

Full Length Research Paper

Stem and leaf anatomy of ten *Geranium* L. species in Iran

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An anatomical study on stem and leaf of ten species of *Geranium* has been performed in order to distinguish tuberous from rhizomatous species. Cross section of stem, number of palisade parenchyma, presence or absence of crystals and shape of epidermal cells in leaf are important characters to distinguish taxa.

Key words: Anatomy, Taxonomy, Geraniaceae, Iran.

INTRODUCTION

The genus *Geranium* L. comprises 400 species in temperate areas and tropical mountains throughout most of the world (Aedo et al., 1998). According to the currently accepted classification (Yeo, 1984), *Geranium* is divided into three subgenera: (i) *Erodioidea* (Picard) Yeo, (ii) *Robertium* (Picard) Rouy and (iii) *Geranium*. The largest subgenera is *Geranium* that comprises over 370 species grouped in at least 10 sections. There is occurring 22 annual and perennial species for this genus in Iran (Rechinger, 1969), but there are not clarified sections for it. Tuberose rootstock, palmatic sect leaves or lacks both of these features (Yeo, 1984), fruit characters (Aedo et al., 2007) and the nodal organization (Kumar, 1977) are important characters for classification this genus in several sections. On the other hands, anatomical research has been directed mainly toward leaf and stem characters (Ramazannejad et al., 2006; Clements, 1929; Evenari, 1949). In this work, ten species of the *Geranium* genus were anatomically examined to determine diagnostic characters to assess taxonomical relationships.

MATERIAL AND METHODS

The material was collected and procured from different places. The list of species is presented in Table 1. Five samples from each species were used. In order to study histofoliar and stem charac-

ters, materials fixed in FAA 50 (Johansen, 1940) and kept in 50%50% ethanol solution (Berlyn and Miksche, 1976). Cross sections of leaf and stem were prepared by hand cutting. Sections were cleaned with sodium hypochlorite, dehydrated and stained with methyl green 0/1% and carmine 1% for 30 s and 15 min respectively, then mounted in gelatin. Observations were carried out with Olympus light microscope. Six characters of stem consist of cross section, pit parenchyma area, number of collenchyma layer, continuity of fibrous layer, arrangement of vascular bundles and the dense of trichomes were evaluated (Figure 1). Also leaf characters such as the shape of epidermal cells, palisade parenchyma, storage crystals, stomatal type, percentage of trichome and chloroplasts density and the number of subsidiary cells of stomata were assessed (Figure 2). The experimental data were analyzed by ANOVA for analysis of variance and the differences were compared at alpha 0.05.

RESULTS

Comparison of important anatomical characters of stem and leaf for separation of taxa are performed. All characters were significantly in $P < 0.05$ (Tables 2a and 2b).

Stem characters

Cross section

The stem in transverse section was more or less circular or to elliptic in tuberous group of species: *G. tuberosum*, *G. stepporum*, *G. persicum*, *G. kotschyi* (Figures 1g, 1h, 1i and 1j) and was irregular, cordate or polygonal in rhizo-

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Table 1. The species of *Geranium* studied and their collecting sites.

Species	Locality and voucher specimen no.
Rhizomatus	
<i>Geranium pyreniacum</i> Brum. F.	Tehran: Tochal, 2500m, Onsori, 2002
<i>G. purpureum</i> Vill. , Hist.	Tehran: Tochal, 3300m, Onsori, 2002
<i>G. collinum</i> Stephan ex willd.	Tehran: Sorkhe-hesar, 2600 m, 2003, Mazooji
<i>G. robertianum</i> L.	Tehran: Pasghale, 1900 m, Onsori 2004
<i>G. rotundifolium</i> L.	Mazandaran: Chalous, 20 km to Ghachsar, 2650 m, Onsori 2005
<i>G. divaricatum</i> Ehrh. In Beitr. Naturk.	Mazandaran: Firozkoh, 1600 m, Mazooji 2006
Tuberous	
<i>G. kotschyi</i> Boiss.	Tehran: Roodehen, 1350 m, Mazooji 2007
<i>G. stepporum</i> Davis.	Tehran: Roodehen, 1900 m, Mazooji 2008
<i>G. persicum</i> Schonb.	Tehran: Tochal, 1400m, Onsori 2009
<i>G. tuberosum</i> L.	Tehran: Velenjak mountain, 1000 m, Mazooji 2010

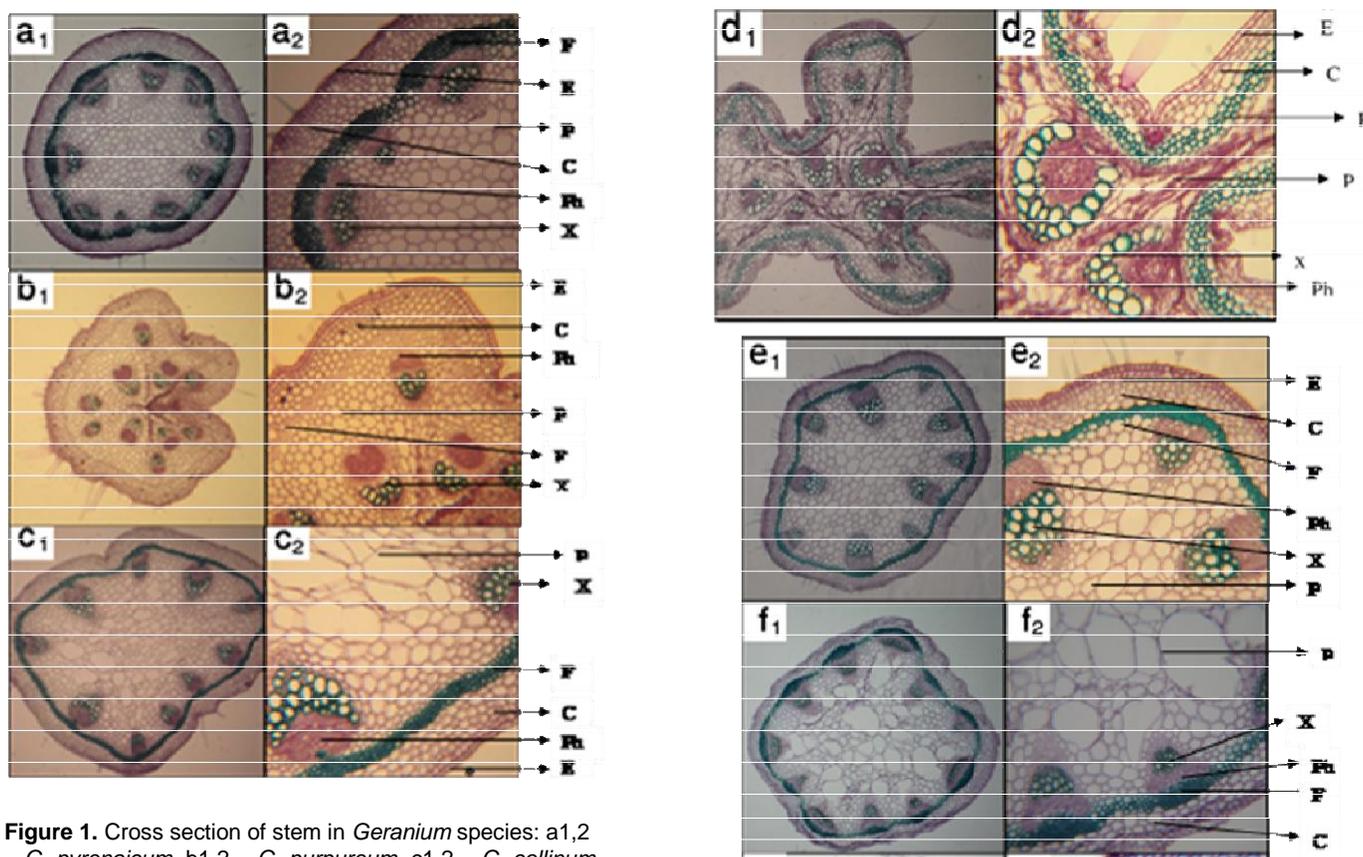


Figure 1. Cross section of stem in *Geranium* species: a1,2 – *G. pyreniacum*, b1,2 – *G. purpureum*, c1,2 – *G. collinum*, d1,2 – *G. robertianum*, e1,2 – *G. rotundifolium*, f1,2 – *G. divaricatum*, g1,2 – *G. kotschyi*, h1,2- *G. stepporum*, i1,2 – *G. persicum*,j1,2 – *G. tuberosum* (20x, 40x). (E: Epidermis, P: Pith, Ph: Phloem, C: Collenchyma, F: Fibre,X: Xylem).

Figure 1. Contd.

matous group consist of *G. pyreniacum*, *G. purpureum*, *G. collinum*, *G. robertianum*, *G. rotundifolium* and *G. divaricatum* (Figures 1a, 1b, 1c, 1d, 1e and 1f).

Vascular cylinder

Nearly all of species posses are vascular cylinder but the number of vascular bundles are different between eight to

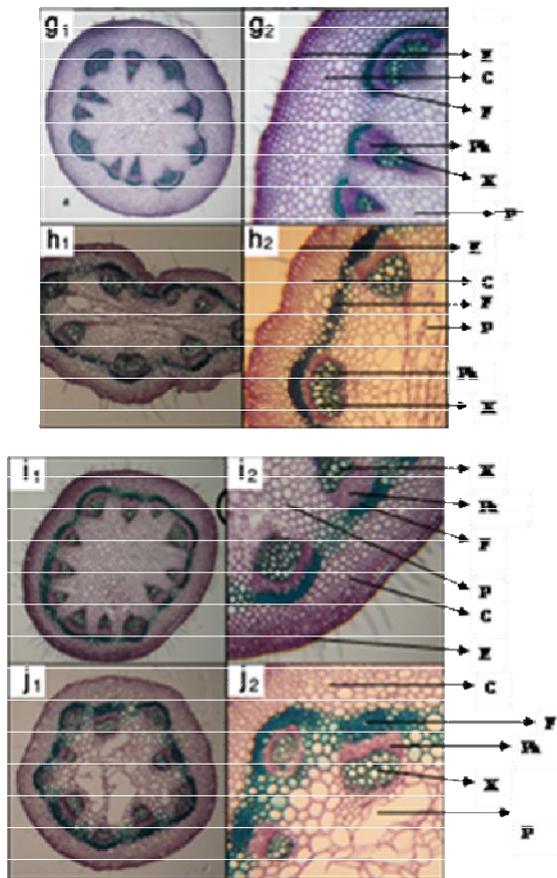


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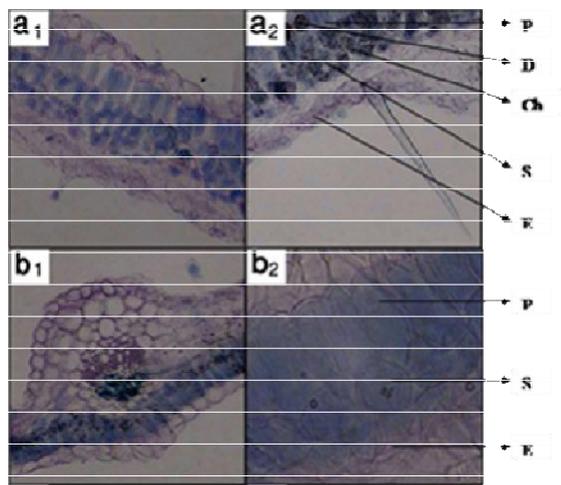


Figure 2. Cross section of leaf in *Geranium* species: a1,2 – *Geranium pyrenaicum*, b1,2 – *G. purpureum*, c1,2 – *G. collinum*, d1,2 – *G. robertianum* e1,2 – *G. rotundifolium*, f1,2 – *G. divaricatum*, g1,2 – *G. kotschyi*, h1,2 – *G. stepporum*, i1,2 – *G. persicum*, j1,2 – *G. tuberosum* (10x, 20x). (P: Parenchyma Palisade, S: Parenchyma Spongy, D: Druse, E: Epi-dermis, Ch: Chloroplast, S: Stomata).

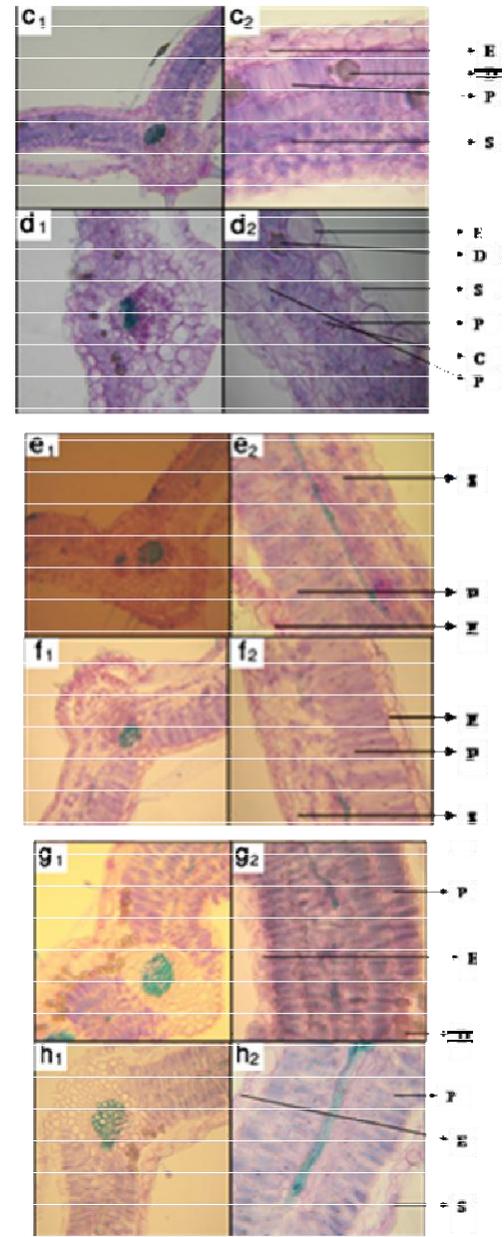


Figure 2. Contd.

eleven in species. The analysis of variance is showed in Table 2a. Also the species had variable size of them (Figures 1 and 3).

Pit parenchyma area

Is very large in tuberose species in comparison to rhizomes ones (Table 2a).

Number of collenchyma layer

Cortical collenchyma presented in all of species but the

Table 2a. Stem characters in *Geranium* species.

Species	Cross section	Number of vascular	Trichome's dense	Pith area	Cortical fibrous	No of collenchyma layer
<i>G. pyreniacum</i>	Polygonal	8.333 ± 0.333	40 ± 1.154	2.833 ± 0.166	Continuity	3.333 ± 0.333
<i>G. purpureum</i>	Cordate	8.433 ± 0.577	73 ± 1.732	0.916 ± 0.083	Continuity	5 ± 0.577
<i>G. collinum</i>	Polygonal	9.666 ± 0.333	26.333 ± 0.881	3.333 ± 0.333	Continuity	6 ± 0.577
<i>G. robertianum</i>	Irregular	9.666 ± 0.333	36.666 ± 0.881	1 ± 0.144	Continuity	4.666 ± 0.333
<i>G. rotundifolium</i>	Elliptic	9.666 ± 0.333	67.666 ± 1.201	2.333 ± 0.166	Continuity	5.666 ± 0.333
<i>G. divaricatum</i>	Polygonal	9.666 ± 0.333	35.666 ± 1.201	6.333 ± 0.44	Not- Continuity	8.333 ± 0.333
<i>G. kotschyi</i>	Circular	10.666 ± 0.333	49.33 ± 0.666	4.666 ± 0.166	Not- Continuity	10.666 ± 0.333
<i>G. stepparum</i>	Circular	10 ± 0.577	118.666 ± 0.881	6.833 ± 0.927	Not- Continuity	8.333 ± 0.333
<i>G. persicum</i>	Circular	11.666 ± 0.333	128.666 ± 4.371	6.166 ± 0.166	Not- Continuity	8.333 ± 0.333
<i>G. tuberosum</i>	Circular	10.333 ± 0.333	40.666 ± 1.855	7.5 ± 0.288	Not- Continuity	8.666 ± 0.666

Table 2b. Leaf characters in *Geranium* species.

Species	Shape of Epidermal cell	Stomata type	No of palisada	Dense of chloroplast	Percentage of stomata (%)	Crystal dense	Subsidiary cell
<i>G. pyreniacum</i>	Polygonal	Anemocytic	1.666 ± 0.333	90 + 2.886	21.166 ± 0.088	4.666 ± 0.333	4.333 ± 0.333
<i>G. purpureum</i>	Polygonal	Anisocytic	1.333 ± 0.333	100 + 7.505	29 + 0.577	8 ± 1	4.666 ± 0.333
<i>G. collinum</i>	Polygonal	Anisocytic	2.333 ± 0.333	53.333 ± 6.009	38.7 + 0.057	3.666 ± 0.333	4.333 ± 0.333
<i>G. robertianum</i>	Polygonal	Anisocytic	1.333 ± 0.333	69.333 ± 2.333	45.75 ± 0.125	7.666 ± 0.881	4.333 ± 0.333
<i>G. rotundifolium</i>	Polygonal	Anemocytic	1.666 ± 0.333	103 + 3.511	13.65 ± 0.028	0 ± 0	5.333 ± 0.333
<i>G. divaricatum</i>	Rectangular	Anemocytic	1.333 ± 0.333	42.666 ± 1.452	18.083 ± 0.044	3.333 ± 0.333	4.666 ± 0.333
<i>G. kotschyi</i>	Rectangular	Anisocytic	5.333 ± 0.333	63.333 ± 1.666	29.083 ± 0.044	15.333 ± 0.333	4 ± 0
<i>G. stepporum</i>	Polygonal	Anisocytic	4.333 ± 0.333	108.333 ± 4.409	17.75 ± 0.028	12 ± 0.577	4.666 ± 0.333
<i>G. persicum</i>	Polygonal	Anisocytic	4.666 ± 0.333	115 + 2.886	23.9 + 0.057	0 + 0	5.333 ± 0.333

number of layers is different between them. Rhizomatous species had 4 - 8 layers of collenchyma but in tuberose species had more than seven (Table 2a).

Continuity of fibrous layer

It was showed specially in rhizomatous species. This character can be separate 2 groups of *Geranium* species (Figure 1).

Trichome's dense

Trichomes were presented in epidermal layer but were more frequent and dense in tuberous species with rhizomatous species. Also the trichomes had one base layer cell (Figures 1 and 3).

Leaf characters

Shape of epidermal cells

Usually consisting of polygonal or rectangular shape. *G.*

divaricatum (e.g. rhizomatous species) have rectangular epidermal cells but other rhizomatous species (e.g. *G. pyreniacum*, *G. collinum*, *G. purpureum*, *G. robertianum* and *G. rotundifolium*) had polygonal shape of epidermal cells. In case of tuberous species, *G. kotschyi* had rectangular shape of epidermal cells but other species had polygonal shape (Table 2b and Figure 2).

Palisade parenchyma

In *G. robertianum*, *G. purpureum* and *G. divaricatum* only one layer was presented. In *G. pyreniacum* and *G. rotundifolium* two layer was showed but other species had three to six layers of palisade parenchyma (Figures 2 and 4).

Storage parenchyma crystals

Presence or absent of crystals is different in taxa. For example, *G. persicum* and *G. rotundifolium* there are no crystals in cortical cells and tuberous ones have more

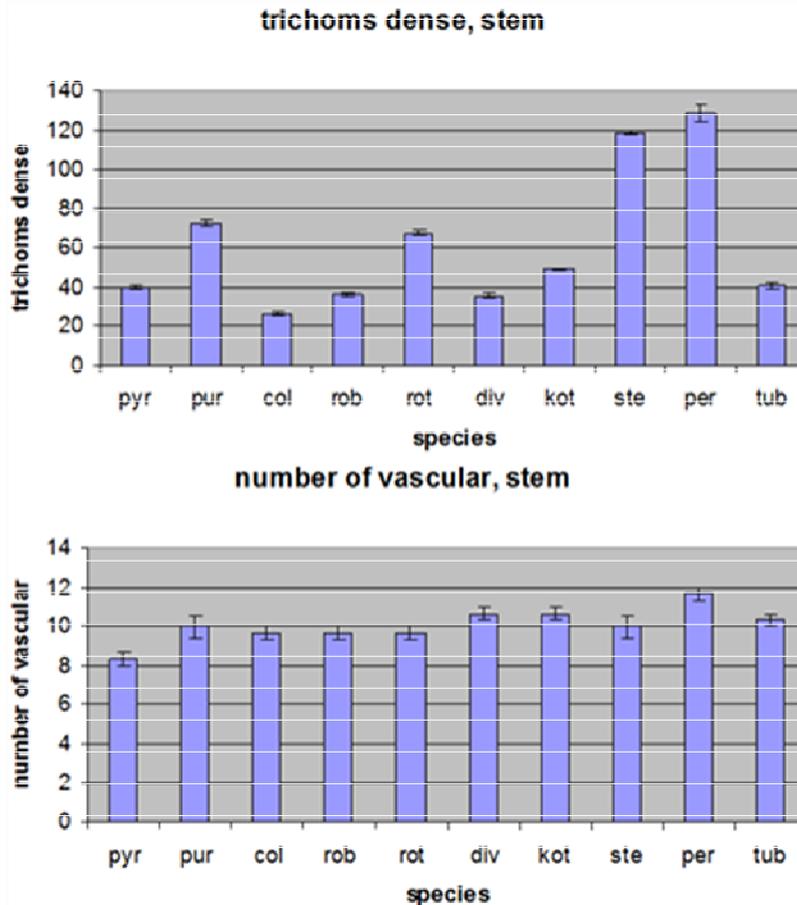


Figure 3. Trichome dense and number of vascular in stems of species . Data are mean \pm S.E.

than crystals in comparison to rhizotamus species (Figures 2e and 2i).

Stomata type

Usually consisting of anemocytic and anisocytic.

Percentage of stomata guard cells

There was variation in dense of stomata gaurd cells between species ranging 13 to 45% average.

Percentage of chloroplast

This character shows high variability within as well as between species (Table 2a).

Subsidiary cells

There were found four to six subsidiary cells presented in

species but the *rhizomatus* species had less subsidiary cells in comparison to tuberous ones.

Field guide

Based on stem and leaf anatomical characters, the guide was written as followed:

- 1- shape of stem in cross section is rounded 2
- shape of stem in cross section is polygonal or irregular.....5
- 2- type of stomata guard cells is anemocyticG. *tuberosom*
- type of stomata guard cells is anisocytic..... 3
- 3- shape of epidermal cell is rectangular G. *kotschyi*
- shape of epidermal cell is polygonal4
- 4- crystal is absent G. *persicum*
- crystal is presentG. *stepporum*
- 5a- palisad parenchyma is one layer.....6
- palisad parenchyma is more than one layer.....8
- 6a- shape of epidermal cell is rectangular..... G. *divaricatum*

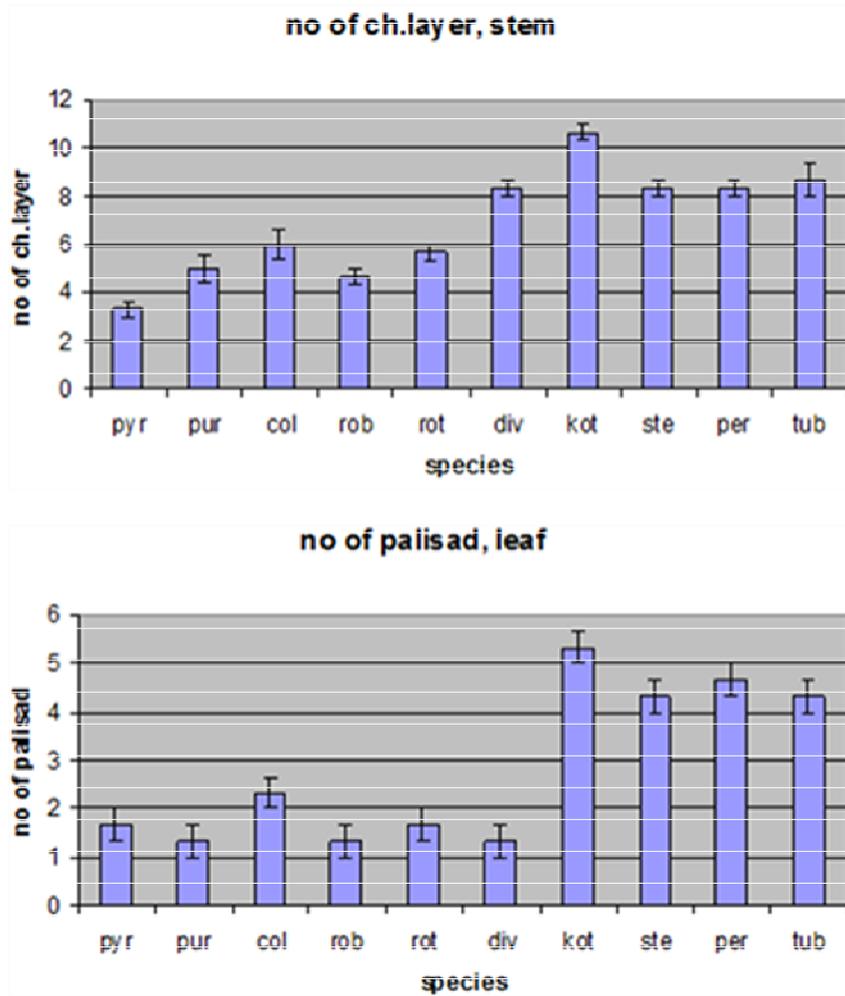


Figure 4. Number of chloroplast in stem and number of palisad in leaf of species. Data are mean \pm S.E.

- shape of epidermal cell is polygonal.....7
- 7a- trichome's dense of stem is low *G. robertianum*
- trichome's dense of stem is high *G. purpureum*
- 8a- type of stomatal guard cell is anemocytic..... 9
- type of stomatal guard cell is anisocytic *G. collinum*
- 9a- storage cristal is absent *G. rotundifolium*
- storage cristal is present *G. pyreniacum*

DISCUSSION

The results obtained from this study showed that, the anatomical characters can separate the taxa and we made an anatomical key for delimitation of them. According to palmatis fruit characters, it seems that tuberous section is a natural group (Aedo, 1998). Our results showed that the cross section of stem support this idea.

In rhizomatous species, the cortical fibrous is conti-

nually except in *G. divaricatum*, but all of tuberous ones have not-continuity layer. According to Chalabian (2007), this character used for distinguishing other genus in Gera-niaceae family. Trichomes are considered relevant in comparative systematic investigations and morpho-diag-nosis (Metcalfe and Chalk, 1988). Our results showed that the trichomes dense of stem is important factor for distinguishing near species such as *G. persicum* and *G. tuberosum* or *G. pyrenaicum* and *G. routondifolium*. According to Ramazannejad et al. (2006). The number and size of vascular bundles are important factors to distinguish species. Our study confirmed to distinguish them by other characters such as pith area. In rhizomatous specie such as *G. robertianum*, *G. purpureum*, the number of vascular bundles have range from seven to nine, but in tuberous ones they have more than nine. Also another factor for separating the species.

In concerning the leaf anatomy, tuberous species exhibits anisocytic stomata (Metcalfe and Chaik, 1950).

G. tuberosum shows anemocytic pattern of stomata, unlike the others. On the other hand, *G. persicum* has similarity to *G. tuberosum* especially in shape of leaves, tuber and stipulate (Schonbeck, 1970). Thus this character is useful for distinguishing these two near species. Presence of palisade parenchyma is also very important character for distinguish species. With reference to the palisade layer, dense of chloroplast and crystal dense have been frequently reported for *Geranium* genus (Carlquist and Bissing, 1976), these are useful to separate near species such as *G. collinum* and *G. robertianum*. Also it seems that, percentage of stomata guard cells is not very significant for comparative the species.

In flora Iranica (Schonbeck, 1970), *Geranium* mentioned with 22 species but they were not distinguished as distinctive sections. Our results showed that anatomical characters are useful for separate taxa in sections level.

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