992. According to karyotype analyses of section *Lathyrostylis* which were conducted by Gunes (2011), *L. cyaneus* and *L. digitatus* (1) belong to the third group (with 8m + 6sm karyotype formula), and according to pollen morphology *L. pallesence* (7) and *L.* s*pathulatus* (11) have the same shape of pollen (spheroidal), according to the research study of Gunes, (2011).

L. armenus (5) and *L. elangatus* (10) demonstrated 56% bootstrap support in this study as given in **Figure 3-63**. Their qualitative data show identical micro characters in epidermal cell shape, cell wall pattern, presence of stomata, stomata position, presence of trichome and trichome type. However, the presence of granular pattern is different as *L.* e*longatus* (10), which has only granule in its abaxial surface, and *L. armenus* (5) lacks granule on both leaf surfaces. *L. cilicicus* (13) exhibits very low bootstrap with these species. As quantitative trait, the length of epidermal cell on both leaf surfaces of *L. cilicicus* (13) in the present study is in agreement with Celep et al., (2011).

Doğan et al., (1992) have indicated similarity level of *L. armenus* (5) and *L. elangatus* (10) in UPGMA as 75 %. Their pollen shape is spheroidal, according to Gunes, 2011. Their karyotype formulas are different as *L. armenus* (5) belongs to the first karyological group, and *L. elangatus* (10) belongs to the second karyological group, according to Gunes, (2011).

L. sativus (23) and *L. cicera* (25) exhibited very weak bootstrap support (39 %) in this study and *L. gorgoni* (26) also is related (28%) to these species in UPGMA as given in **Figure 3-63**. *L. sativus* (23) and *L. cicera* (25) have identical epidermal cell shape, presence and position of stomata, trichome type and granular pattern. Only the wall pattern is different. *L. cicera* (25) and *L. gorgoni* (26) both have an elongated cell shape with straight wall pattern (in the abaxial surface), which is in agreement with Bässler, (1966).

Molecular analysis (RAPD) of eight *Lathyrus* species indicates that *L.* s*ativus* (23) is most similar to *L. gorgoni* (26). On the other hand, *L. odoratus* was the most distant from *L. sativus* (23). Also, *L. gorgoni* (26) and *L. cicera* (25) are equidistant from *L. sativus* (23) in terms of genetic divergence (Croft, 1999). *L. sativus* (23) and *L. cicera* (25) were investigated by Schaefer et al (2012) and bear a close relationship (unmeasured bootstrap support) under *Lathyrus* section. *L. gorgoni* (26) bears a close relationship to *L. sativus* (23) and *L. cicera* (25) in their study.

The molecular based on internal transcribed spacer and cp DNA sequence Kenicer et al., (2005) mentioned *L. sativus* (23) and *L. cicera* (25) to exhibit a very strong bootstrap value (100 %). *L. gorgoni* (26) was not considered in their research. AFLP were used to examine systematic relationships in *Lathyrus* section by Badr et al (2002). In their phylogenetic tree (parsimony tree and distance based tree of Neighbor Joining) *L. sativus* (23), *L. cicera* (25), and *L. gorgoni* (26) show a close relationship on a clade (that is unsupported by bootstrap value). Asmussen and Liston, (1998), in strict consensus tree analyses of combined rpoC and IR cp DNA restriction site data, showed that *L. cicera* (25), *L. gorgoni* (26), *L. amphicarpus* and *L. sativus* (23) are supported by 94% bootstrap value. *L. cicera* (25) and *L. gorgoni* (26) have 85% bootstrap value, and *L. amphicarpus* and *L. sativus* (23) have 82% bootstrap value in *Lathyrus* section.

L. cicera (25) and *L. sativus* (23) had the same style type (Cle–type) in micromorphological study conducted by Oskuyiean et al (2011). *L. gorgoni* (26) has Sse-type as style type. Seed protein of 14 species of *Lathyrus* was used by El-

Shanshoury (1997), and *L. sativus* (23) and *L. cicera* (25) are classified into the first group in PCA and *L. gorgoni* (26) as in a different group. In the UPGMA phenogram, which was conducted by Abou El-Enain et al (2007) based on morphological characters of the seed surface, *L. sativus* (23), *L. cicera* (25) , *L. annuus* (20) and *L. gorgoni* (26) were studied in addition to 44 other *Lathyrus* species, and in the cladogram obtained these species belong to subclade *Lathyrus* (their bootstrap value is unmeasured). *L. sativus* (23), *L. cicera* (25) and *L. gorgoni* (26) are included in subgroup (b2). *L. cicera* (25) is most closely aligned with *L. sylvestris* (about 0.80 similarity), and *L. sativus* (23) is most closely related to *L. annuus* (20) (about 0.80 similarity).

In this study, *L. annuus* (20) and *L. undulatus* (27) show 71 % bootstrap support as shown in **Figure 3-63** and both belong to section *Lathyrus*. Their qualitative data in leaf surface in the meaning of cell wall pattern, presence of stomata and its position on epidermis, and granular pattern are exactly the same. Cell shape and trichome presence, and type are different in these species.

L. annuus (20) and *L. hierosolymitanus* in Badr et al., (2002) and Asmussen and Liston, (1998) show very strong bootstrap value (100 %). Although *L. undulatus* (27) is not considered in these studies. Schaefer et al., (2012) also showed that bootstrap value between *L. annuus* (20) and *L. hierosolymitanus* is very strong (95 %) and *L. undulatus* (27) and *L. rotundifolius* and *L. miniatus* show a very strong relationship (100 %). *L. annuus* (20) and *L. undulatus* (27) are related in their study with unmeasured bootstrap. Doğan et al., (1992) show strong bootstrap (95 %) between *L. annuus* (20) and *L. hierosolymitanus. L. undulatus* (27) is in sect. *Gorgonia* in their study.

Based on karyological research (Gunes and Cirpici, 2008) *L. annuus* (20) and *L. undulatus* (27) have different chromosome types and chromosome total length, but their arm ratio (chromosome) is close in value (1.86 for *L. undulatus* and 1.78 for *L. annuus).*

In the distance based on the Euclidean similarity index of UPGMA as given in **Figure 3-63**, *L. boissieri* (6) is separated from *Lathyrostylis* section and it is closer to species of the section *Pratensis*. *L. boissieri* (6) has an isodiametric and slightly elongated cell shape with straight wall pattern, which is specific among the species examined. One of the phenon in the UPGMA tree (**Figure 3-22**) obtaining qualitative data is *L. boissieri* (6). In the UPGMA quantitative data (**Figure 3-18**) *L. boissieri* (6) is close to *L. brachypterus* (14). Further analyses is required to evaluate its exact placement. Also, *L. haussknechtii* (15) is a closer relative to *L. sativus* (23), *L. cicera* (25), and *L. gorgoni* (26) than to be *Lathyrostylis* section. This species was known as variety of *L. brachypterus* (14) according to Davis, (1970) but it is accepted as a different species by Çıldır, 2011. Therefore, *L. haussknechtii* (15) is confirmed as a different species in the current study.

Among the species examined in the present study, *L. pallesence* (7) and *L. cyaneus* var. *cyaneus* (4) overlap due to their qualitative leaf data in both type of microscope (LM, SEM), and due to their quantitative leaf data of light microscope, both species are close. These species belong to sect. *lathyrostylis*. *L. armenus* (5) and *L. elongatus* (10) also overlap due to their qualitative data obtained by the light microscope method, and are close in SEM method. Also, *L. satdaghensis* (9), *L. karsianus* (2), *L. atropatanus* (8), and *L. variabilis* (12) overlap, according to their qualitative data with both types of microscope.

UPGMA produced (Euclidean) based on qualitative micro morphological traits in leaf surface is largely in agreement with existing estimates of phylogeny from Kupicha, (1983); Asmussen and Liston, (1998); Kenicer et al., (2005) and Schaefer et al., (2012). So these results support the hypothesis that qualitative microtraits in leaf surface reflect an evolutionary relationship.

Quantitative characters (epidermal cell size, stomata size and trichome length) were measured for both leaf surfaces (**Table 3-5**, **Table 3-6**) and were used in the PAST 3.05 software program in order to obtain UPGMA. Adaxial and abaxial quantitative data are used separately or together (Appendix B, Figure B1, B2, B4), and

relationships of species tend to disagree with existing phylogenetic estimate of relationships. When quantitative data are used individually (Appendix B, Figure B3), samples of the species are scattered across the tree. Missing data may have influenced clustering a little (not all quantitative data are found in one photograph, so it can imply missing data). This also suggests that quantitative traits are variable within species, implying they are not phylogenetically determined and may be environmentally determined or fall within the natural variation of the species.

3.2.2 Calyx Surface

Inner surface of calyx lacks stomata and trichomes among the species examined. All qualitative and quantitative micromorphological character results of the outer surface of calyx (presence of stomata, stomata size, trichome length and types) from the use of the scanning electron microscope are given in **Table 3-9** and **Table 3-10**. Photographs are shown between Figure 3-65 and Figure 3-88.

Table 3-9 Micromorphological traits (qualitative) of calyx surface among the 28 examined species (every species observed between 10 -15 samples)

Species (numbers)	Presence of Stomata	Trichome Type
L.digitatus **(1)**	POS	II , III
L.karsianus **(2)**	POS	II , III
L.tukhtensis **(3)**	POS	II , III
L.cyan **var.** *cya* **(4)**	POS	II , III
L.armenus **(5)**	POS	II , III
L.boissieri **(6)**	POS	II , III
L.pallesence **(7)**	POS	II , III
L.atrapatanus **(8)**	POS	III
L.satdaghens **(9)**	POS	II , III
L.elangatus **(10)**	POS	II, III
L.spathulatus **(11)**	POS	II, III
L.variabilis **(12)**	POS	III
L.cilicicus **(13)**	POS	II, III
*L.brachypteru***(14*)**	POS	II, III
*L.haussknechtii***(15)**	NEG	III
*L.aureus (***16)**	POS	II, III

Table 3-9 continued

L.vernus(17)	POS	III
*L.laxiflorus(*18)	POS	II , III
L.roseus(19)	POS	II
L.annuus(20)	POS	II
L.niger(21)	POS	II, III
L.clymenum(22)	POS	II, III
*L.sativus(*23)	POS	II, III
L.pratensis(24)	POS	II, III
L.cicera(25)	POS	II, III
L.gorgoni(26)	POS	II
L.undulatus(27)	POS	II
L.czeczottianus(28)	POS	III

POS (positive), presence of stomata; NEG (negative), absence of stomata; II, glandular trichome; III, non – glandular trichome

Table 3-10 Mean values of Quantitative Calyx surface characters (missing data are shown with -1)

species (numbers)	Non gland length	Gland length	Stomatal width	Stomatal length
L.digitatus (1)	135.91	79.84	14	3.07
L.karsianus(2)	94.63	61.35	9.46	2.47
L.tukhtensis(3)	94.61	51.43	13.42	4.21
L.cyan var. *cya(*4)	98.68	46.78	10.47	3.61
L.armenus(5)	141.99	88.11	13.25	4.31
L.boissieri(6)	126.55	63.79	12.1	4.23
L.pallesence(7)	119.53	77.56	13.6	4.2
L.atrapatanus(8)	178.54	−1	8.4	2.7
L.satdaghens(9)	216.56	65.81	11.16	3.38
L.elangatus(10)	120.36	55.74	11.81	3.55
L.spathulatus(11)	141.91	92.64	13.77	3.17
L.variabilis(12)	99.31	−1	11.38	2.85
L.cilicicus(13)	162.22	82.15	10.93	3.58
L.brachypterus(14)	178.6	67.57	12.28	3.26
L.hausknechtii(15)	103.96	−1	−1	−1
L.aureus(16)	185.17	65.9	12.88	3.66
L.vernus(17)	131.21	−1	11.8	3.62
L.laxiflorus(18)	493.9	72.68	13.18	2.66

Table 3-10 continued

*L.**roseus*** **(19)**	−1	95.2	12.43	2.45
*L.**annuus*** **(20)**	−1	54.97	14.38	2.75
*L.**niger*** **(21)**	192.62	85.2	15.72	3.47
*L.**clymenum*** **(22)**	183.85	65.02	10.17	3.57
*L.**sativus*** **(23)**	284.25	60.55	9.17	2.24
*L.**pratensis*** **(24)**	345.28	67.43	11.78	2.89
*L.**cicera*** **(25)**	242.84	65.01	12.14	4.59
*L.**gorgoni*** **(26)**	−1	64.82	9.25	1.87
*L.**undulatus*** **(27)**	−1	87.05	16.15	3.65
*L.**czeczottian*** **(28)**	881.68	−1	14.58	4.15

3.2.2.1 Micromorphological Properties of Calyx Surface

3.2.2.1.1 Stomata

All species examined have stomata in their calyx except *L. haussknechtii*, which belongs to *Lathyrostylis* section (See **Table 3-9**). Stomata density is different between species. For example, *L. tukhtensis, L. annuus*, *L. pratensis* and *L. cicera* have many stomata on their calyx. Some others have very few stomata in their calyx, such as *L. roseus, L. niger,* or *L. gorgoni*. However, stomata density is influenced by environmental factors, and so it was not considered in the present study.

Stomatal width ranges between 8.4 µm in *L. atropatanus* and 16.15 µm in *L. undulatus*. Stomatal length ranges between 1.87 µm in *L. gorgoni* and 4.59 in *L. cicera* as shown in **Table 3-10**. Except for *L. atropatanus,* all others belong to section *Lathyrus*. Stomata position on the calyx surface does not indicate any significant difference between the examined *Lathyrus* species.

3.2.2.1.2 Trichomes

Trichomes present as two types in the calyx: glandular and non-glandular (photographs seen in Figure 3-65 and Figure 3-90). Non glandular trichomes are a typical bicellular type accompanied by an elongated terminal (tapering gradually toward the apex). Glandular hairs are of capitate type, which is unicellular, and

contains a stalk, neck cell, and cutinized secretory head. The length of the stalk is less than, more than, or equal to the length of the head. The presence of both glandular types is a common condition in the calyx surface among the species examined. *L. atropatanus, L. variabilis, L. haussknechtii, L. vernus,* and *L. czeczottianus* have only a non-glandular trichome type. Some species like *L. roseus, L. annuus, L. gorgoni* and *L. undulatus* have only a glandular trichome in their calyx as shown in **Table 3-9**. Some species like *L. digitatus, L. spathulatus,* and *L. variabilis* have only trichomes on the margin of the calyx but not on the surface. Others like, *L. karsianus, L. armenus, L. boissieri, L. atropatanus, L. satdaghensis, L. niger,* and *L. czeczottianus* have many trichomes on the margin and surface of the calyx. Most species examined which have both kinds of trichomes have many non–glandular trichomes and few glandular trichomes, except *L. laxiflorus, L. clymenum,* and *L. sativus.*

The longest non- glandular trichome length on calyx belongs to *L. czeczottianus* at 881.68 µm. Also, this species has the longest non-glandular trichome in the adaxial leaf surface. The second and the third longest non-glandular trichome lengths in the calyx belong to *L. laxiflorus* and *L. pratensis.* All of these species are in the *Pratensis* section. The shortest non–glandular trichome length on the calyx belongs to *L. karsianus* and *L. tukhtensis* with 94.63 µm and 94.61 µm respectively. Both of these are seen in the *Lathyrostylis* section. The longest glandular trichome length belongs to *L. roseus* at 95.2 µm (*Orobon* section). (*L. roseus* has the longest glandular trichome in the abaxial leaf surface), and the shortest glandular trichome length belongs to *L. cyaneus* var. *cyaneus* at 46.78 µm (*Lathyrostylis* section). Therefore, sect. *Lathyrostylis* has species with the shortest non–glandular and glandular trichome length among the *Lathyrus* species examined, and sect. *Pratensis* has species with the longest non-glandular trichome on the calyx surface among the species examined.

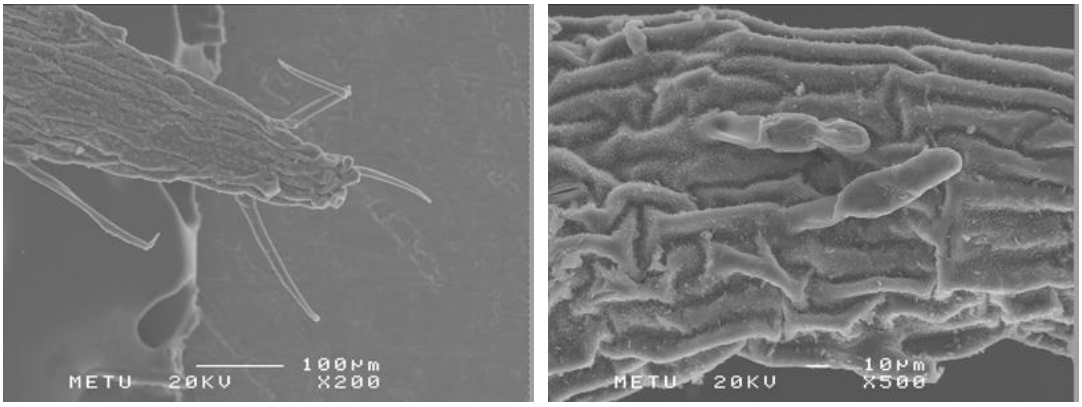

Figure 3-65. Non glandular trichome (left) and glandular trichome (right) on the calyx surface of *L. digitatus*

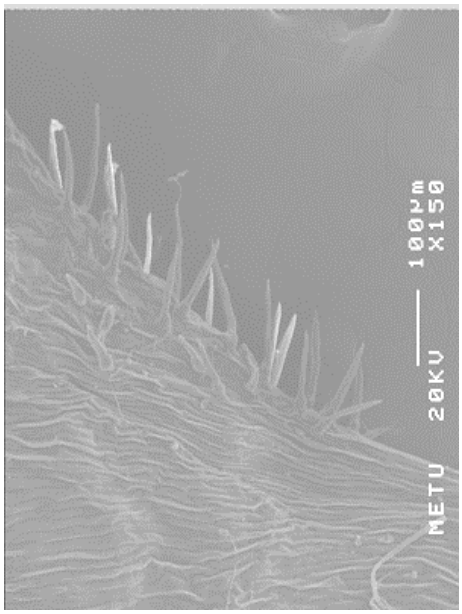

Figure 3-66. Non glandular and glandular trichome on the calyx surface of *L. karsianus*

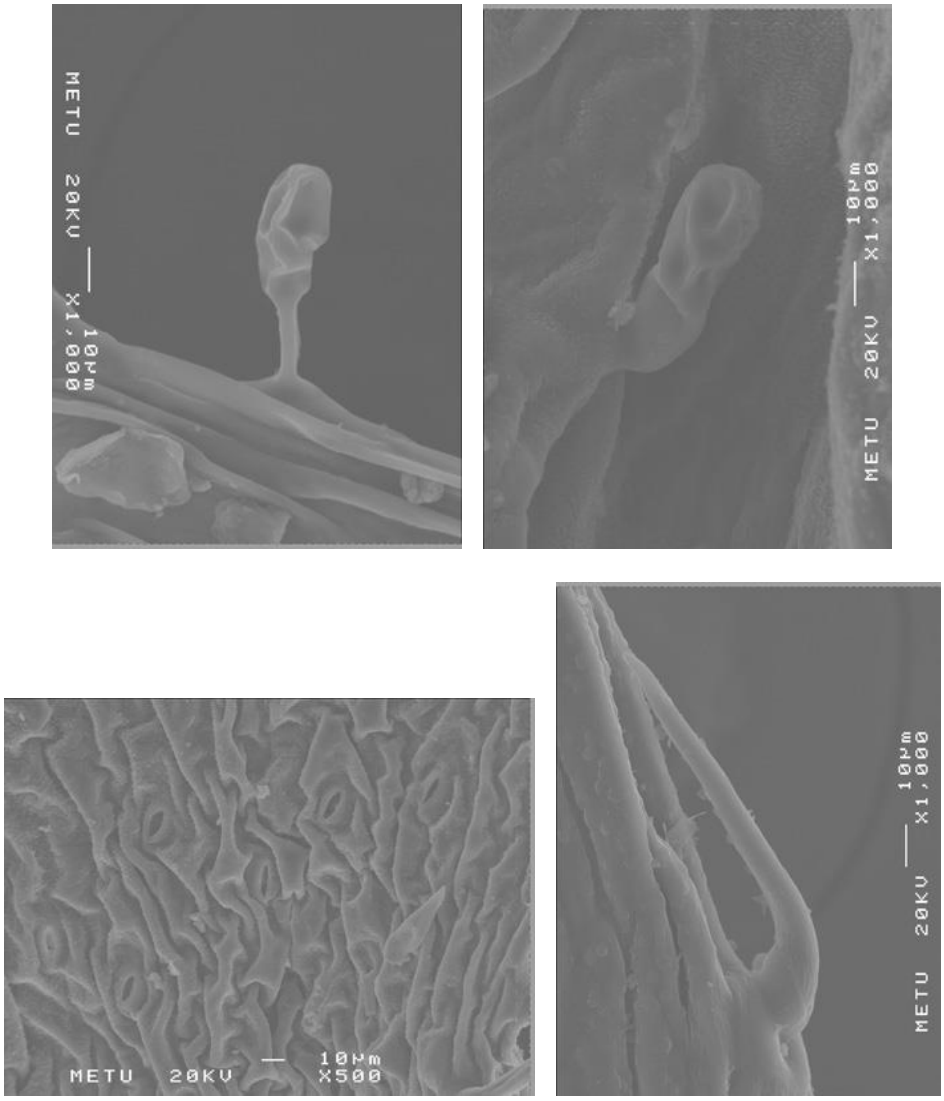

Figure 3-67. Glandular trichome (above), calyx surface (below , left) and non-glandular trichome of *L tukhtensis.*

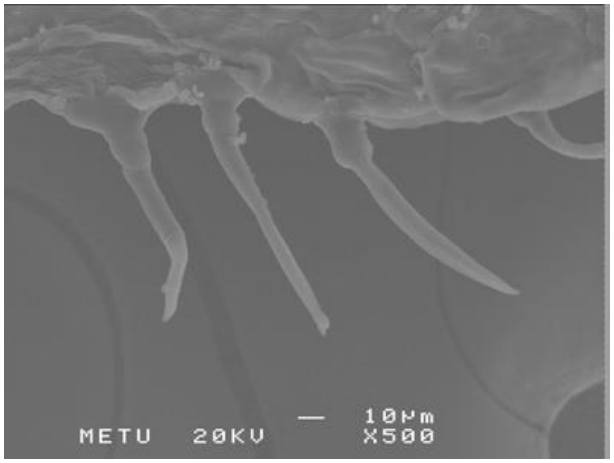

Figure 3-68. Non-glandular trichome of *L. cyaneus* var. *cyaneus*

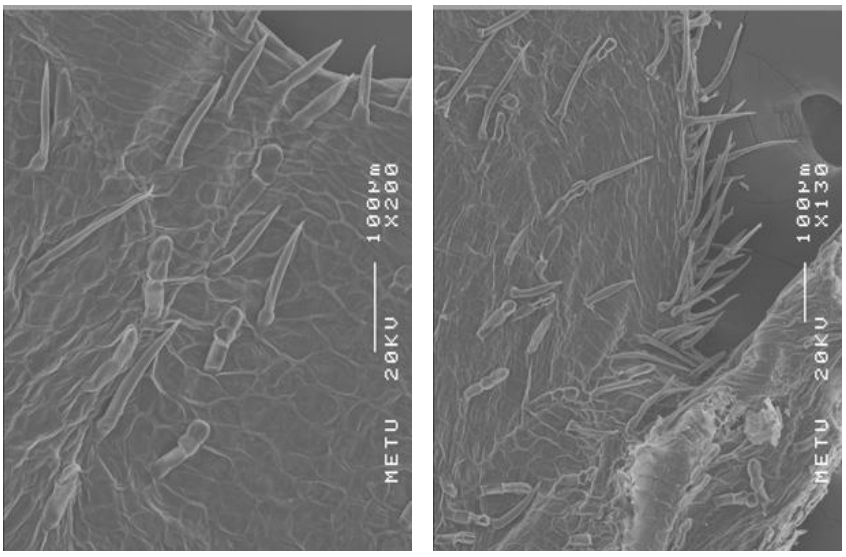

Figure 3-69. Various type of non-glandular and glandular trichomes on the calyx surface of *L. armenus*

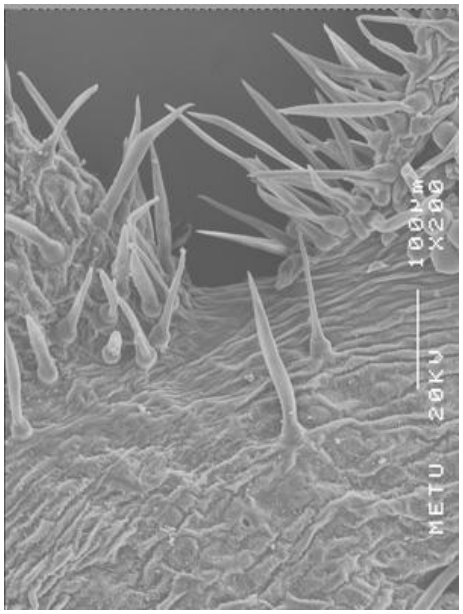

Figure 3-70. Non-glandular trichomes on the calyx surface of *L. boissieri*

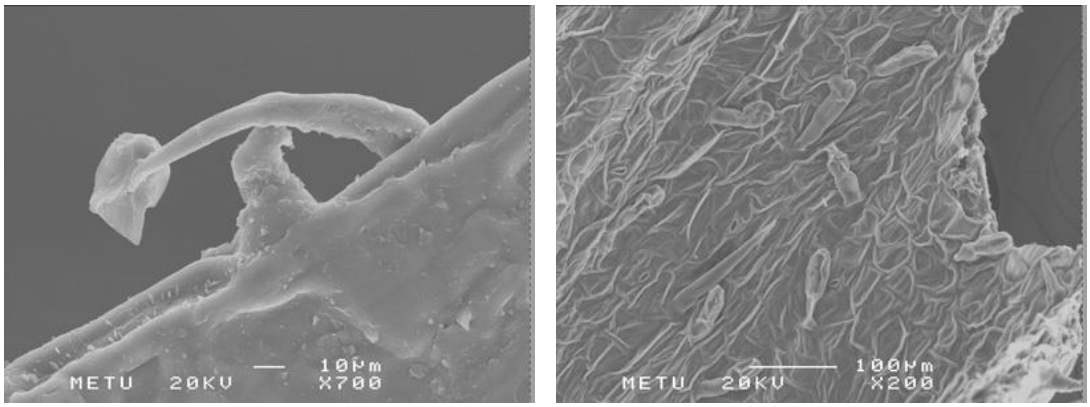

Figure 3-71. Non-glandular trichome (left), acicular and glandular trichome (right) on the calyx surface of *L. pallesence*

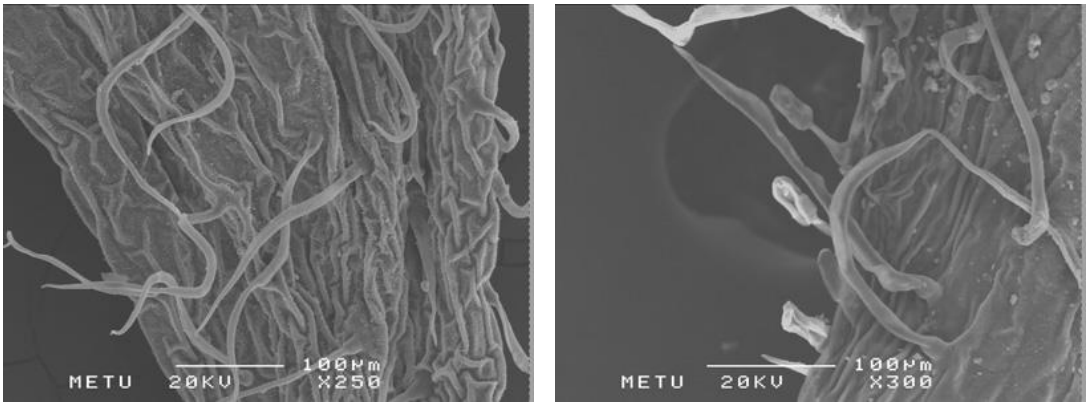

Figure 3-72. Very long non-glandular trichome (left) of *L. atropatanus* and glandular and non-glandular trichome (right) on the calyx surface of *L. satdagensis*

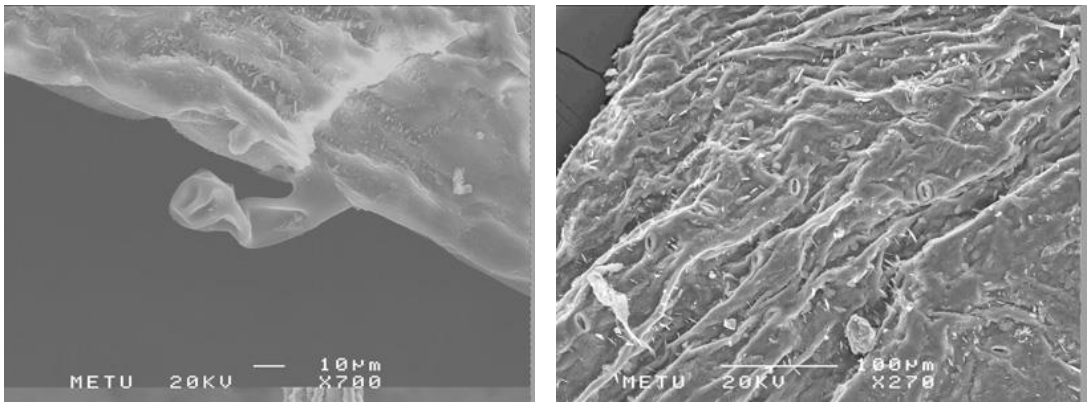

Figure 3-73. Glandular trichome (left) on the calyx surafce of *L. elangatus* and calyx surface with stomata (right)

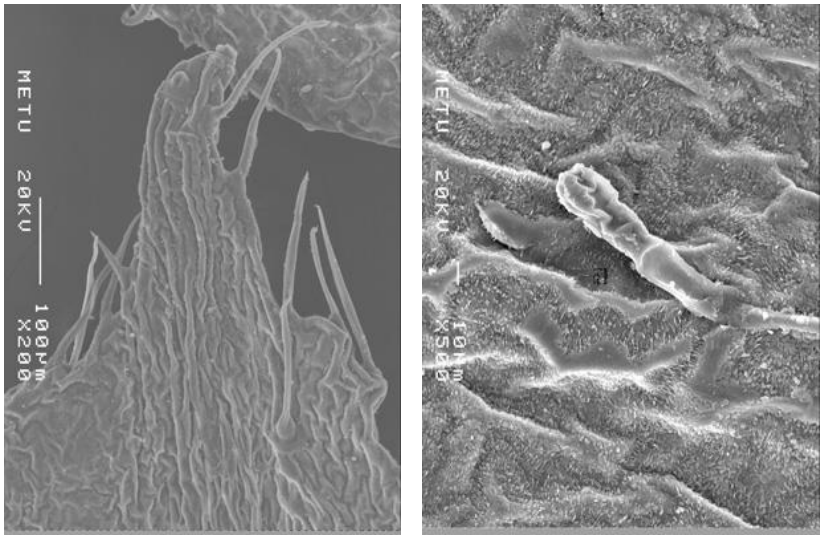

Figure 3-74. Non-glandular trichome (left) and glandular trichome on the calyx surface of *L. spathulatus*

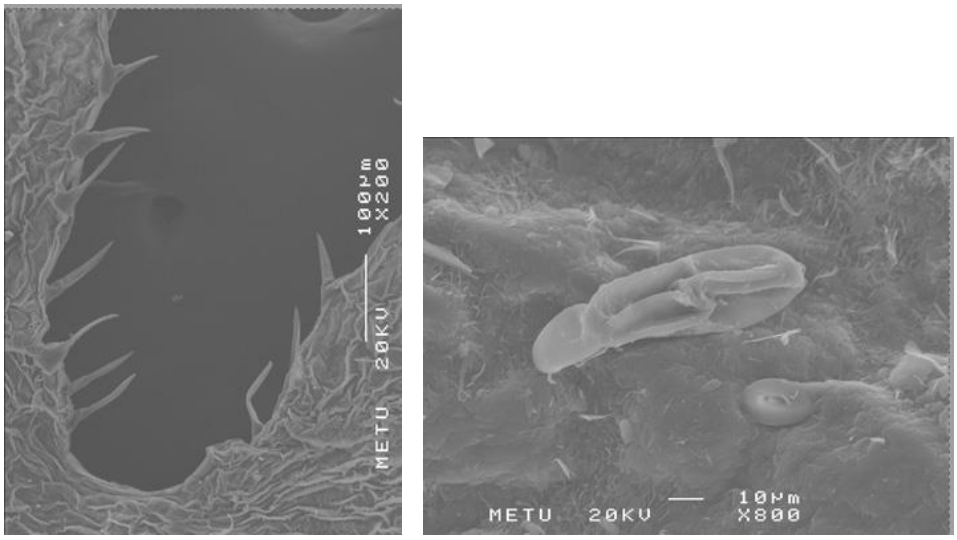

Figure 3-75. Non-glandular trichome (left) of *L. variabilis* and glandular trichome (right) on the calyx surface of *L. cilicicus*

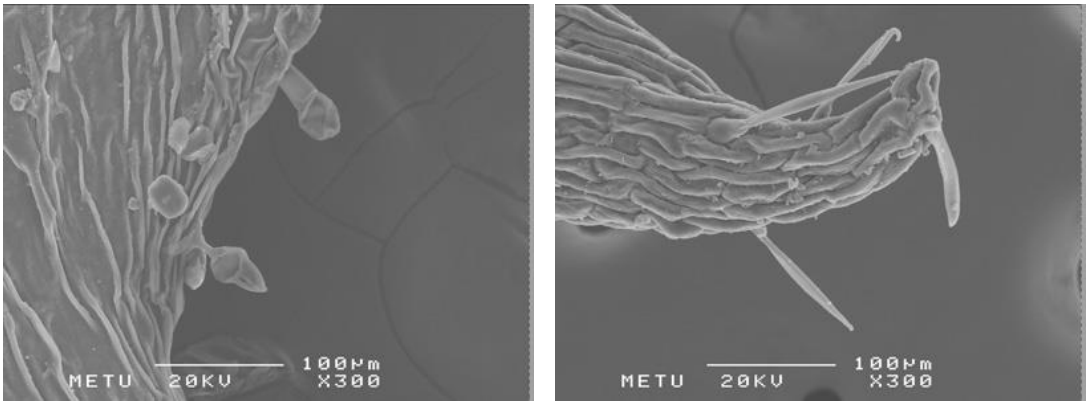

Figure 3-76. Glandular trichome (left) of *L. brachypterus* and non-glandular trichome (right) on the calyx surface of *L. haussknechtii*

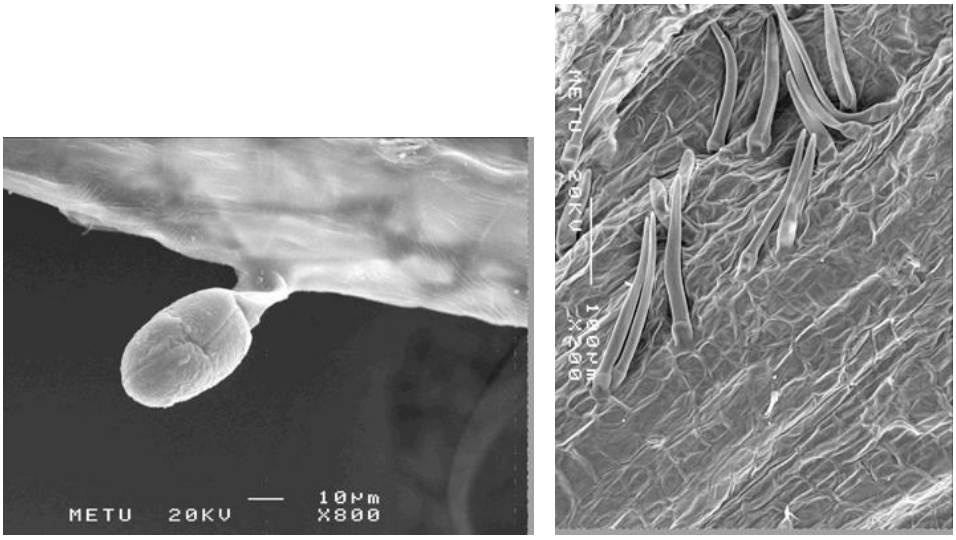

Figure 3-77. Glanduar trichome (left) , non-glandular trichome (right) on the calyx surface of *L. aureus*

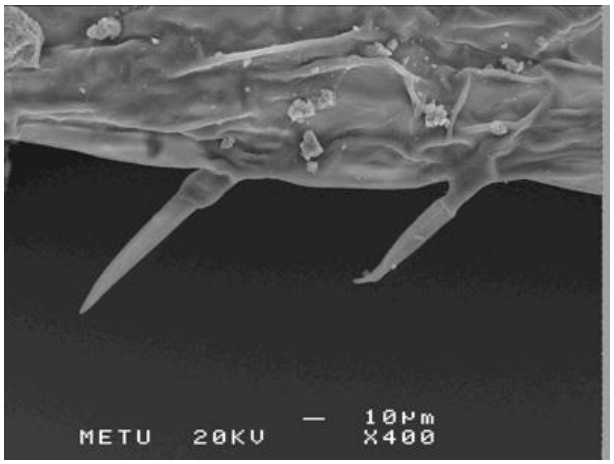

Figure 3-78. Non-glandular trichome of *L. vernus*

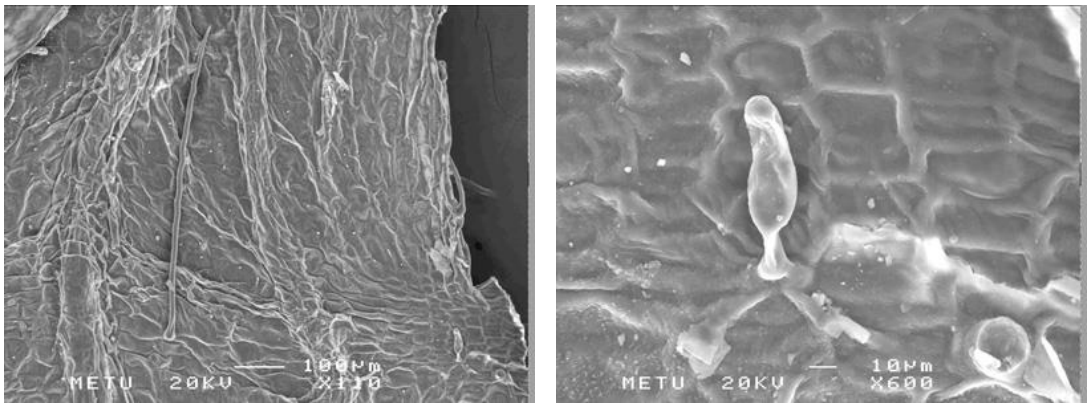

Figure 3-79. Very long non-glandular trichome (left) and glandular trichome (right) on the calyx surface of *L. laxiflorus*

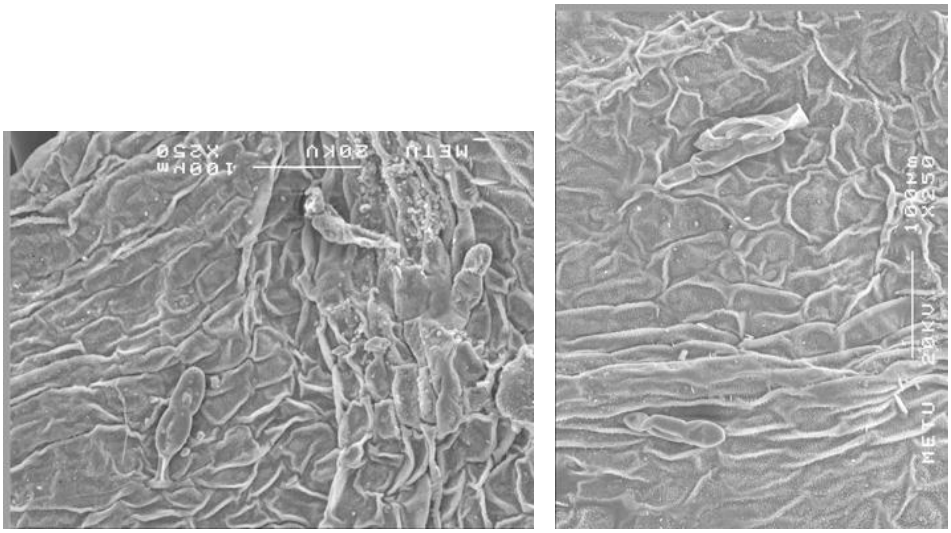

Figure 3-80. Glandular trichomes on the calyx surface of *L. roseus*

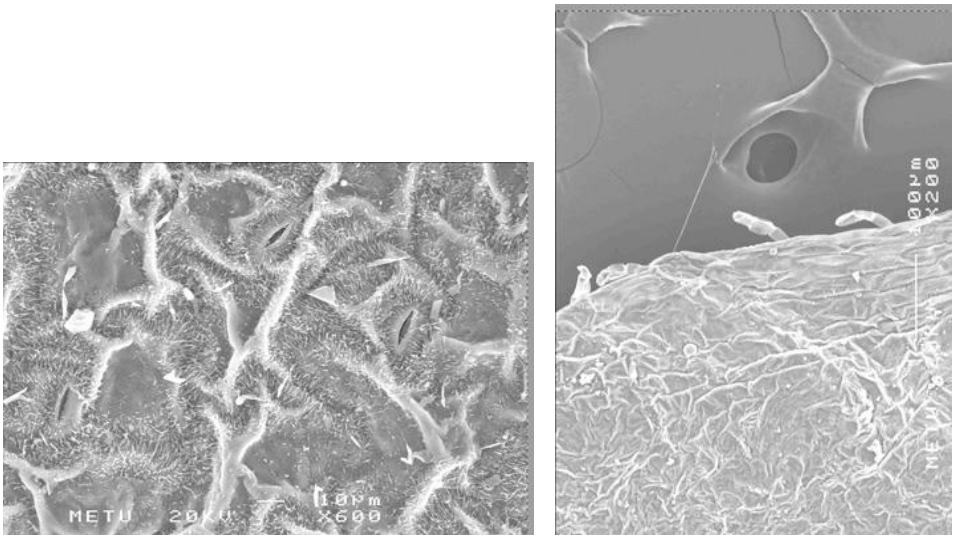

Figure 3-81. Surface of calyx with stomata (left) and glandular trichome (right) on the calyx surface of *L. annuus*

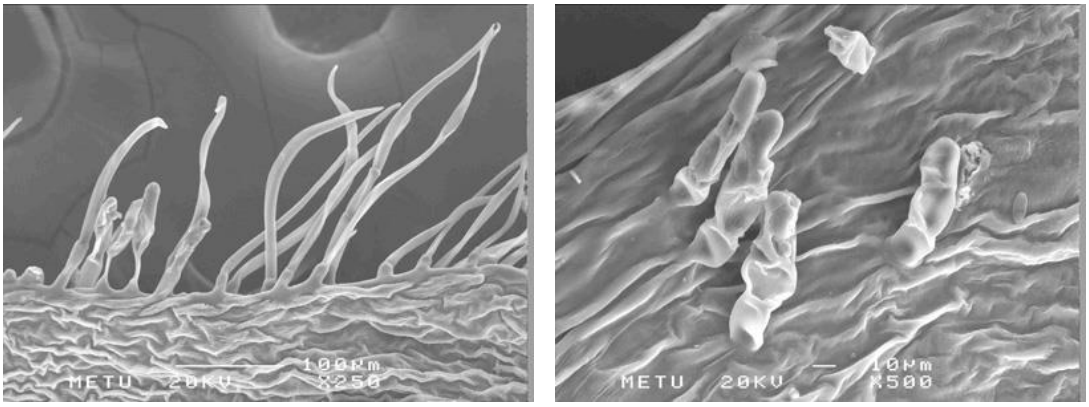

Figure 3-82. Non-glandular, glandular trichome (left) and glandular trichome (right) on the calyx surface of *L. niger*

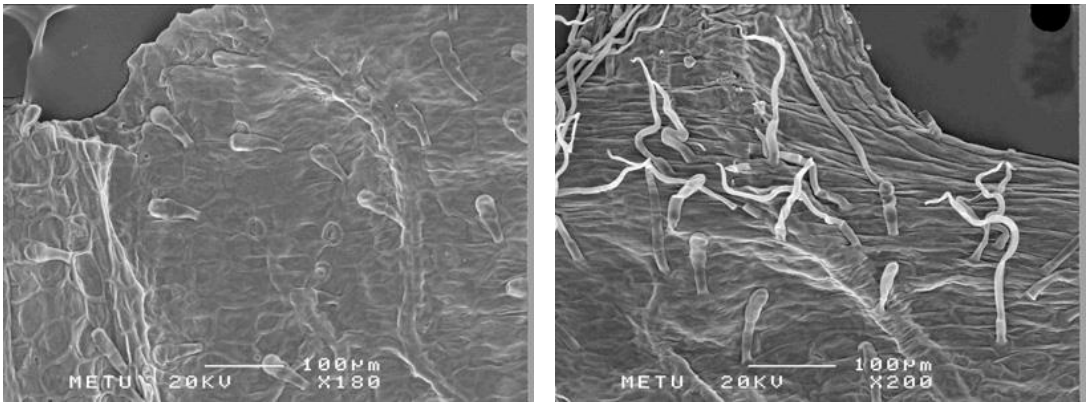

Figure 3-83. Glandular trichome (left) and non-glandular, glandular trichome (right) on the calyx surface of *L. clymenum*

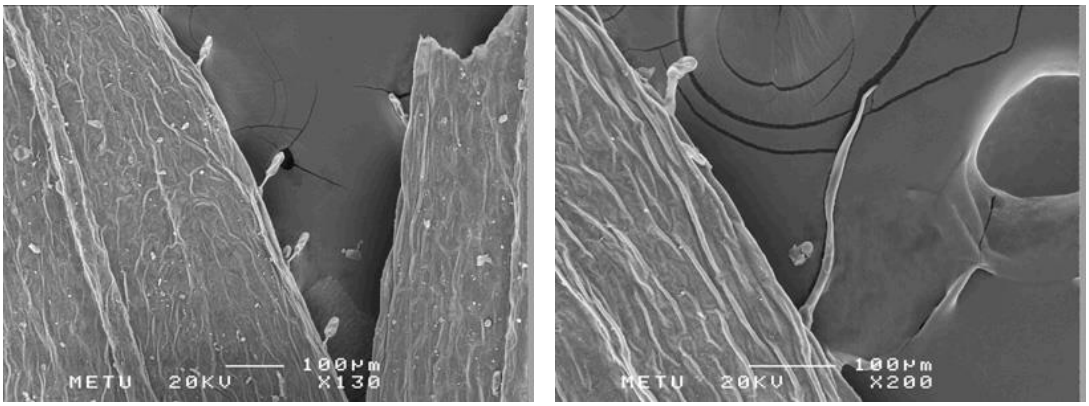

Figure 3-84. Glandular trichome (left) and non-gandular, glandular trichome (right) on the calyx surface of *L. sativus*

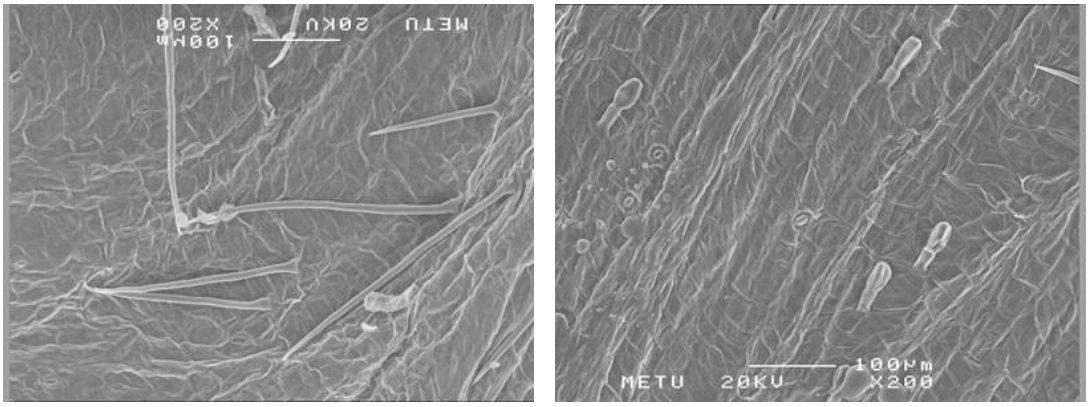

Figure 3-85. Non-glandular, glandular trichome (left) and glandular trichome (right) on the calyx surface of *L. pratensis*

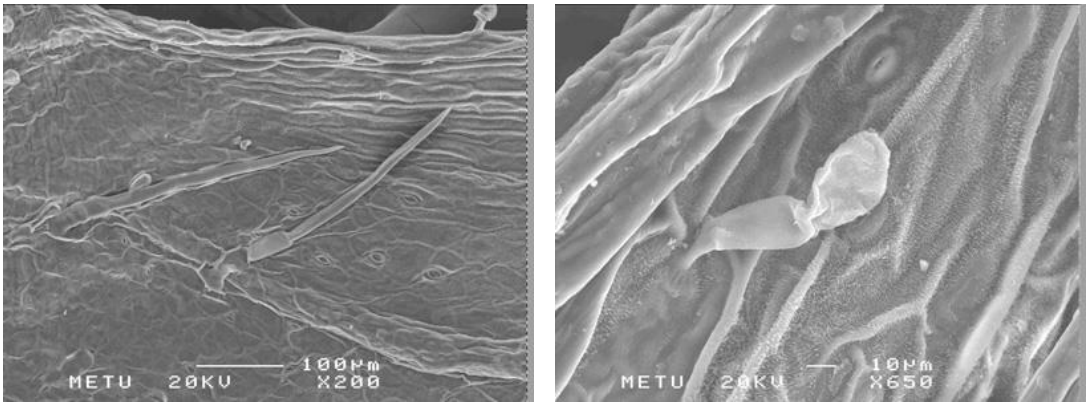

Figure 3-86. Non-glandular trichome (left) of *L. cicera* and glandular trichome(right) on the calyx surface of *L. gorgoni*

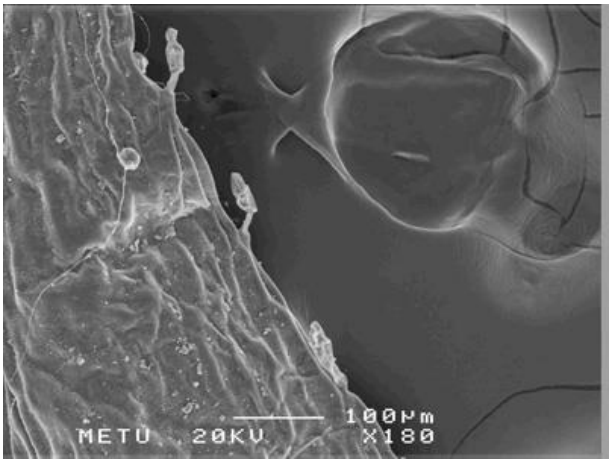

Figure 3-87. Glandular trichome on the calyx surface of *L. undulatus*

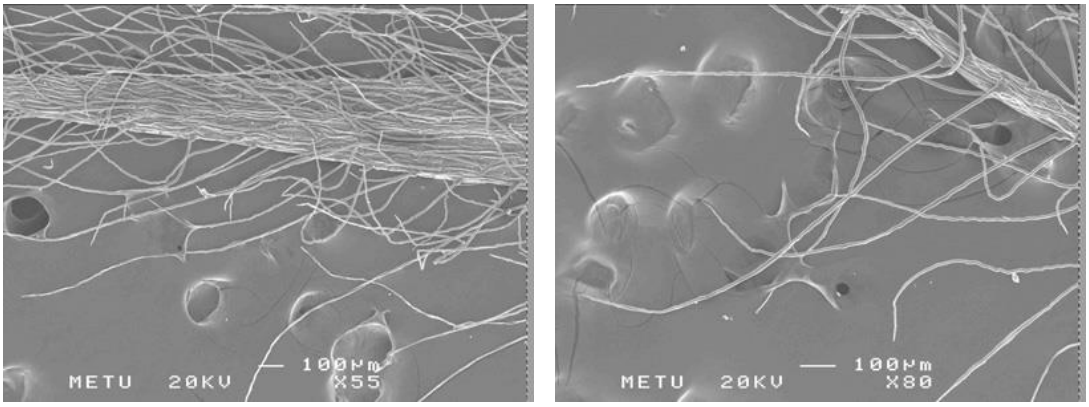

Figure 3-88. Very long non- glandular trichome on the calyx surface of *L. czeczottianus*

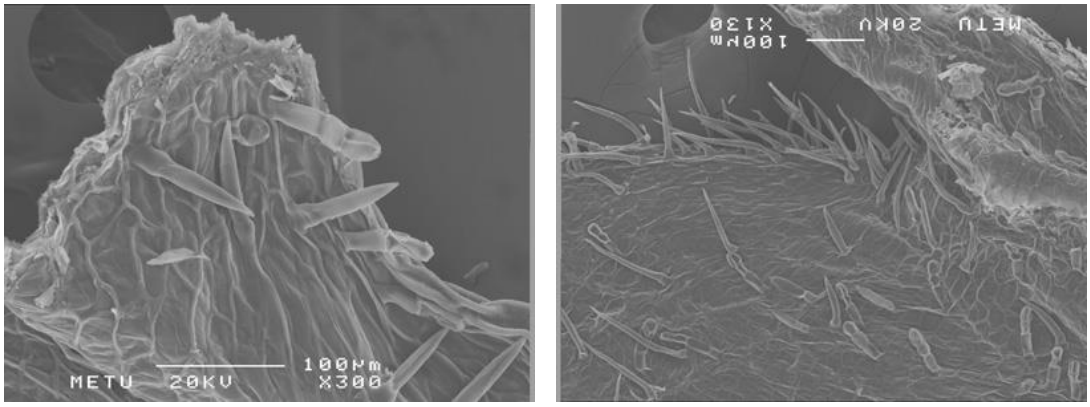

Figure 3-89. Non-glandular (acicular) trichome on the calyx surafce of *L. armenus*

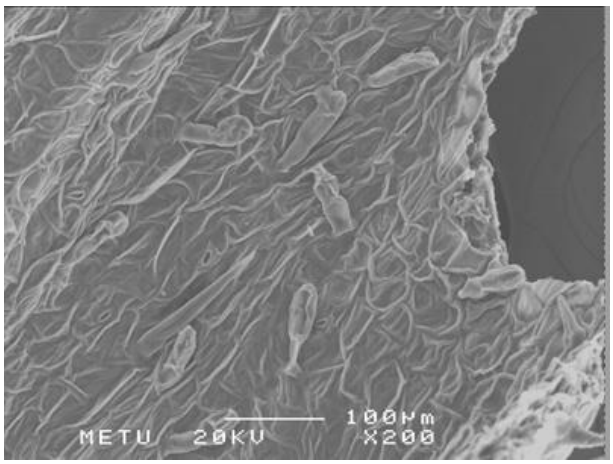

Figure 3-90. Non-glandular (acicular) trichome on the calyx surface of *L. pallesence*

3.2.2.2 Ordination Analyses and Phenetic Cluster results for Micromorphological traits of Calyx

Observed (qualitative) characters of the calyx surface (presence of stomata, trichome type) were coded as 0, 1, and 2 and scored into numerical value as a data matrix in order to obtain PCA (as ordination analyses) and UPGMA (as clustering analyses) using the PAST 3.05 software program as shown in **Table 3-11**.

Table 3-11 Data matrix of micromorphological traits (qualitative) of Calyx among 28 species of *Lathyrus*

Species	P. stomata	T.type
L.digitatus **(1)**	1	1
L.karsianus **(2)**	1	1
L.tukhtensis **(3)**	1	1
L.cyan **var.** *cya* **(4)**	1	1
L.armenus **(5)**	1	1
L.boissieri **(6)**	1	1
L.pallesence **(7)**	1	1
L.atrapatanus **(8)**	1	0
L.satdaghens **(9)**	1	1
L.elangatus **(10)**	1	1
*L.spathulatus(***11)**	1	1
L.variabilis **(12)**	1	0
L.cilicicus **(13)**	1	1
L.brachypteru **(14)**	1	1
L.haussknecht **(15)**	0	0
L.aureus **(16)**	1	1
L.vernus **(17)**	1	0
L.laxiflorus **(18)**	1	1
L.roseus **(19)**	1	2
L.annuus **(20)**	1	2
L.niger **(21)**	1	1
L.clymenum **(22)**	1	1
L.sativus **(23)**	1	1
L.pratensis **(24)**	1	1
L.cicera **(25)**	1	1
*L.gorgoni (***26)**	1	2
*L.undulatus***(27)**	1	2
*L.czeczottianus***(28)**	1	0

Eigenvalues and their corresponding % of total variance that is accounted in the PCA are given in **Table 3-12**. The first eigenvalue is a very high percentage (91.44 %) of total variance.

Table 3-12 Eigenvalues and % variances of each component for qualitative calyx data in PCA.

Principal Component (PC)	Eigenvalue	% variance
1	0.336255	91.442
2	0.0314702	8.5581

Scatter plot (X axis is PC1, Y axis is PC2) in the PCA is shown in the **Figure 3-91**. In the loading plot, **Figure 3-92**, A is presence of stomata, B is trichome type.

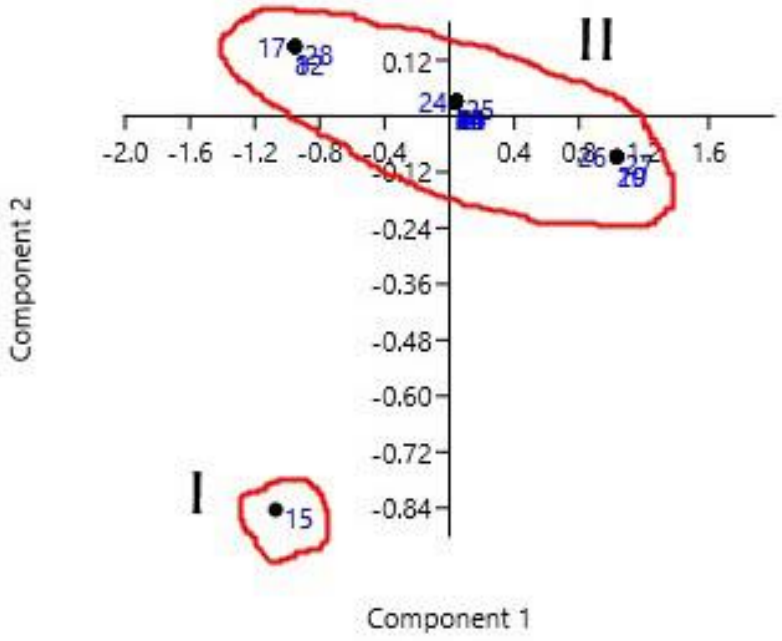

Figure 3-91 A two dimensional scatter plot of qualitative calyx data constructed in the PCA.

Group I covers : *L.haussknechtii* (15), Group II covers : *L.digitatus* (1), *L.karsianus* (2), *L.tukhtensis* (3), *L.cyaneus* var. *cyaneus* (4), *L.armenus* (5), *L.boissieri* (6), *L.pallesence* (7), *L.atropatanus* (8), *L.satdagensis* (9), *L.elangathus* (10), *L.spathulatus* (11), *L.variabilis* (12), *L.cilicicus* (13), *L.brachypterus* (14), *L.aureus* (16), *L.vernus* (17), *L.laxiflorus* (18), *L.roseus* (19), *L.annuus* (20), *L.niger* (21), *L.clymenum* (22), *L.sativus* (23), *L.pratensis* (24), *L.cicera* (25), *L.gorgoni* (26), *L.undulatus* (27) , *L.czeczottianus* (28)

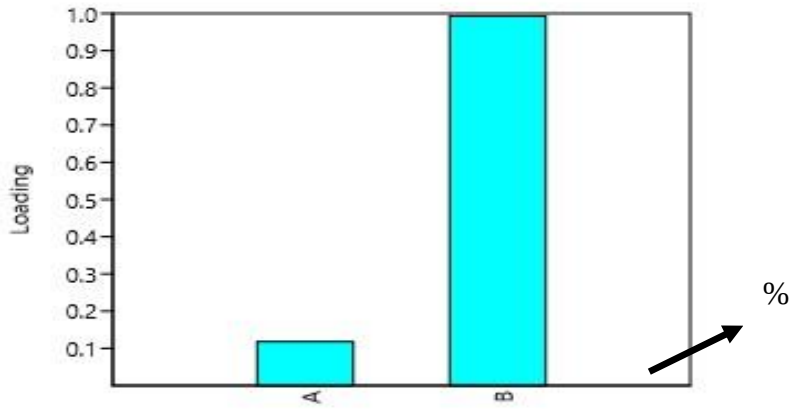

Figure 3-92 Loading plot of qualitative calyx data constructed in the PCA.

A: is presence of stomata, B: is trichome type

Percent of the total variation that is accounted by the first axis (% 91.44) is shown by the arrow.

There are two groups in the PCA. *L. haussknechtii* (15) is in one group (I), and the rest of species are in the second group (II). Some species overlap in the second group. All of the species examined have stomata in their calyx surface except *L. haussknechtii*. Its trichome is typical non–glandular.

Trichome type plays a much more important role than the presence of stomata due to the loading plot (**Figure 3-92**).

3.2.3 Mixed data (Leaf, Calyx)

Qualitative data in the calyx surface has been mixed with qualitative data of the leaf to evaluate characters. In total, sixteen qualitative characters of leaf and calyx have been analyzed. Eigenvalues and their corresponding % of total variance that is accounted in the PCA are provided in **Table 3-13**.

Table 3-13 Eigenvalues and % variance of each component for qualitative mixed data in PCA.

Principal Component (PC)	Eigenvalue	% variance
1	2.76652	44.49
2	1.22699	19.732
3	0.862484	13.87
4	0.573088	9.2162
5	0.262359	4.2192
6	0.138698	2.2305
7	0.115917	1.8641
8	0.0891822	1.4342
9	0.0626274	1.0072
10	0.0530993	0.85393
11	0.03184	0.51204
12	0.0214514	0.34497
13	0.00988503	0.15897
14	0.00411552	0.066184
15	0.010804	0.017375
16	0.0491658	0.079067

The first of the four variances are the highest percentage (44.49 %, 19.73 %, 13.87 %, and 9.21 %) of the total variance. The scatter plot for qualitative mixed data is shown in **Figure 3-93** and is very similar to the scatter plot for qualitative leaf data shown in **Figure 3-61**.

In the loading plot, from A to N relates to qualitative data in leaf, O is the presence of stomata of calyx surface, and P is trichome type of calyx surface.

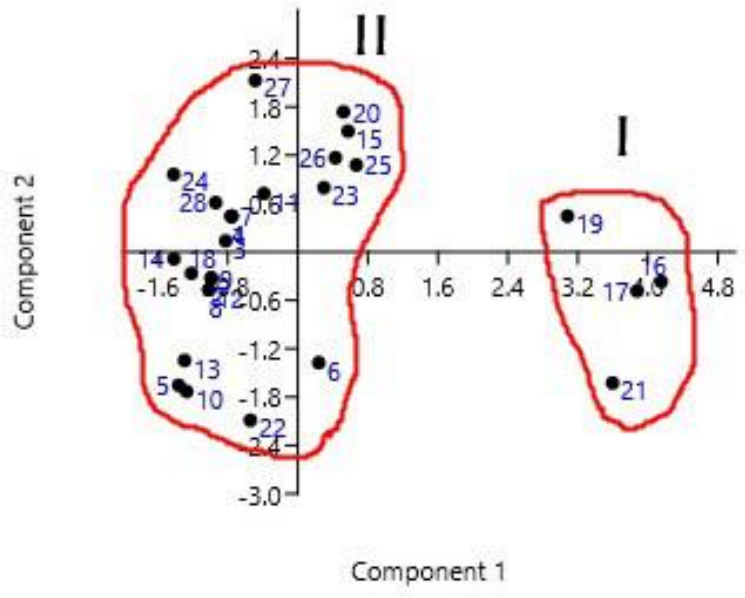

Figure 3-93 A two dimensional scatter plot of mixed qualitative data in the PCA.

Group I covers *: L.aureus* (16), *L.vernus* (17), *L.roseus* (19), *L.niger* (21) Group II covers*: L.digitatus* (1), *L.karsianus* (2), *L.tukhtensis* (3), *L.cyaneus* var. *cyaneus* (4) , *L.armenus* (5), *L.boissieri* (6), *L.pallesence* (7), *L.atropatanus* (8), *L.satdagensis* (9), *L.elangatus* (10),*L.spathulatus* (11),*L.variabilis* (12),*L.cilicicus* (13), *L.brachypterus* (14),*L.haussknechtii* (15), *L.laxiflorus* (18), *L.annuus* (20), *L.clymenum* (22), *L.sativus* (23), *L.pratensis* (24), *L.cicera* (25), *L.gorgoni* (26), *L.undulatus* (27) , *L.czeczottianus* (28)

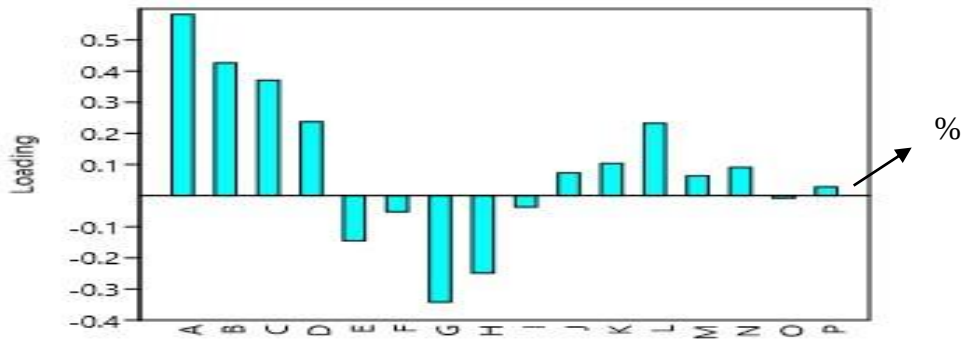

Figure 3-94 Loading plot of qualitative mixed data constructed in the PCA.

A :is epidermal cell shape of adaxial, B :is epidermal cell of abaxial, C :is cell wall pattern of adaxial, D :is cell wall pattern of abaxial, E :is presence of stomata of adaxial , F :is presence of stomata of abaxial , G :is stomoata position of adaxial, H :is stomata position of abaxial, I :is presence of trichome of adaxial, J :is presence of trichome of abaxial, K :is trichome type of adaxial, L :is trichome type of abaxial, M :is granular pattern of adaxial, N :is granular pattern of abaxial , O :is presence of stomata of calyx and P :is trichome type of calyx.

Percent of the total variation that is accounted by the first axis (% 44.49) is shown by the arrow.

There are two groups in the PCA. *L. aureus* (16), *L. vernus* (17), *L. roseus* (19) and *L. niger* (21) comprise one group, and the remainder of species comprise the second group. In the first group *L. aureus* (16), *L. vernus* (17), and *L. niger* (21) belong to sect. *Orobus*. *L. roseus* (19) belong to sect. *Orobon*. Therefore grouping in the scatter plot is very similar to the PCA of qualitative leaf data. According to the loading plot, A, B, C, D, G, H, L holds the most important place. The presence or absence of stomata (O) and trichome type in the calyx surface (P) result in less impact than other characters.

The phenogram tree constructed in the UPGMA cluster analyses employing Euclidean (Cophen corr, 0.92) is given in **Figure 3-95**. UPGMA cluster analyses using Bray Curtis (Cophen corr, 0.87) is shown in **Figure 3-96**.

The phenon line at 3.8 (Euclidean similarity index, **Figure 3-95)** creates two groups or phenon in the UPGMA tree, and results are almost identical in the tree which is obtained by leaf data as given in **Figure 3-63**. *L. aureus* (16), *L. vernus* (17), *L. roseus* (19), and *L. niger* (21) are in one phenon, and the remainder of species are in the second phenon. Bootstrap value was increased in UPGMA with qualitative mixed data in some nodes compared to UPGMA obtained with leaf qualitative data. For example, *L. annuus* (20) and *L. undulatus* (27) have been supported with 81 % bootstrap in mixed data as their support value with only leaf data is 71 %. Therefore, adding qualitative data of calyx to leaf data results in the same grouping with higher bootstrap support. There are some differences between both phenograms with mixed data as shown in **Figure 3-95** and **Figure 3-96**. For example, *L. boissieri* (6) holds a different place in both phenograms. Also, some species of sect. *Lathyrostylis*, such as *L. karsianus* (2), *L. satdaghensis* (9) and *L. atropotanus* (8), *L. variabilis* (12) overlap, and all of these species have a distance about 1 in UPGMA (Euclidean similarity index) with mixed data. All of these species overlap in UPGMA (Euclidean similarity index) and have distance 0 in phenogram with leaf qualitative as given in **Figure 3-63**. According to qualitative calyx data as given in **Figure 3-91**, *L. haussknechtii* (15) is in one group, and the remaining of species are in the second group. Its placement in qualitative leaf and mixed data (leaf and calyx) is close to *L.*

sativus (23), *L. cicera* (25) and *L. gorgoni* (26). Further analyses are needed to evaluate its exact placement.

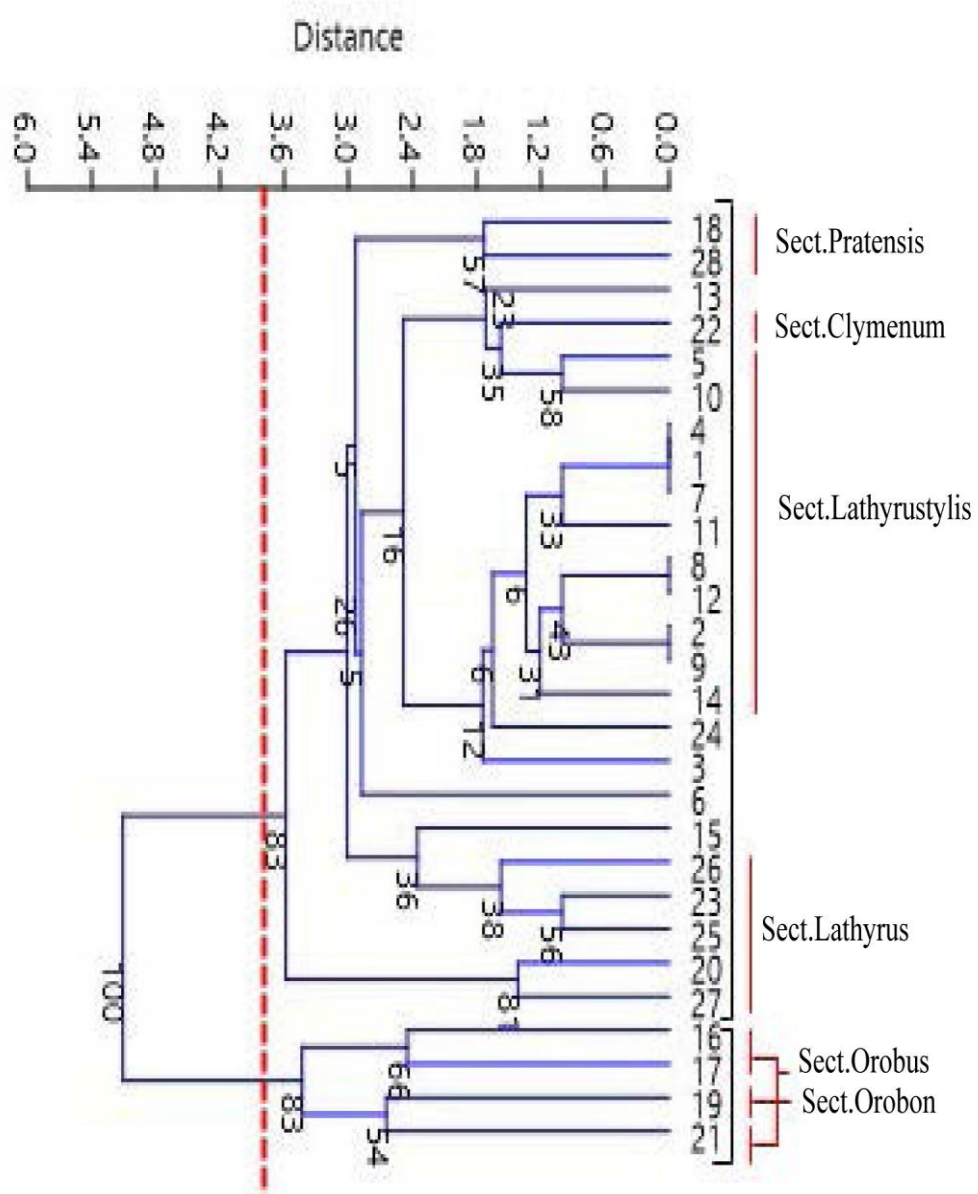

Figure 3-95 UPGMA using Euclidean similarity index with mixed data (calyx and leaf).

Red dash line represents phenon line (at 3.8). Solid vertical red lines represent sections.*L.digitatus* (1), *L.karsianus* (2), *L.tukhtensis* (3), *L.cyaneus* var. *cyaneus* (4), *L.armenus* (5), *L.boissieri* (6), *L.pallesence* (7), *L.atropatanus* (8), *L.satdagensis* (9), *L.elangatus* (10), *L.spathulatus* (11), *L.variabilis* (12), *L. cilicicus* (13*), L. brachypterus* (14), *L.haussknechtii* (15), *L.aureus* (16), *L.vernus* (17), *L.laxiflorus* (18), *L. roseus* (19), *L.annuus* (20), *L.niger* (21), *L.clymenum* (22), *L.sativus* (23), *L.pratensis* (24), *L.cicera* (25), *L.gorgoni* (26), *L.undulatus* (27) , *L.czeczottianus* (28).

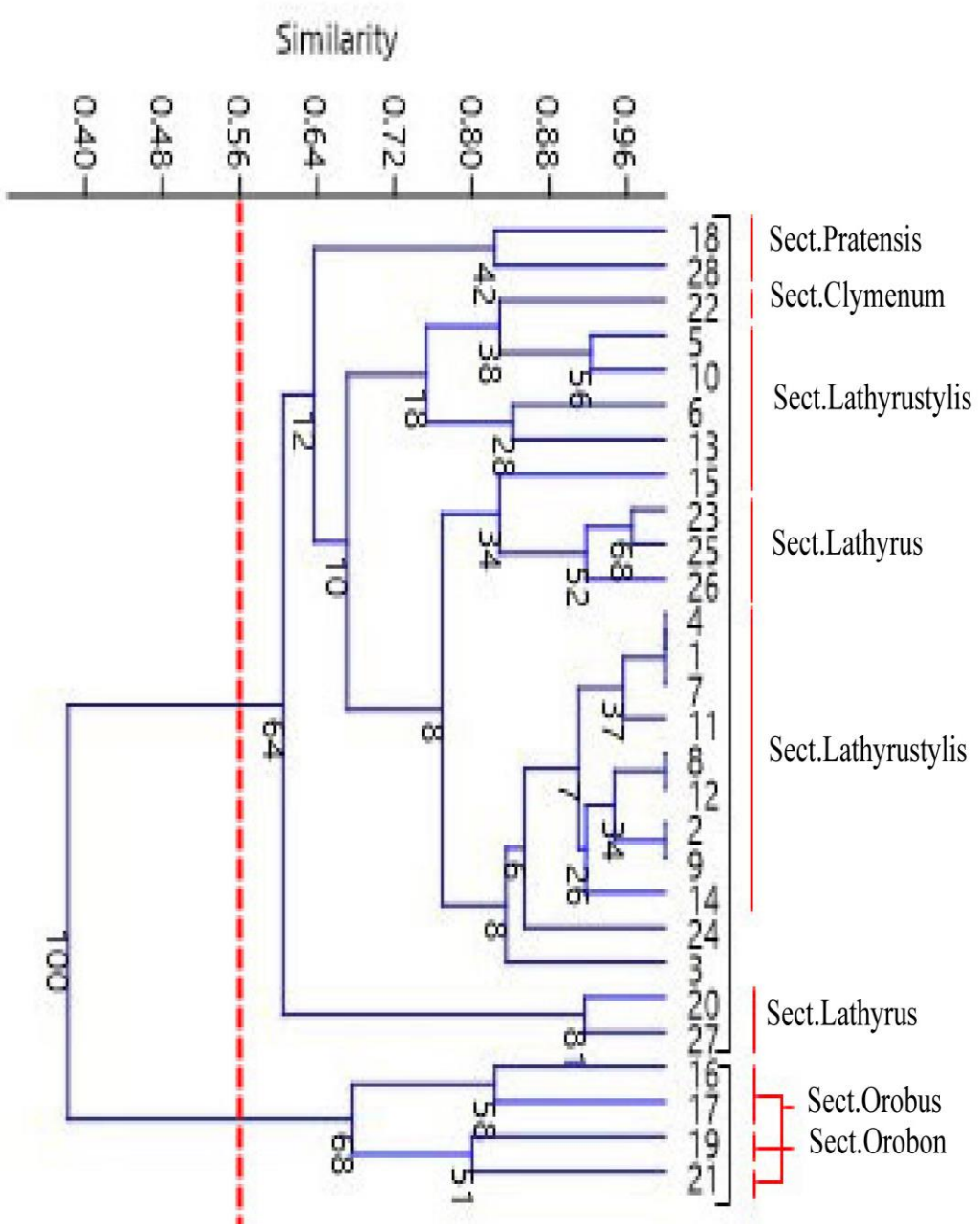

Figure 3-96 UPGMA using Bray Curtis index with mixed data (calyx and leaf).

Red dash line represents phenon line (at 0.56). Solid vertical red lines represent sections. *L.digitatus* (1), *L.karsianus* (2), *L.tukhtensis* (3), *L.cyaneus* var. *cyaneus* (4) , *L.armenus* (5), *L.boissieri* (6), *L.pallesence* (7), *L.atropatanus* (8), *L.satdagensis* (9), *L.elangatus* (10), *L. spathulatus* (11), *L.variabilis* (12), *L. cilicicus* (13), *L.brachypterus* (14), *L.haussknechtii* (15), *L.aureus* (16), *L.vernus* (17), *L.laxiflorus* (18), *L.roseus* (19), *L. annuus* (20), *L.niger* (21), *L.clymenum* (22), *L.sativus* (23), *L.pratensis* (24), *L.cicera* (25), *L.gorgoni* (26), *L.undulatus* (27) , *L.czeczottianus* (28)

The calyx and corolla surface of *L. digitatus* (1), *L. sativus* (23) have been analyzed by Cildir et al (2012). Both species have only non-glandular trichome in their calyx surfaces, but in the present study these species contain glandular and non-glandular trichomes in their calyx. There has been so far little micromorphological research on calyx of *Lathyrus*.

In general, UPGMA produced with Euclidean similarity index in **Figure 3-95** and Bray curtis similarity index in **Figure 3-96** based on qualitative characters of mixed data (leaf , calyx) is in agreement with the existing estimates of phylogeny from Kupicha, (1983); Asmussen and Liston , (1998); Kenicer et al., (2005) and Schafer et al., (2012).

Finally, a comparison between the results of the present study and the two previous studies: one based on molecular characters (Schaefer et al, 2012) the other based on morphological characters (Kupicha, 1983) can be made by referring to **Table 3-14.** These two studies are used because they harbor many of the species employed in the present study: Schaefer et al's, (2012) study covered 17 of the 28 species used in the present study; and Kupicha's, (1983) study covered 27. Also, Kupicha's, (1983) study constitutes the only worldwide treatment of the genus *Lathyrus*, and Scheafer et al's, (2012) work is the most recent molecular-based research study of this genus. Comparisons are made on the basis of the assignments of the species to their sections. **Table 3-14**, proves that micromorphological characters used in the present study are useful to classify 23 (out of 28) *Lathyrus* species studied in their previously assigned sections. Further analyses are required to evaluate species such as *L. boissieri, L. haussknechtii, L. roseus, L. clymenum and L. pratensis*.

Quantitative charatcters (stomatal width, stomatal length, non-glandular trichome length, and glandular trichome length) have been measured for calyx surface as given in **Table 3-10** and were used in the PAST 3.05 software program in order to obtain UPGMA. Similarly to quantitative data obtained from the leaf, *Lathyrus* species tend to disagree with existing phylogenetic estimate of relationships when this data is used (Appendix B, Figure B5). The same is also true when quantitative data is used as individual (Appendix B, Figure B6). All samples are scattered across the tree. Missing data could have influenced the phenogram a little, but it could also imply

that quantitative data may differ among species in response to something other than a simple DNA relationship as the UPGMA tree does not reflect an evolutionary pattern. This therefore suggests that the quantitative traits are variable within species and there may be environmental factors which are affecting the outcome.

Table 3-14 Comparative analyses of the examined *Lathyrus* species in relation to their sections in different studies.

Numbers represent species‘s numbers of the present study.

	Schaefer et al, (2012)	**Kupicha, (1983)**	**Sections**	**Present study**
	1 3 7 11	1 8 2 9 3 10 4 11 5 12 6 13 7 14	Lathyrostylis	1 8 2 9 3 10 4 11 5 12 6 13 7 14 15
	16 17 21	16 17 21	Orobus	16 17 21
	19	19	Orobon	19
	18 28 24	18 28 24	Pratensis	18 28 24
	20 23 25 26 27	20 23 25 26 27	Lathyrus	20 23 25 26 27
	22	22	Clymenum	22
Common sp	**17**	**27**		–
Total # of species	**119**	**153**		**28**

Common sp: Species those are common with the present study.Total # of species: total species that are used in the studies. Blue, black, orange and Green arrows indicate species that are in unexpected sections. *L.digitatus* (1), *L.karsianus* (2), *L.tukhtensis* (3), *L.cyaneus* var. *cyaneus* (4), *L.armenus* (5), *L.boissieri* (6), *L.pallesence* (7), *L.atropatanus* (8), *L.satdagensis* (9), *L.elangatus* (10), *L.spathulatus* (11), *L.variabilis* (12), *L.cilicicus* (13), *L.brachypterus* (14), *L.haussknechtii* (15), *L.aureus* (16),

L.vernus (17), *L.laxiflorus* (18), *L.roseus* (19), *L.annuus* (20), *L.niger* (21), *L.clymenum* (22), *L.sativus* (23), *L.pratensis* (24), *L.cicera* (25), *L.gorgoni* (26), *L.undulatus* (27) , *L.czeczottianus* (28). Arrows show species (*L. boissieri, L. haussknechtii, L. roseus, L. clymenum, L. pratensis*) which are not placed in their sections in this study.

4. CONCLUSION

Micromorphological characters of both leaf surfaces of the 15 species (sect. *Lathyrostylis*) were investigated in this study using a light microscope. Many more micromorphological characters of both the leaf surfaces and the outer surface of the calyx of the 28 species (sect. *Lathyrostylis, Orobon, Orobus*, *Pratensis, Clymenum, Lathyrus*) were studied using a scanning elctron microscope (SEM).

A multivariate statistical approaches through an ordination and cluster analyses was used, thereby objectively achieving the discrimination of the species and sections.

There are two main groups in the UPGMA tree obtained and in the PCA of qualitative characters of leaf surface and mixed data (leaf and calyx). *L. aureus, L. vernus, L. roseus, L. niger* are in one group and the remaining species are in the second group. *L. aureus, L. vernus* and *L. niger* belong to sect. *Orobus,* while *L. roseus* belongs to sect. *Orobon.* Thus, it seems that, in accordance with the characters, methods, species and sections used in the study, *Orobus* and *Orobon* are the most distinct sections. In all of the analyses, *L. pallesence* and *L. cyaneus* var. *cyaneus* overlap based on their micromorphological characters.

The constructed UPGMA based on the Euclidean similarity index of qualitative micro morphological characters on the leaf surface and mixed data is largely in agreement with the existing estimates of phylogeny from Kupicha, (1983); Asmussen and Liston, (1998); Kenicer et al., (2005) and Schaefer et al., (2012). Therefore these results support the premise that qualitative microcharacters on the leaf surface reflect an evolutionary relationship. These characters help to classify *Lathyrus* species at the sectional level. Epidermal cell shape, cell wall pattern, stomata position on both leaf surfaces and trichome type of the abaxial leaf surface seem to have a higher level of discriminating power compared to other variables employed.

Relationships of the species based on quantitative characters of both the leaf surface and the outer surface of the calyx tend to disagree with existing phylogenetic estimates of relationships. Missing data may have influenced the clustering. In addition, the variability of quantitative characters within species maybe more affected by environmental factors than by qualitative characters. Therefore, environmental effects might have blurred phylogenetic relationships when quantitative data was used.

Several suggestions for future study can be proposed. First of all, micromorphological characters of the corolla should be included in the study. In this way, a higher number of discriminating characters could perhaps be found. Secondly, more populations of the species examined could be analyzed to reassess the phenograms obtained here. In this way, the robustness of the phenograms can be increased. Thirdly, *L. aphaca* has been studied in many molecular studies (for example, Asmussen and Liston, 1998) and exhibits a close relationship with *L. pratensis*, so its micromorphological characters should be involved in the study. Then, perhaps, *L. pratensis* could be correctly allocated in its section. Also, more species of sect. *Clymenum* should be investigated in order to evaluate its placement in the tree. In other studies, some other species like *L. filiformis, L. hierosolymitanus,* and *L. amphicarpus* exhibit strong linkage with some species of the present study. But as they belong to other flora (Israel, Bulgaria, and Portugal), they were not considered here. Maybe in the future, their micromorphological characters should also be included in a study with a larger span. Such a study would reveal more general results covering western Eurasian species of the genus *Lathyrus.* Light microscope studies with fresh material would give more accurate results, especially related to stomata index. Lastly, most of the species of sect. *Lathyrostylis* have not been previously studied using molecular markers. In the future it can be expected that molecular studies involving a higher number of species from this section as well as other *Lathyrus* species will provide more data for better comparisons with the morphological data. It must also be emphasized that the inclusion of distant species to be used as outgroups will provide more directionality within the phenograms.

It can be concluded that in the future, the addition of new species and many populations of the species, and the examination of species in the genus *Lathyrus* by morphological and molecular characters, especially in the presence of out-group species, will reveal the true phylogeny of the genus. However, this task is not easy to accomplish, and therefore the big picture needs to be completed in small steps. In this context the present study provides some results by using micromorphological characters of the leaf and calyx of Turkish *Lathyrus* species. This study, also, serves as a first attempt to assess the taxonomic values of *Lathyrus* micromorphological characters.

A diagnostic key using leaf micromorphological characters is presented in below. It was not possible to find any significant difference between some *Lathyrus* species from the point of view of leaf micromorphology. Therefore, these species are presented in one branch.

Group A

1. No stomata on adaxial leaf surface; stomata position on abaxial leaf surface is even

   2. Epidermal cell shape of both leaf surface are isodiametric, cell wall pattern of both leaf surface are deeply sinuous, Sect. *Orobus.*

      3. Only non- glandular trichome type on abaxial leaf surface, *L. niger*

      3. Both non- glandular and glandular trichome type on both leaf surfaces

         4. Stomata position on adaxial leaf surface is even, no stomata on abaxial leaf surface, *L. vernus*

         4. Stomata position on abaxial leaf surface is even, no stomata on adaxial leaf surface, *L. aureus*

2. Epidermal cell shape of both leaf surface are isodiametric slightly elongated, cell wall pattern of both leaf surface are deeply sinuous, and have only glandular trichome on abaxial leaf surface, Sect. *Orobon*. *L. roseus*

Group B

1. Stomata presence on both leaf surfaces with sunken position

5. Epidermal cell shape of abaxial leaf surface is elongated

6. Cell wall pattern of adaxial leaf surface is wavy or straight, cell wall pattern of abaxial leaf surface is deeply sinuous, Sect. *Clymenum*. *L. clymenum*

6. Cell wall pattern of abaxial leaf surface is straight, Sect. *Lathyrostylis,* no trichome on both leaf surface, glabrous, *L. armenus*, *L. elongatus*, with only non-glandular trichome type on adaxial leaf surface, *L. cilicicus, L. boissieri,* both trichome type on abaxial leaf surface, *L. tukhtensis* Only non–glandular trichome on adaxial leaf surface and both trichome types on abaxial leaf surface, *L. digitatus, L. cyaneus* var *cyaneus, L. pallesence,* with straight cell wall pattern on both leaf surface, *L. spatulathus.* Only non-glandular trichome on both leaf surface, *L. karsianus, L. atropoatnus, L. satdaghensis, L. variabilis, L. brachypterus*

7. Glandular trichome on abaxial leaf surface, epidermal cell shape are elongated on both leaf surface, *L. annuus,* epidermal cell shape are slightly elongated on both leaf surface, *L. undulatus*

7. Glandular trichome on both leaf surface, epidermal cell shape are elongated on both leaf surface, *L. sativus, L. gorgoni, L. haussknechtii, L. cicera*

5. Epidermal cell shape of abaxial leaf surface is slightly elongated, Sect. *Pratensis*

8. Cell wall pattern are deeply sinuous on adaxial and wavy or straight in abaxial leaf surface, no trichome on abaxial leaf surface, *L. laxiflorus*, non -glandular trichome on both leaf surface, *L. czeczottianus*

8. Cell wall pattern are wavy or straight on both leaf surface, both type of trichome on abaxial leaf surface, *L. pratensis*

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APPENDIX A

INFORMATION OF *LATHYRUS SPECIES EXAMINED*

Table A 1 Voucher number and collection information of *Lathyrus* Species

Species	Date of collection	Collector	Place of collecting	Voucher number
L.digitatus	11.05.2008	F. Gunes	Sogucak village / savas tepe/ balik esir	169
L.karsianus	09.07.2007	F. Gunes	Sarikamis / kars	970
L.tukhtensis	28.07.2007	F. Gunes	Akhisar village – kale / gumushane	1327
L.cyaneus var cyan	11.08.2007	F. Gunes	Boga tepe village/ kars	1512
L.armenus	21.06.2008	F. Gunes	Sogan village road/ 200m from the main road/ Agri	1866
L.boissieri	07.06.2009	F. Gunes	Siverek – karacadag road, 37.km borders of field	2268
L.pallesence	06.06.2009	F. Gunes	Guney kahya village , eleskirt/ agri	2246
L.atrapotanus	14.06.2008	F. Gunes	Van – hakkari road, guzel dere gate way/ van	1848
L.satdaghensis	07.06.2008	F. Gunes	Yuksek ova – daglicay / Hakkari	1828

Table A 1 continued

L.elangatus	27.04.2005	F. Gunes	Mersin – guzel oluk road, Mersin	59
L.spathulatus	29.04.2008	F. Gunes	Alman pinari, osmaniye	1605
L.variabilis	26.04.2008	F. Gunes	Dogan sehir –gol basi road, 40.km ,5 km after erkenek, Malatya	1541
L.cilicicus	11.06.2009	F. Gunes	Hadin, taskent road 3. Km, Konya	2314
L.brachypterus	01.06.2008	F. Gunes	Binboga daglari, elbistan , kahramanmaras	1808
L.haussknechtii	12.06.2008	F. Gunes	Pirresit dagi, muradiye, van	1835
L.aureus	07.06.1976	T. Ekim	Eski sehir Turkmen D. Fargus alti	2448
L.vernus	30.03.1983	A.Guner	Artvin: muratliya 1. Km. 100-150 m , sarp kayalik ve kumlu yamac., staphylea , caliliklari ve acikliklari	4665
L.laxiflorus	07.06.1976	T. Ekim	Eskisehir: Turkmen Q. yaygin	3143

Table A 1 continued

L.roseus	14.06.1981	R.Ilarslan	Tokat,artova yenice koy,kayalidere tepesi, karsikorman ici	1280
L.annuus	23.05.1985	M. Demirors	Zonguldak, hisaronu kale ici, ca (50m)	1703
L.niger	05.06. 1968	Y.Akman	Hatay –erzin amanos daglari, ca (1100 m)	219
L.clymenum	30.05.1978	O. Ketenoglu	Kastamonu, inebolu ya 10 km kala, ca (100m)	652
L.sativus	27.05.1986	A.Atik	Konya, Kadin hanladik guneyi ,karadag kuzey dagi yamaclari , ca (1550 m)	353
L.pratensis	17.07.1985	M. Demirors	Karabuk, keltepe bolge binalari cevresi , ca(1175m)	1655
L.cicera	12.05.1982	Y. Akman, E Yurdakulol, M Demirors	Kastamonu- ilgaz-kursunlu yol ayirimi, ca (900 m)	12377
L.gorgoni	21.04.1958	K. Karamonoglu	Tarsus, A7 kanali banketlerinde	5329
L.undulatus	02.06.2001	E. Yurdakulol	Istanbul , pasa koy , omerli , baraj golu alti (200 m)	3682
L. czeczottianus	12.06.1975	Y. Akman	Isik dagi , kara cam alti	25

APPENDIX B

PHENOGRAMS

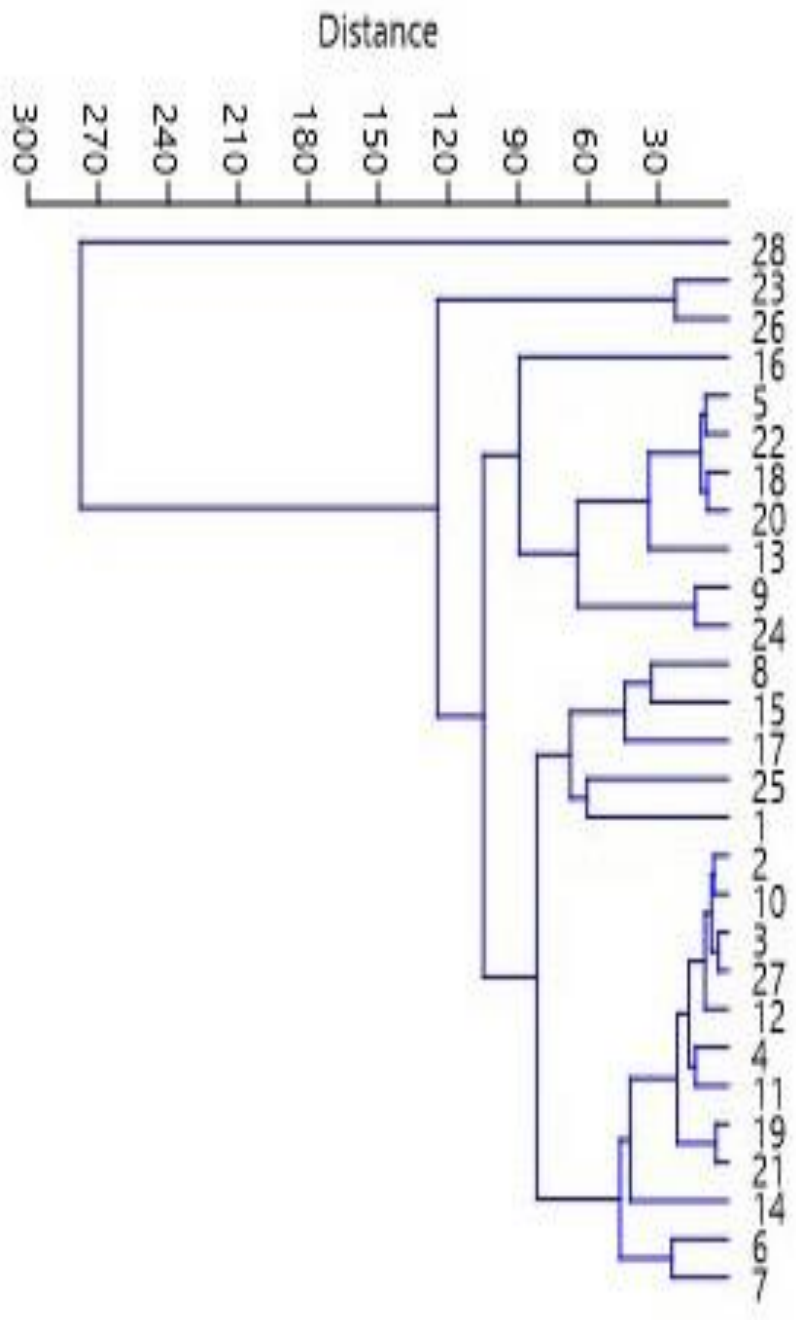

Figure B-1 UPGMA using Euclidean similarity index with quantitative data of adaxial leaf surface

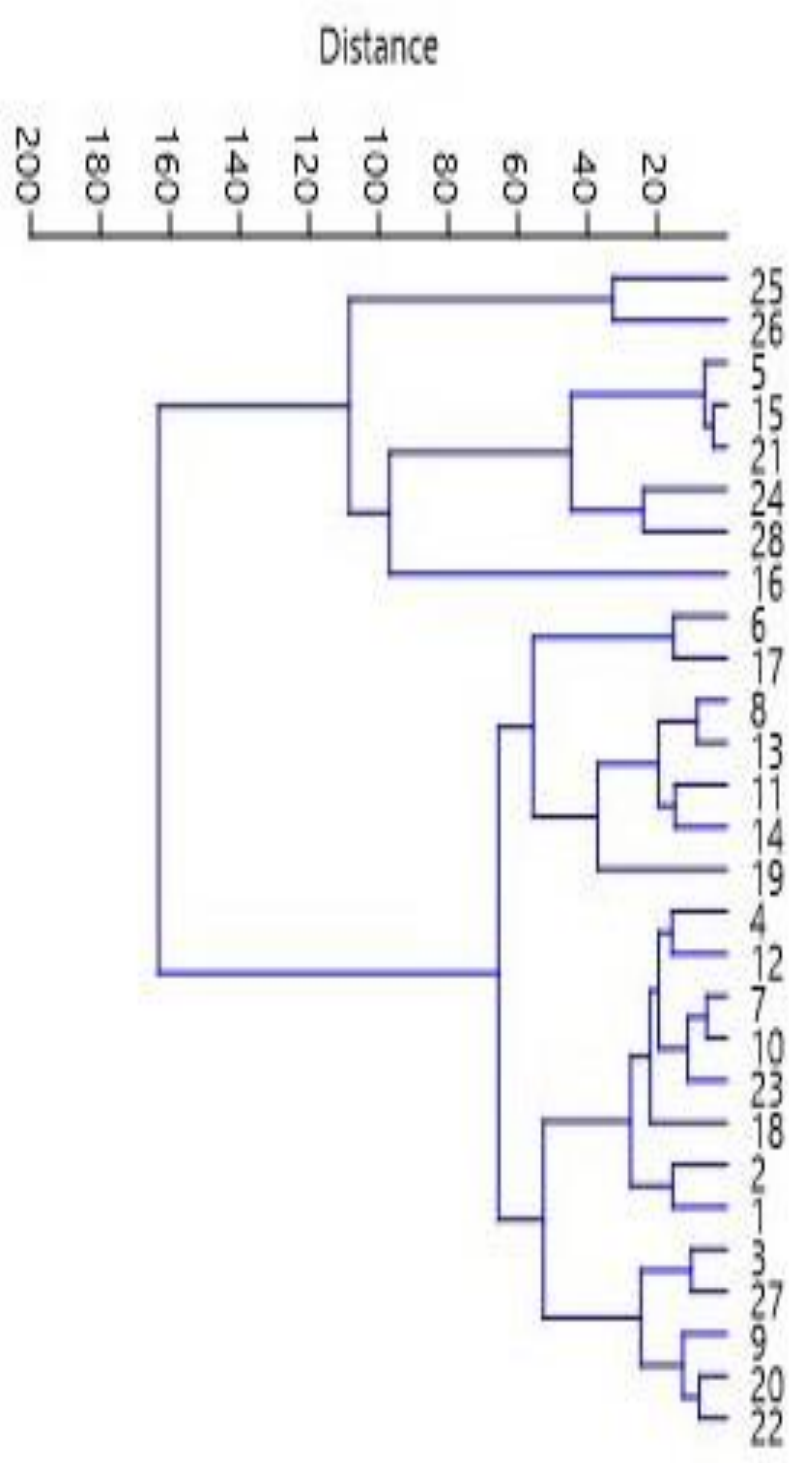

Figure B-2 UPGMA using Euclidean similarity index with quantitative data of abaxial leaf surface

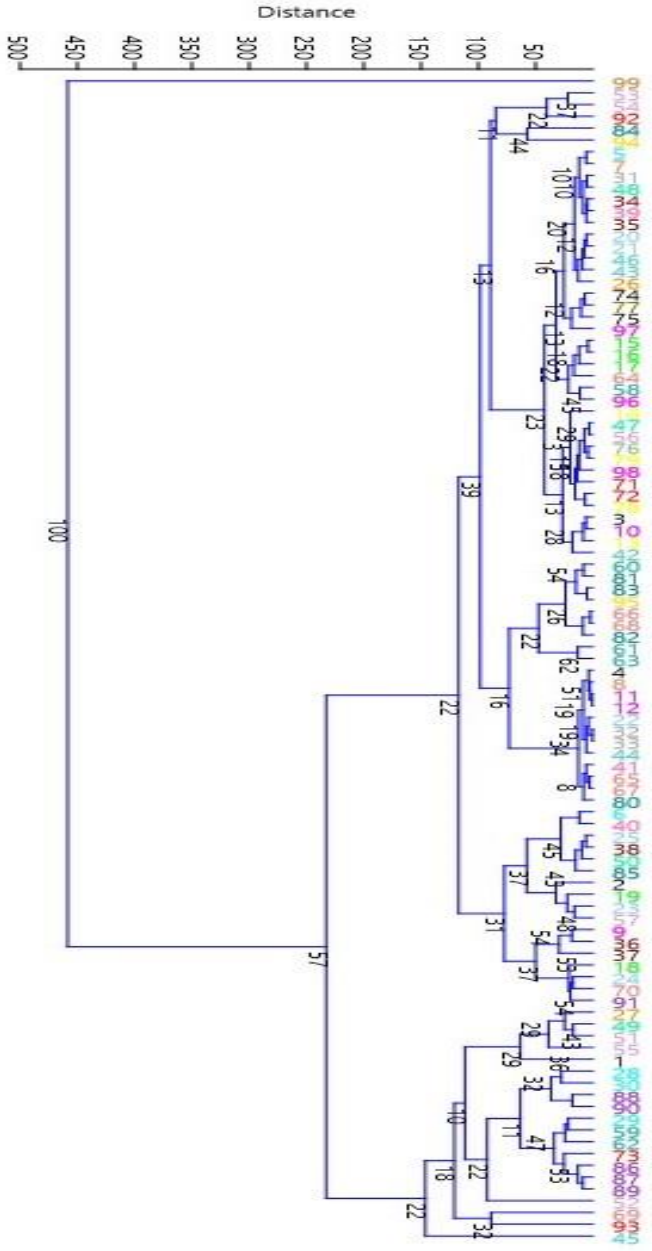

Figure B-3 UPGMA using Euclidean similarity index with individual quantitative data of adaxial leaf surface

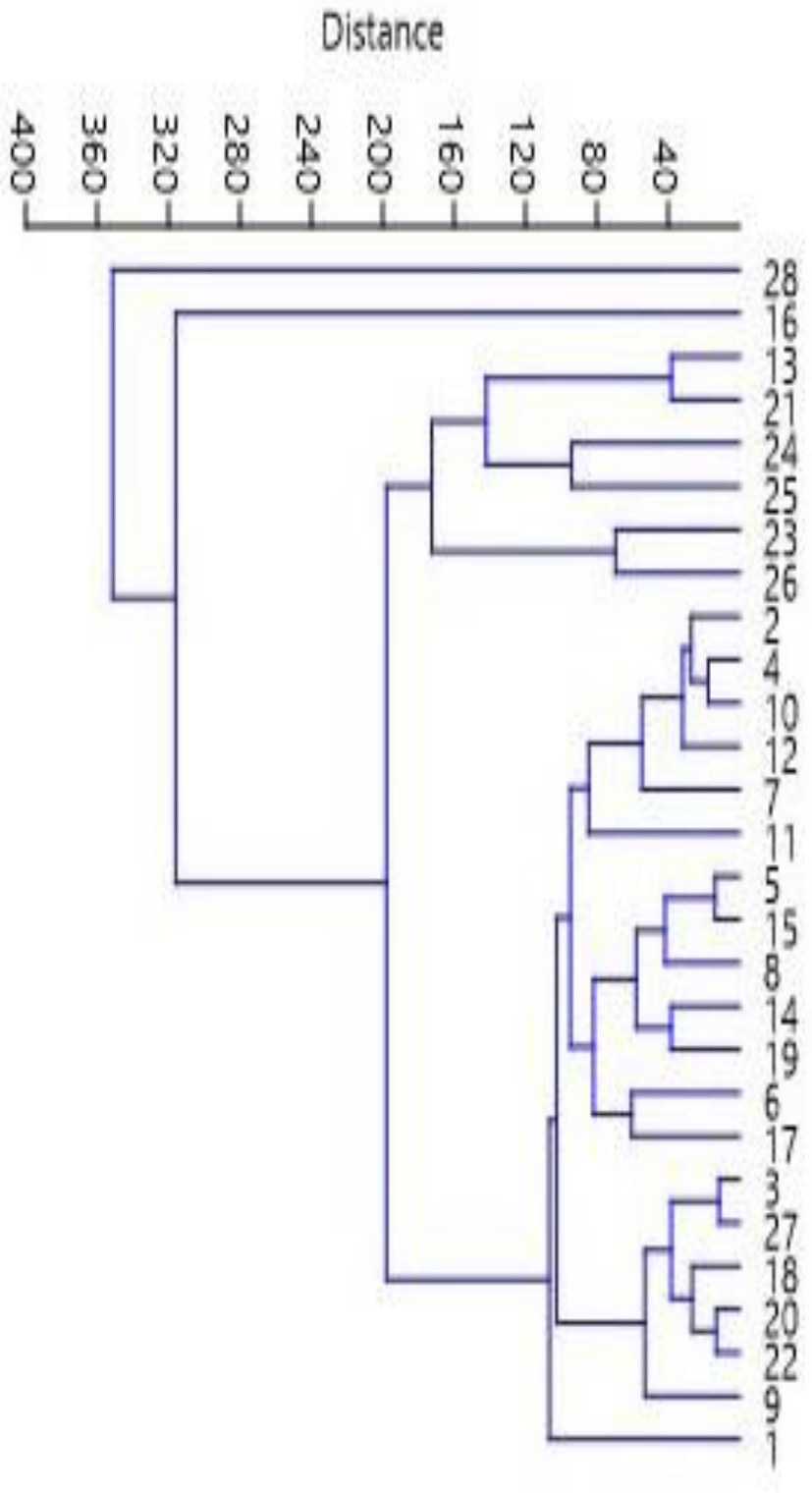

Figure B-4, UPGMA using Euclidean similarity index with quantitative data of both leaf surface

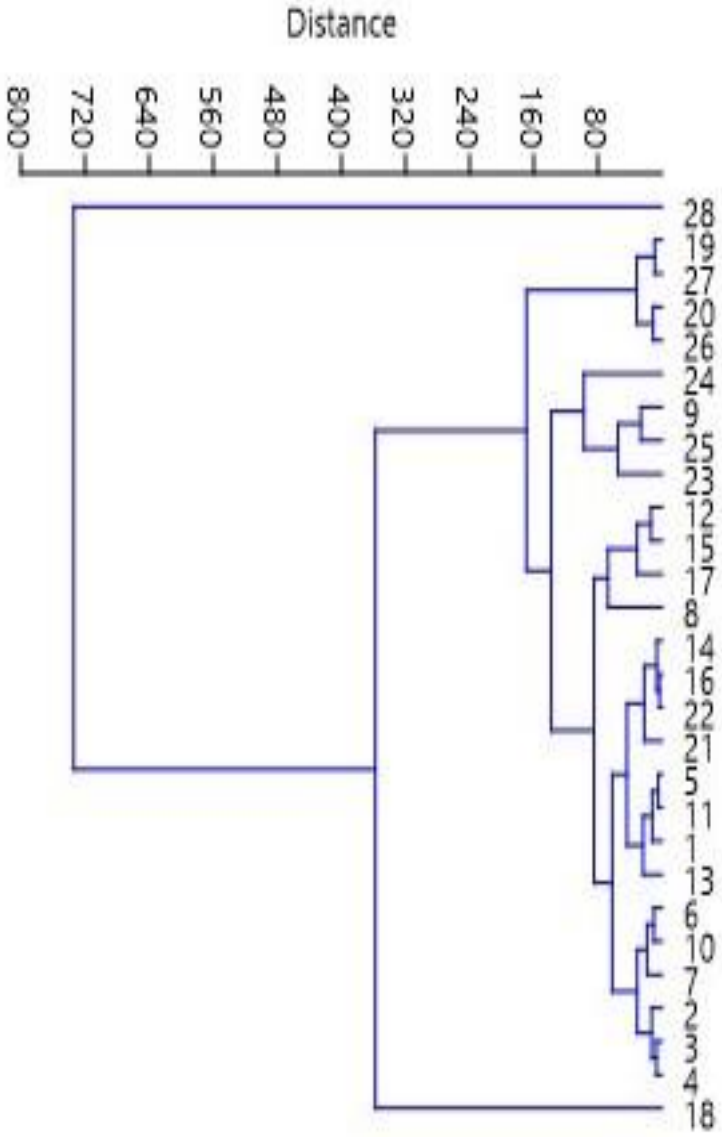

Figure B-5, UPGMA using Euclidean similarity index with quantitative data of calyx

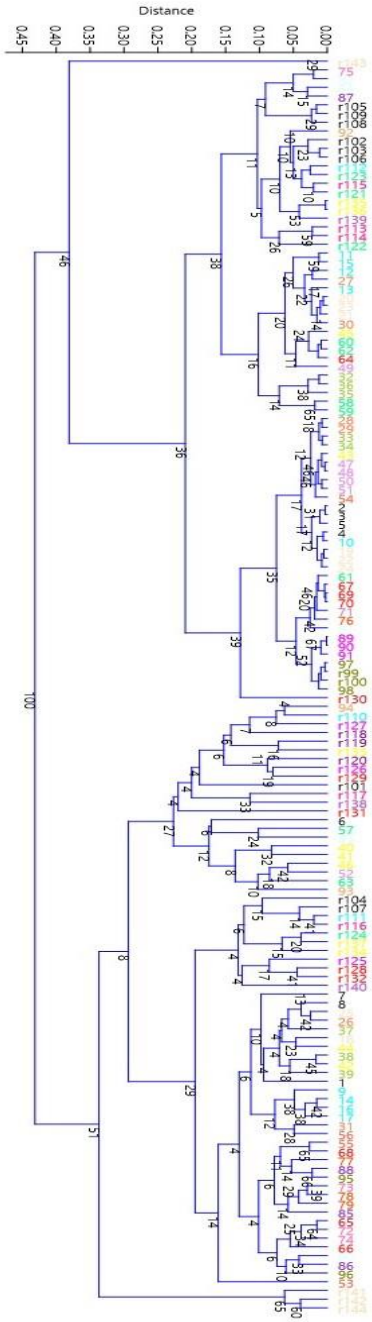

Figure B-6, UPGMA using Gower similarity index with individual quantitaive data of calyx

CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name: Mazaheri, Rodin

Place of Birth: Iran / Urumia

Email: mazaherirodin@gmail.com

EDUCATION

Degree	University	
Master of Science	Ankara University	84.52/100
Bachelor of Science	Urumia university	14.60/20

WORK EXPERIENCE

Year	Place
2010- 2014	Australian embassy, Translator
2008- 2010	UNHCR, External interpreter
2004- 2006	Urumia University, Lecturer