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Genetic identification and taxonomic studies on six species of *Pelargonium* in Egypt

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ABSTRACT

This study was performed on six species belong to genus Pelargonium (family: Geraniaceae) grown in Egypt; namely; Pelargonium graveolens, P. radula, P. fragrance, P. peltatum, P. zonale and P. grandiflorum. The aim of this study is to explore the taxonomic relationships between these species by using randomly amplified polymorphic DNA (RAPD) markers to elucidate the pattern of genetic diversity among these individuals of different species of Pelargonium. In addition, the morphological, anatomical and lamina surface characters under Scanning Electron Microscope (SEM) are studied. Numerical analysis was also used to determine interrelationships among the investigated species.

Key words: *Pelargonium*, Anatomy, Morphology, RAPD, Scanning Electron Microscope (SEM), Taxonomy**.**

INTRODUCTION

Geraniaceae are represented by 5 genera and 750 species, cosmopolitan in distribution, but mostly in temperate areas. The family is divided into two tribes *i.e.*, Geranieae (*Geranium* L., *Erodium* L., *Monsonia* L. and *Sarcocale*) and Pelargonieae (*Palargonium* L. Herit ex Aiton) [1].

Genus *Pelargonium* L. 'Herit is originally from South Africa and includes over 175 species according to some authors or, even more than 300 species after others. The plants, known improper as "geranium" have entered Europe in the eighteenth century and have spread rapidly in all its areas. They are herbaceous plants, perennial, pubescent, rarely high, stems soft, succulent and rich foliage [2, 3]. Most of the known species are endemic to the Western Cape. Although this plant is indigenous to South Africa, it is widely cultivated in Egypt, India and China, and to a lesser extent in Central Africa, Madagascar, Japan, Central America and Europe.

Nowadays, the majority of *Pelargonium* species stand out through vigorous plants, with variated colors (red, scarlet, white, pink, purple etc.), fragrant, simple, double and sometimes very large flowers. It is among the top 20 aromatic plants that are particularly rich in essential oils; therefore most of the chemical studies were concentrated on this fraction [4].

The species of this genus are very diverse, general appearance, color of flowers, but especially after leaf morphology. Geraniums are classified in the family Geraniaceae ornamental plants, most species being bred for their decorative flowers and some liked the smell of the leaves. Fragrancerant leaves of these species contain geraniina a rate higher or lower. This substance is used to obtain oils with therapeutic properties [5]. A lot of medicinally important attributes have been assigned to the plants of *Pelargonium* species. They are rich source of monoterpenes,

sesquiterpenes, coumarins, tannins, phenolic acids, cinnamic acids, flavones, flavonoids and flavonols derivatives [6].

With increased sophistication of classification systems it has become increasingly important to have more elaborative means for identification. The leaf has not lost its importance as a taxonomic tool but rather has proved to be more useful when a fuller understanding of all its characteristics are known and appreciated. Surface sculpturing by using Scanning Electron Microscope (SEM) technique may aid in solving problems of identity or relationship concerning taxa at various levels [7].

Salimpour *et al.* [8] studied the anatomical structure of stem and leaf of ten species of *Geranium* 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. [9] studied the internal description of leaves, petioles and stems for five species of family Myrtaceae to determine their taxonomic importance and clarity the interrelationship among them. [10] establish the main characteristics and differences that occur between *Pelargonium zonale*, *P. hispidum*, *P. grandiflorum*, *P. peltatum* and *P. radens*, regarding the leaf-stalk, blade and trichomes.

Lyubov *et al.* [11] establish the chemotaxonomic and anatomical features of four species of the genus *Geranium* L. of flora of Ukraine; namely, *Geranium robertianum* L., *G. sibiricum* L., *G. sanguineum* L. and *G. macrorrhizum* L. The aim of this study is to distinguish the taxonomic relationship between six species of *Pelargonium* grown in Egypt; namely, *Pelargonium graveolens*, *P. radula*, *P. fragrance*, *P.peltatum*, *P. zonale* and *P. grandiflorum*. Randomly Amplified Polymorphic DNA (RAPD) markers were used to determine the level of polymorphism within the studied *Pelargonium* species. Morphological descriptions and Scanning Electron Microscope (SEM) survey of the leaf, as well as leaf and petiole anatomy of the studied species were investigated.

MATERIALS AND METHODS

Fresh material of six species of *Pelargonium*; namely, *Pelargonium graveolens* L. Herit, *P. radula* L., *P. fragrance* L., *P.peltatum* L., *P. zonale* L. and *P. grandiflorum* (Andr.) were examined. These species were planted at Research Station of Faculty of Pharmacy, Cairo University, Giza, Egypt during the two autumn growing seasons of 2014 and 2015. The present investigation aimed to represent the taxonomical relationships among the six studied species. Taxonomic evidences and characters which might explore these relationships were gathered from the following different sources during this study; randomly amplified polymorphic DNA (RAPD) markers were used to determine the level of polymorphism within the studied species, morphological description for each species, Scanning Electron Microscope (SEM) of the surfaces of leaves and finally the anatomical information of leaf and petiole.

Genomic DNA isolation and PCR amplification

DNA was extracted from field-grown leaves using the CTAB protocol described by [12]. Six different primers (C1, P13, N8, B12, H5 and P8) were used in this study for DNA amplification according to other literature [13] (Table 1).The polymerase chain reactions (PCR) were carried out in 25 µl volume containing 50 ng of genomic DNA template, 30 pmoles/µl primers, 0.2 µM each of dATP, dCTP, dGTP and dTTP, 10 x buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, and 2 units of Taq polymerase (Thermo Fisher Scientific, MA, USA).

Table (1): The sequences of the six arbitrary primers used in the finger print analysis

Primers		Sequences
1.	**C1**	TTCGAGCCAG
2.	**P13**	GGAGTGCCTC
3.	**N8**	ACCTCAGCTC
4.	**B12**	CCTTGACGCA
5.	**H5**	AGTCGTCCCC
6.	**P8**	GGAGCCCAG

PCR amplifications were carried out in a Applied Biosystem thermal cycler programmed as follows: an initial strand separation at 94 °C (5 min) followed by 40 cycles with the following temperature profile: 94°C (1 min), 36°C (1 min), 72°C (1.5 min) and a final extension at 72°C (7 min). Amplification products were visualized by electrophoresis in 1.5% agarose gels stained with Ethidium Bromide then photographed with a SynGene gel documentation unit and the DNA banding patterns were scored.

All these data were manually scored comparing with the pictures where a binary data was organized and only the polymorphic fragment were scored as band present (1) and band absent (0). Absent and present binary data of 8

individuals and170 polymorphic loci were used as the basis for the analysis. In the present study, based on [14] the band-based approach was used for the analysis in the individual level and allele frequency-based approaches (for population level). Dice coefficient [15] was used to calculate the similarity among 12 individuals. The cluster analysis was prepared through un weighted pair group method with arithmetic mean (UPGMA) based on Dice index [16]. Bootstrap values (based on 1000 re-sampling) was used to estimate the reliability of the clustering pattern. This analysis was carried out in Free Tree [17]. The NTSYS-pc version 2.20 [18] was used to prepare Principal Co-ordinates Analysis (PCoA) of the correlations matrix to test the relationship among *Pelargonium* species.

Preparing samples for the leaves surface scan features (SEM).
The upper and lower surfaces of leaf of each species were examined by using Scanning Electron Microscope (SEM). The leaf samples were prepared before examination as follows:
a. The fixation according to the method of [19].
1. The specimens were immediately fixed by immersion in 4% gluteraldehyde in 0.1 m sodium cacodylate buffer, pH 7.3 for at least 4 hrs.
2. The specimens were then washed for 1.5 hrs (or overnight) with three changes of the same buffer.
3. Then post fixation was carried out in 1% osmium tetraoxide (OsO_4) in the same buffer for at least 2 hrs or until the specimens become black in color, followed by washing the specimens three times in the same buffer for 30 min.
b. The Dehydration
1. Dehydration of the specimens was carried out through a graded series of ethanol; 50% (10 min.); 70% (10 min.); 80% (10 min.); 90% (10 min.) and absolute (3x10 min.).
2. Up to this stage of preparation the temperature was at 0-4 ^{o}C, the next steps were carried out at room temperature.
3. Finally the specimens were completely dried through the Critical Point Dryer with CO_2 liquid.
4. The specimens were mounted on copper stubs with double-sides adhesive tape and coated with gold using Sputter Coater S 150A Edwards-England.
5. The specimens were examined under JXA- 840A Electron Probe Microanalyzer- JEOL-JAPAN.

For anatomical studies, fresh leaves and petioles were killed and fixed at least for 48 hrs. in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and85 ml ethyl alcohol 70%). The tested materials were washed in 50% ethyl alcohol, dehydrated in a normal butylalcohol series, embedded in paraffin wax of melting point 56 ^{o}C, sectioned to a thickness of 20 microns, double stained with crystal violet-erythrosin, cleared in xylene and mounted in Canada balsam [20]. Photomicrographs of the anatomical sections were taken and analyzed microscopically.

RESULTS AND DISCUSSION

DNA finger printing of *Pelargonium*
Molecular markers are efficient tools for genotype identification and estimation of relatedness through DNA finger printing. Short primers of arbitrary nucleotide sequence, called RAPD markers, were used to amplify genomic DNA segments, and that polymorphism can be detected between the amplification products of the different isolates through examination of agarose gel [21]. Six random primers are used as RAPD markers, for analysis of genomic DNA diversity among different six *Pelargonium* field-grown cutting leaf samples (C1, P13, N8, B12, H5, and P8).

RAPD markers profile and densitograms results of *Pelargonium* samples are mentioned in Table (2) and Fig. (1), respectively. The results revealed that 170 reproducibly scorable genomic DNA bands were generated by six random decamer primers. The amplified DNA fragments obtained in this study ranged from 3000 to 100 base pairs (bp) in size. The number of amplified DNA fragments was scored for each primer; the highest number of amplified DNA fragments was 38 and 33 (primers B12 and P8, respectively). The other four primers amplified 99 DNA fragments, with an average of 24 amplicons per primer across the six *Pelargonium* samples.

Table (2): Total number of amplicons, monomorphic and polymorphic amplicons as revealed by RAPD primers among the six *Pelargonium* species

Primer	Total Number of Amplicons	Polymorphic amplicons	Monomorphic amplicons	% of Polymorphism
C1	27.0	25.0	2.0	92.6%
P13	27.0	27.0	0.0	100%
N8	21.0	19.0	2.0	90.5%
B12	38.0	38.0	0.0	100%
H5	24.0	17.0	7.0	70.8%
P8	33.0	33.0	0.0	100%
Total	**170**	**159**	**11**	**93.5%**

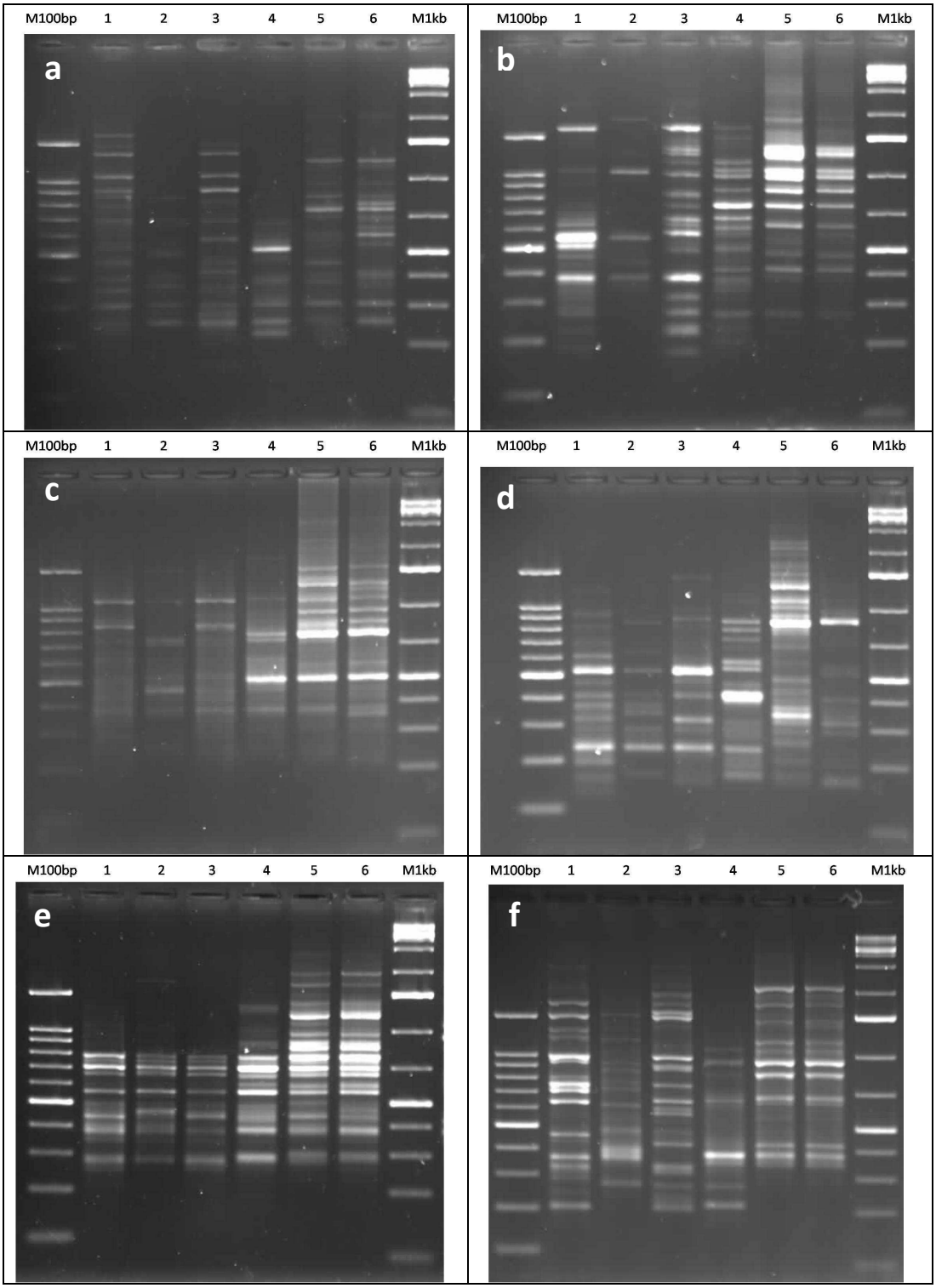


Figure 1: RAPD profile for 6 *Pelargonium* species as detected with primers a (C1), b (P13), c (N8), d (B12), e (H5) and f (P8)
1- *P. fragrance,* 2- *P. radula,* 3- *P. graveolens,* 4- *P. peltatum,* 5- *P.zonale* and 6- *P. grandiflorum.*

The UPGMA tree (Fig. 2) is obtained by cluster analysis of the similarity indexes using the UPGMA method based on the values for the genetic distance. It reveals that the six *Pelargonium* species could be separated into two clusters. Based on the cluster analysis (Fig. 2), the *Pelargonium* species were grouped into 2 clusters, whereby the genotypes in cluster 1 are more closely related than the individuals in the other cluster. The first cluster was the smallest, including 2 species, *P. zonale and P. grandiflorum* .The rest of the *Pelargonium* species were placed in the second cluster, *P.fragrance, P.graveolens, P.peltatum, P.radula*.

The Genetic similarities among the 6 *Pelargonium* species were estimated based on the number of common fragment. Similarity values among individuals ranged from 0.1792 to 0.5842 on the Dice index (data not shown). It also showed that the similarity values between *P. radula* and *P. zonale* is 0.1792; whereas, the similarity values between *P. zonale* and *P. grandiflorum* was the highest (0.5842).

Numerical Analysis

Data of the studied characters of the genetic distances among six different *Pelargonium* species by RAPD-PCR amplification, morphological, anatomical and the surface scan descriptions of the leaf and were applied in clustering analysis by using the numerical analysis technique "NTSYS-PC.", version1.5 program [22]. The final results of this technique are represented in a form of dendrograms (Fig.2 and Table 3). The dendrograms represent the levels of similarity or dissimilarity distance between studied species.

From the illustrated dendrogram of genetic and morphological characters (Fig.2) it could be stated that the 6 studied species distributed according to the similarity or dissimilarity distance through two main clusters. The very far cluster which started at the highest similarity level (0.1312) included two species; *P. grandiflorum* and *P.zonale*. The other cluster included the rest of the studied species and started at similarity level (0.3146). At that level (0.3009), the species *P. radula* split individually away from the other species and started to join them at level. At that level (0.25) the species *P. peltatum* join the remaining species. At level (0.1884) there are two species; namely, *P. graveolens* and *P. fragrance*.

From the above mentioned results, it is worthy to note that the two species *P. grandiflorum and P.zonale* join the rest of the studied species lately at the highest similarity level (0.3146). The reason for that is because this species have many morphological and surface scan features vary from the others. Some of these varied characters are; leaf palmatum, dull, petiole length 5.6-6.9 cm., rugose upper epidermis and favulariate lower epidermis of leaf.

At the beginning of this study (Table 3) it was thought that *P. grandiflorum* could be similarly close to *P.zonale*. The reason for that is because both species are similar in some characters of leaf. But after numerically analyzed all their studied characters (anatomical for leaf and petiole), it was found that both species have many characters differ from each other. This could be one of the reasons for inability of both species to form one sharing cluster.

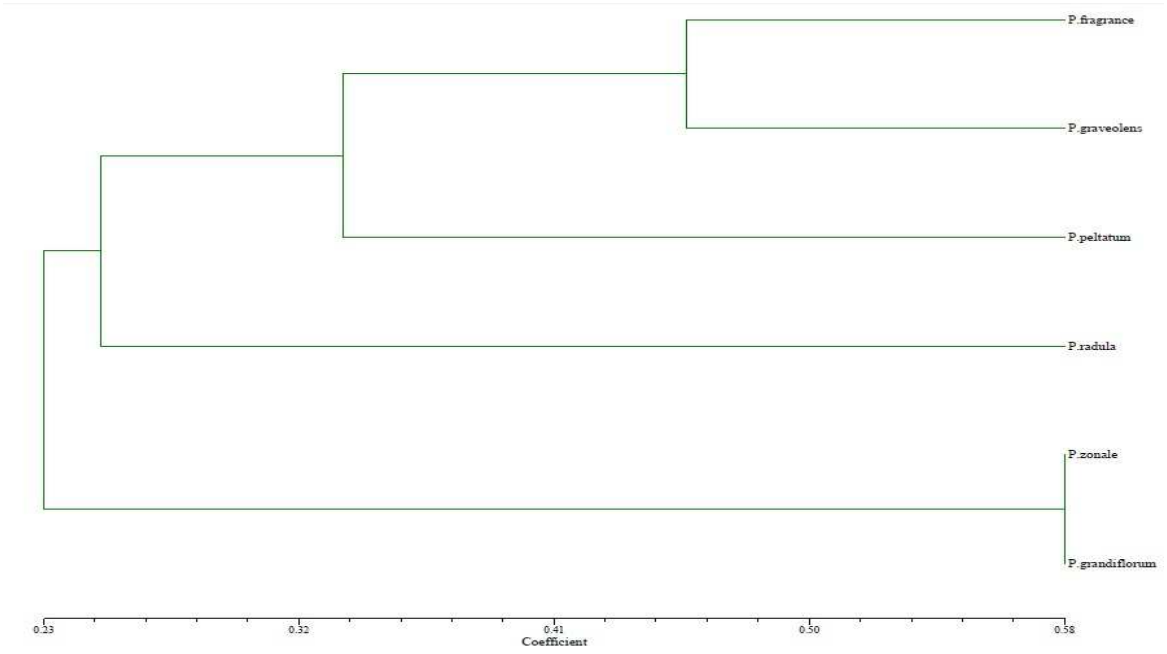


Figure 2: UPGMA dendrogram based on Nei's (1972) genetic distances among six different *pelargonium* species by RAPD-PCR amplification. Bar on the bottom indicates similarity index based on S.M. coefficients

Table 3: Genetic distance dendrogram for *Pelargonium* species by RAPD-PCR

Species	*P. fragrance*	*P. radula*	*P. graveolens*	*P. peltatum*	*P. zonale*	*P. grandiflorum*
P. fragrance	1.0000					
P. radula	0.2706	1.0000				
P. graveolens	0.4526	0.2135	1.0000			
P. peltatum	0.3404	0.2632	0.3263	1.0000		
P. zonale	0.2400	0.1792	0.1923	0.2883	1.0000	
P. grandiflorum	0.2222	0.1895	0.2119	0.3100	0.5842	1.0000

Leaf morphological characters

Data presented in Table (4) and Fig. (3) indicate that all studied species are simple, petioled and alternated leaves.

As shown in Table (4) and Fig. (3) *P. grandiflorum*, *P. peltatum* and *P. zonale* have palmatum leaf shape, whereas *P. graveolens* has palmatum lobed shape. On the other hand *P. radula* has pinnatifidum lobed leaf shape and *P. fragrance* has praemorsum shape.

P. peltatum showed the maximum leaf length and width (12.4-13.4×7.5-8.9 cm) over all studied species, whereas, *P. fragrance* showed the minimum values (5.8-6.3×2.4-3.1 cm), and *P. radula* and *P. graveolens* recorded intermediate values (7.8-8.3×7.9-8.4 cm) and (6-8×4.5-6 cm), respectively. Both *P. grandiflorum* and *P. zonale* were nearly the same for length and width (8.9-10.2×7-8.4 cm and 8.9-10.2×6.8-7.9 cm), respectively. Both *P. radula* and *P. graveolens* have green leaves; *P. fragrance* has dull and pale green leaves. *P. grandiflorum* has dull grayish green and have a brownish zonal marking. While, *P. peltatum* has lustrous dark green, some of leaves; distinctive zonal markings, but this is not a common. *P. zonale* has dark green, dark horseshoe-shaped mark is often present.

It is obvious from data that, both *P.grandiflorum* and *P. zonale* were quite similar in petiole length, 5.6-6.9 and 5.8-6.4 cm, respectively. Whereas, *P.peltatum* exhibited the highest value 7.5-8.3 cm. on the other hand, petiole length of *P. fragrance* was intermediate 4.1-4.7 cm, while *P.graveolens* and *P.radula* recorded the lowest petiole length, 2.6-3.1 and 3.2-3.5 cm. Both species; *P. grandiflorum* and *P.graveolens* have rose leaf sectioned, while *P.fragrance* has old spice, *P.radula* has lemon leaf sectioned. *P.peltatum* and *P.zonale* have strawberry and camphor leaf sectioned, respectively.

Table (4). Morphological information of the leaves of the six studied species of *Pelargonium*

Characters	*P.fragrance*	*P.radula*	*P. grandiflorum*	*P. graveolens*	*P. peltatum*	*P. zonale*
Shape	praemorsum	pinnatifidum lobed	palmatum	palmatum lobed	palmatum	palmatum
Length cm.	5.8-6.3	7.8-8.3	8.9-10.2	6-8	12.4-13.4	8.9-10.2
Width cm.	2.4-3.1	7.9-8.4	7-8.4	4.5-6	7.5-8.9	6.8-7.9
Colour	dull& Pale green	green	dull grayish green have a brownish zonal marking	green	lustrous Dark green, Some of leaves ; distinctive zonal markings, but this is not a common	dark green, dark horseshoe-shaped mark is often present
Petiol cm.	4.1-4.7	3.2-3.5	5.6-6.9	2.6-3.1	7.5-8.3	5.8-6.4
Leaf sectioned	old spice	lemon	rose	rose	strawberry	camphor
Texture	glaucous, tiny,	hard and rigid	dull , coarsely toothed	leathery	semi-succulent, trailing	succulent, smooth
Apex	mucronatus	mucronatus	obtusus	obtusus	obtusus	obtusus
Base	cordatus	cordatus	cordatus	hastatus	cordatus	cordatus

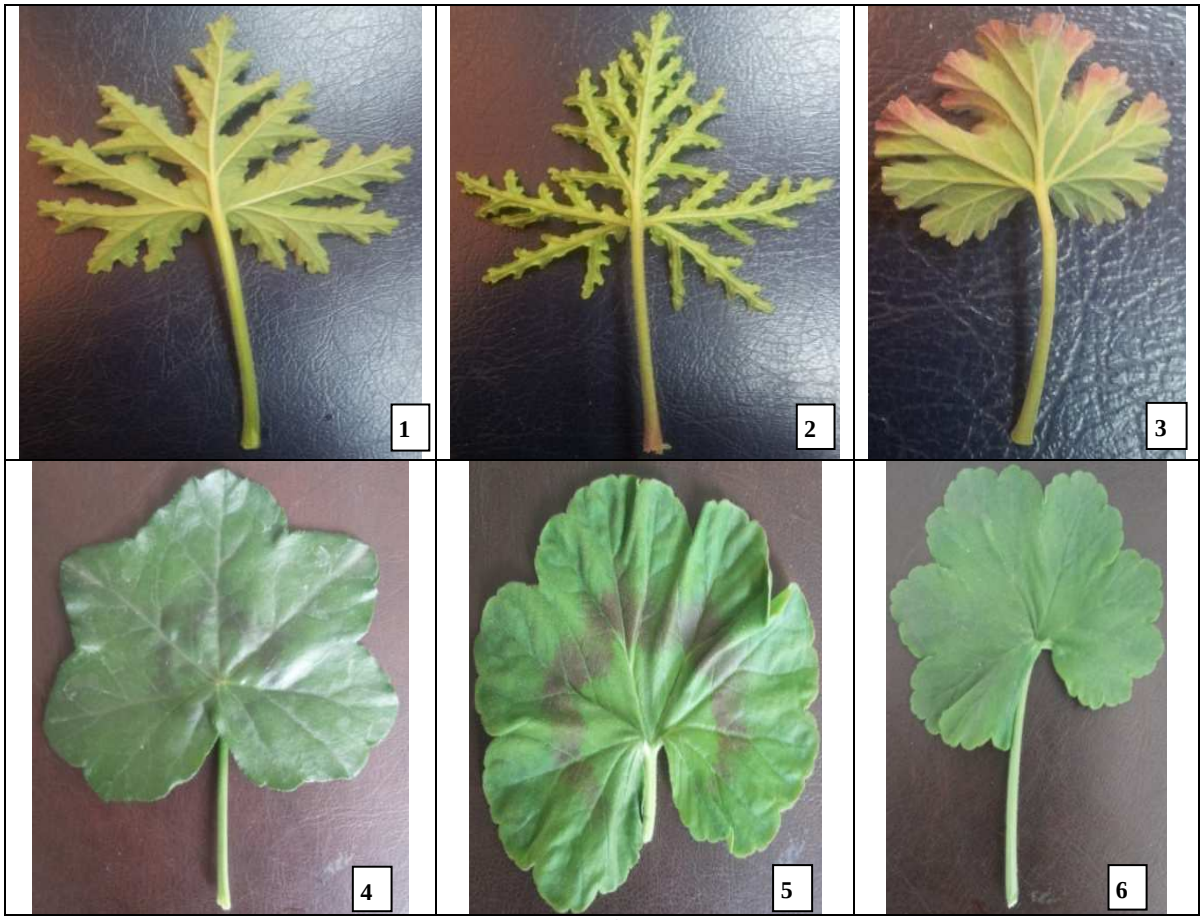


Fig. (3): Photographs showing leaves of the six studied *Pelargonium* species
1- *P. graveolens*, 2- *P. radula*, 3- *P. fragrance*, 4- *P.peltatum*, 5- *P. zonale* and *6- P. grandiflorum*.

Leaf texture of *P. fragrance* has glaucous and tiny, *P.* radula showed hard and rigid texture, *P.grandiflorum* has dull and coarsely toothed leaf texture. On the other hand, *P.graveolens* has leathery, while *P.peltatum* has semi-succulent, trailing texture, but *P.zonale* has leaf texture with succulent and smooth.

P. fragrance and *P. radula* have leaves apex with mucronatus shape, whereas leaves of *P. grandiflorum*, *P. graveolens*, *P. peltatum* and *P. zonale* show obtusus apex shape. It is obvious that all studied species have cordatus lamina base shape, except *P. graveolens* with hastatus lamina base shape.

Scanning Electron Microscopy of leaf
A- Upper lamina surface
As shown in Fig. (4) and Table (5) *P.radula*, *P.peltatum* and *P.zonale* are rugose in sculpture of lamina upper surface, while *P. grandiflorum* has rugose-faulariate sculpture. *P. fragrance* has indistinct and *P.graveolens* has weak reticulate sculpture of lamina upper surface. All studied species have anomocytic type of stoma with superficial level, except, *P.graveolens* has anomocytic with raised level. Whereas *P. fragrance* has indistinct stoma. *P.peltatum* exhibited actinocytic ones with superficial level.

Trichome glandular (digitalis type) were observed in *P.radula*, *P.peltatum* and *P.zonale*, while digitalis and menhta in *P.graveolens*. *P. grandiflorum* exhibited vasaka ones, on the other hand, *P. fragrance* has vasaka and belladonna glandular trichome.

Non glandular trichomes (datura type) are noticed on upper epidermis of *P.radula* and *P.zonale*. However, *P. fragrance* and *P. grandiflorum* exhibited nux vomica type, while in *P.peltatum* is nux vomica at margin. Digitalis type is found in *P.graveolens*.

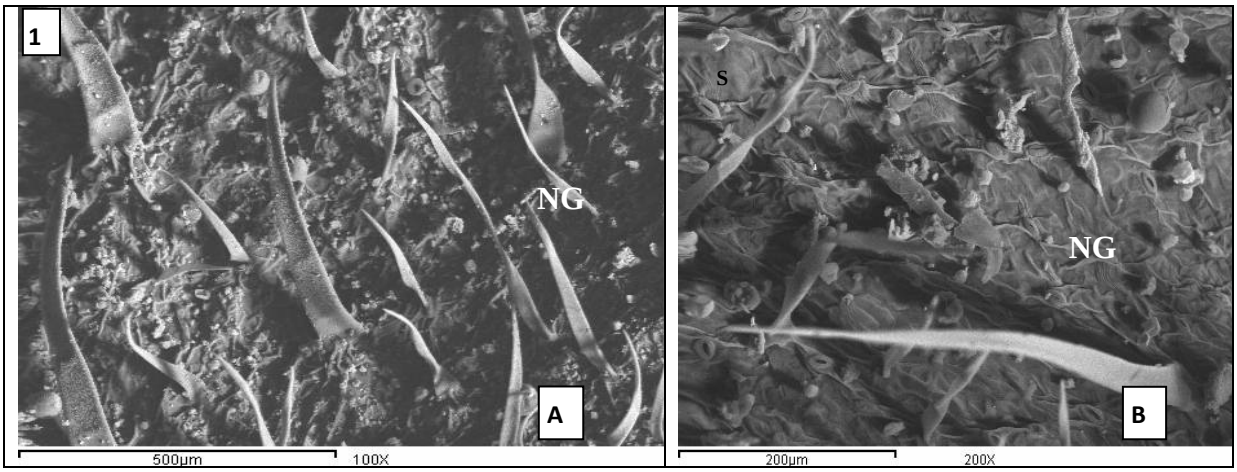
Table (5). Micromorphological information of the leaves of the six studied species of *Pelargonium* using SEM

	Characters	*P.fragrance*	*P.radula*	*P. grandiflorum*	*P. graveolens*	*P. peltatum*	*P. zonale*
Upper epidermis	Sculpture	indistinct	Rugose	Rugose-Faulariate	Weak reticulate	Rugose	Rugose
	Type of Stomata	indistinct	Anomocytic	Anomocytic	Anomocytic	Actinocytic	Anomocytic
	Stomatal leveling		Superficial	Superficial	Raised	Superficial	Superficial
	Trichome (Glandular)	Vasaka& Belladonna	Digitalis	Vasaka	Digitalis& menhta	Digitalis	Digitalis
	Trichome (nonglandular)	Nux vomica	Datura	Nux vomica	Digitalis	Nux vomica at margin	Datura
Lower epidermis	Sculpture	Rugose	Rugose	Rugose-Faulariate	Rugose	indistinct	Faulariate
	Type of Stomata	Anomocytic	Anomocytic	Anomocytic	Anomocytic	indistinct	Anomocytic
	Stomatal leveling	semiraised	Semi-raised	Superficial	Superficial		Superficial
	Trichome (Glandular)	Vasaka & Belladonna	Digitalis	Vasaka	Vasaka	DigitalisNux vomica at margin	Digitalis
	Trichome (nonglandular)	Nux vomica	Datura	Nux vomica	Digitalis		Nux vomica

B- Lower lamina surface

It is obvious from Fig. (4) and Table (5) that *P. fragrance*, *P.radula* and *P.graveolens* are rugose in sculpture of lamina lower surface, while *P. grandiflorum* has rugose-faulariate sculpture. *P.peltatum* has indistinct and *P.zonale* has faulariatein sculpture of lamina lower surface.

The three species; *P. grandiflorum*, *P.graveolens* and *P.zonale* have anomocytic type of stoma with superficial level. Whereas *P. fragrance* and *P.radula* are anomocytic with semi-raised level. On the other hand, *P.peltatum* has indistinct stoma. Trichomes glandular (vasaka type) are observed in *P. grandiflorum* and *P.graveolens*, while type vasaka and belladonna is noticed in *P. fragrance*. Digitalis type is exhibited in *P.radula*, *P.peltatum* and *P.zonale*. Non glandular trichomes (nux vomicatype) are noticed on lower epidermis in *P. fragrance*, *P.radula* and *P.zonale*. *P.peltatum* exhibited nux vomica at margin, while in *P.radula* is datura type. Digitalis type is found in *P.graveolens*. Lyubov *et al*. [11] stated that some species of the genus *Geranium* L.have stomata of anomocytic type. The stomata on the abaxial surface (for all species, except G. *sanguineum*) are larger by size and by quantity per 1mm^2, compared with adaxial surface. There are glandular and simple trichomes on lamina. The number of glandular trichomes on abaxial surface is higher relatively to adaxial surface.



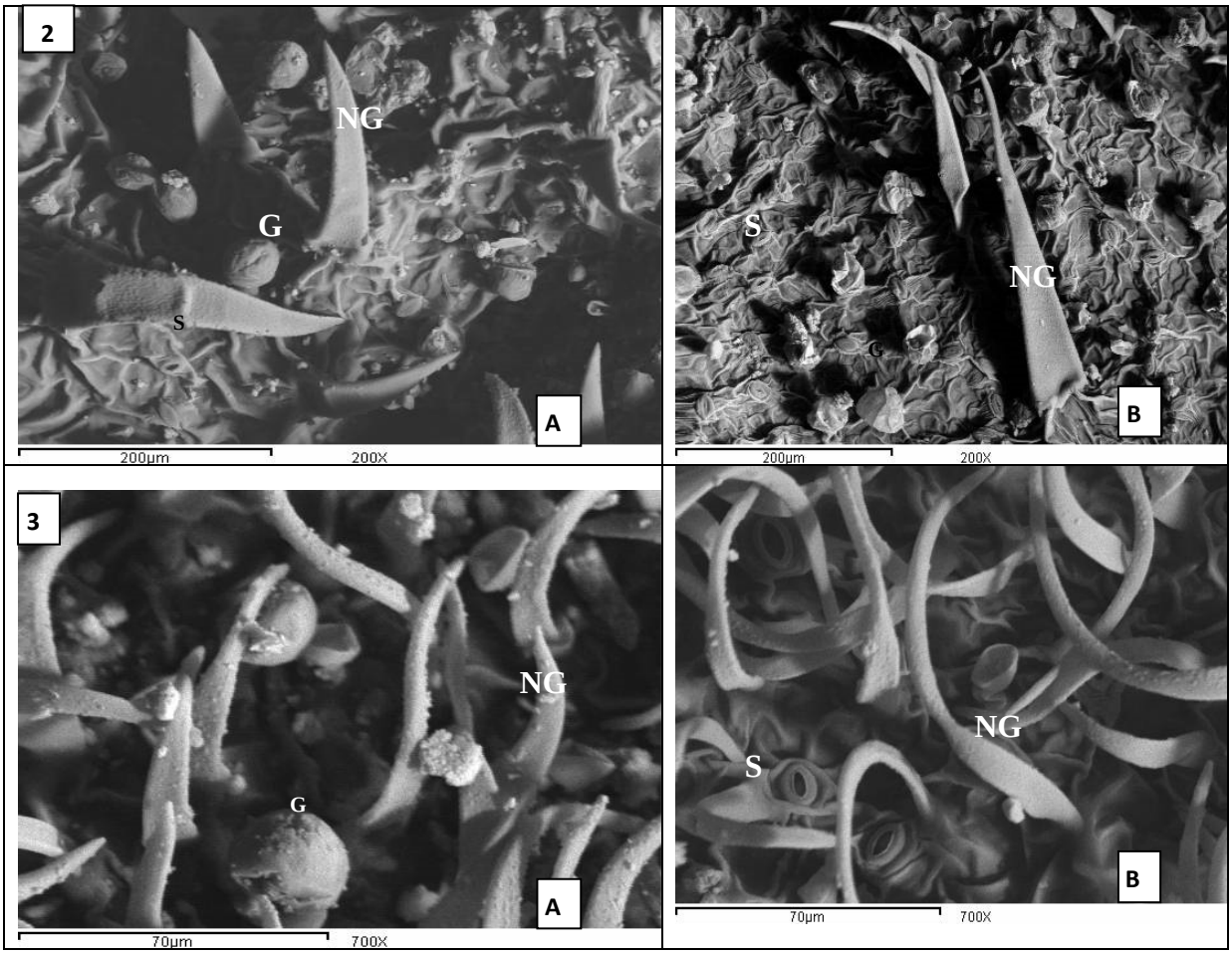
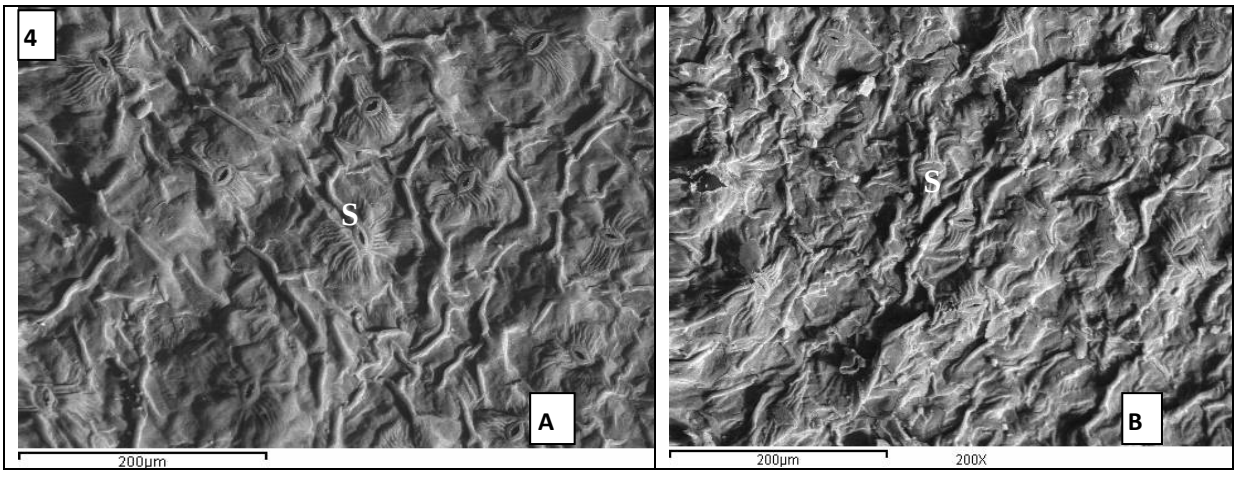


Fig. (4): Scanning electron micrographs of leaf blade of studied *Pelargonium* species.
A: Surface of upper epidermis, B: Surface of lower epidermis
1- *P.graveolens,* 2- *P. radula* and 3- *P. fragrance.*

(Cont.)



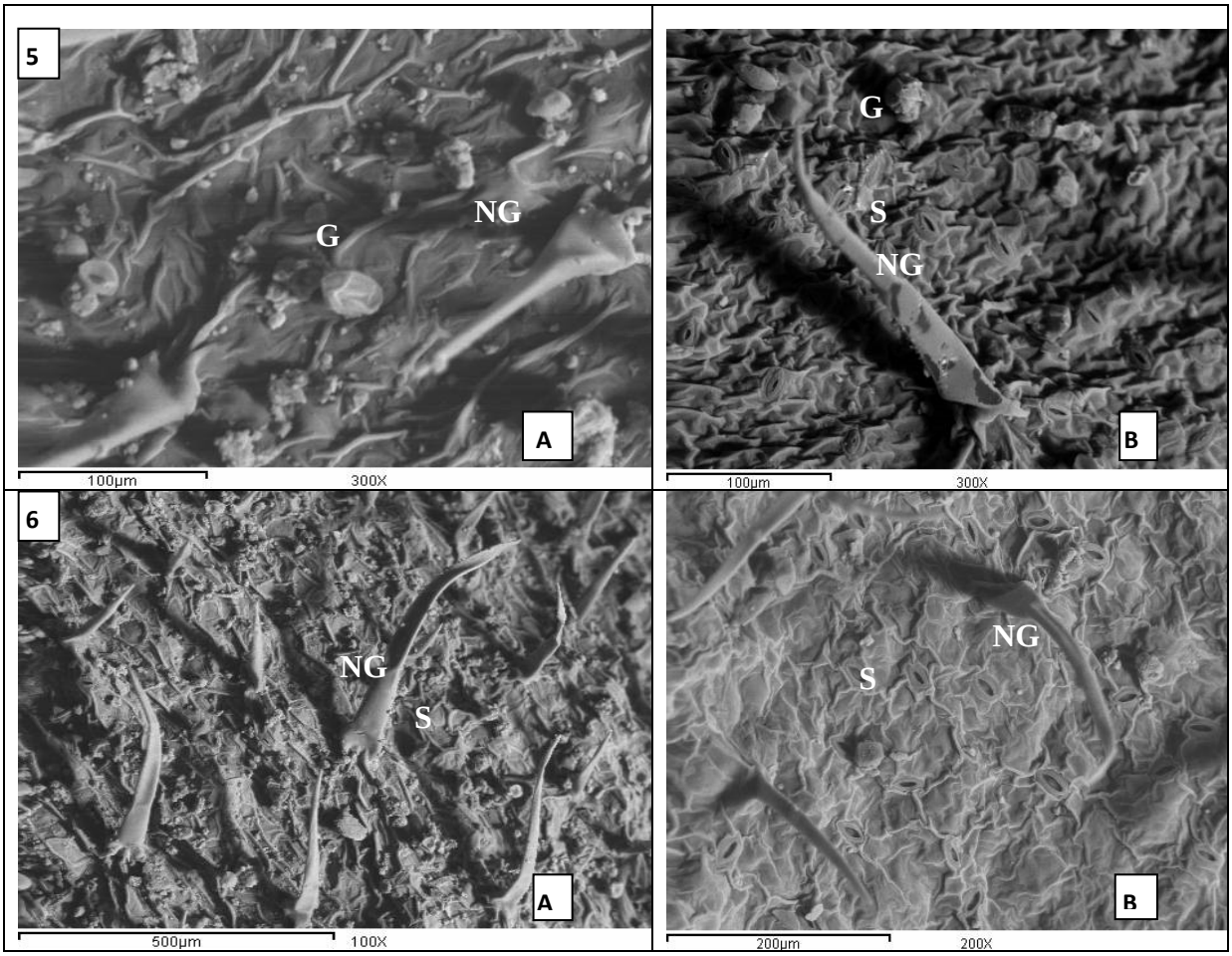


Fig. (4): Cont.
4- *P. peltatum*, 5- *P. zonale* and *6- P. grandiflorum.*
Details: S, stoma; G, glandular trichomes; NG, non glandular trichomes.

Anatomical characters

a- Lamina anatomy

The anatomical features of the leaf of studied *Pelargonium* species as shown in Figure (5) illustrate that the epidermis in both upper and lower sides is composed of single row of rectangular cells covered by thin cuticle, while upper epidermis cells were bigger than the lower ones in all studied species (Table 6). Multi cellular and glandular trichomes are recognized on both surfaces.

The mesophyll composed of single raw of well developed palisade parenchyma with relatively large intercellular spaces in *Pelargonium graveolens*, *P. radula* and *P. fragrance*. While in *P.peltatum*, *P. zonale* and *P. grandiflorum*, the palisade tissue composed of two layers of compact cells with no intercellular spaces and extended above vascular bundle. Lamina of all species under studied develop one kind of oxalate calcium crystals (druses) except *P. zonale* which lack of crystals. Multilayers of spongy parenchyma are found next to palisade tissue. The spongy parenchyma cells are loosen and irregularly shaped with wide intercellular spaces. Also noticed a considerable amount of oil secretory glands in lamina of *P.graveolens*, *P. radula* and *P. fragrance*. Main vascular bundle oriented in the center area is central, with xylem surrounded by phloem and the later bounded by caps of fibers. While other lateral bundles are distributed in leaf blade of *P.peltatum* and *P. zonale*.

Salimpour *et al.* [8] studied leaf anatomy of ten *Geranium* L. species and stated that *Geranium divaricatum* and *G. kotschyi* have rectangular epidermal cells but *G. pyreniacum*, *G. collinum*, *G. purpureum*, *G. robertianum* and *G. rotundifolium* had polygonal shape of epidermal cells. Palisade parenchyma in *G. robertianum*, *G. purpureum* and *G. divaricatum* only one layer was presented. In *G. pyreniacum* and *G. rotundifolium* two layers was showed but other species had three to six layers of palisade parenchyma. Presence or absence of crystals is different in taxa, *G. persicum* and *G. rotundifolium* there are no crystals in cortical cells and *G. kotschyi* , *G. stepporum* , *G. persicum* and *G. tuberosum* have more than crystals. Stomata type, usually consisting of anemocytic and anisocytic.

Lyubov *et al.* [11] studied the anatomical differences between some species of the genus *Geranium* L. They reported that the leaves of considered species of the genus *Geranium* are amphistomatous, with dorsiventral mesophyll. The

epidermis cells have strongly flexuous shape. The upper and lower epidermis are single layer, covered with cuticle. In all studied species the thickness of upper epidermis is larger than the thickness of lower one. The palisade mesophyll consists of two layers of cells and takes a larger percentage of the leaf structure compared to the spongy mesophyll. The latter contains small intercellular spaces. The vascular bundles are collateral type. The strengthening tissue is represented by sclerenchyma fibers of primary phloem and by collenchyme facing of vascular bundle on abaxial side of the leaf.

b- Petiole anatomy

It is clear from Fig. (6) that the petiole of *P. fragrance*, *P.peltatum* and *P. grandiflorum* is circle, while in *P.graveolens*, *P. radula* and *P. zonale* is semi-circular in shape.

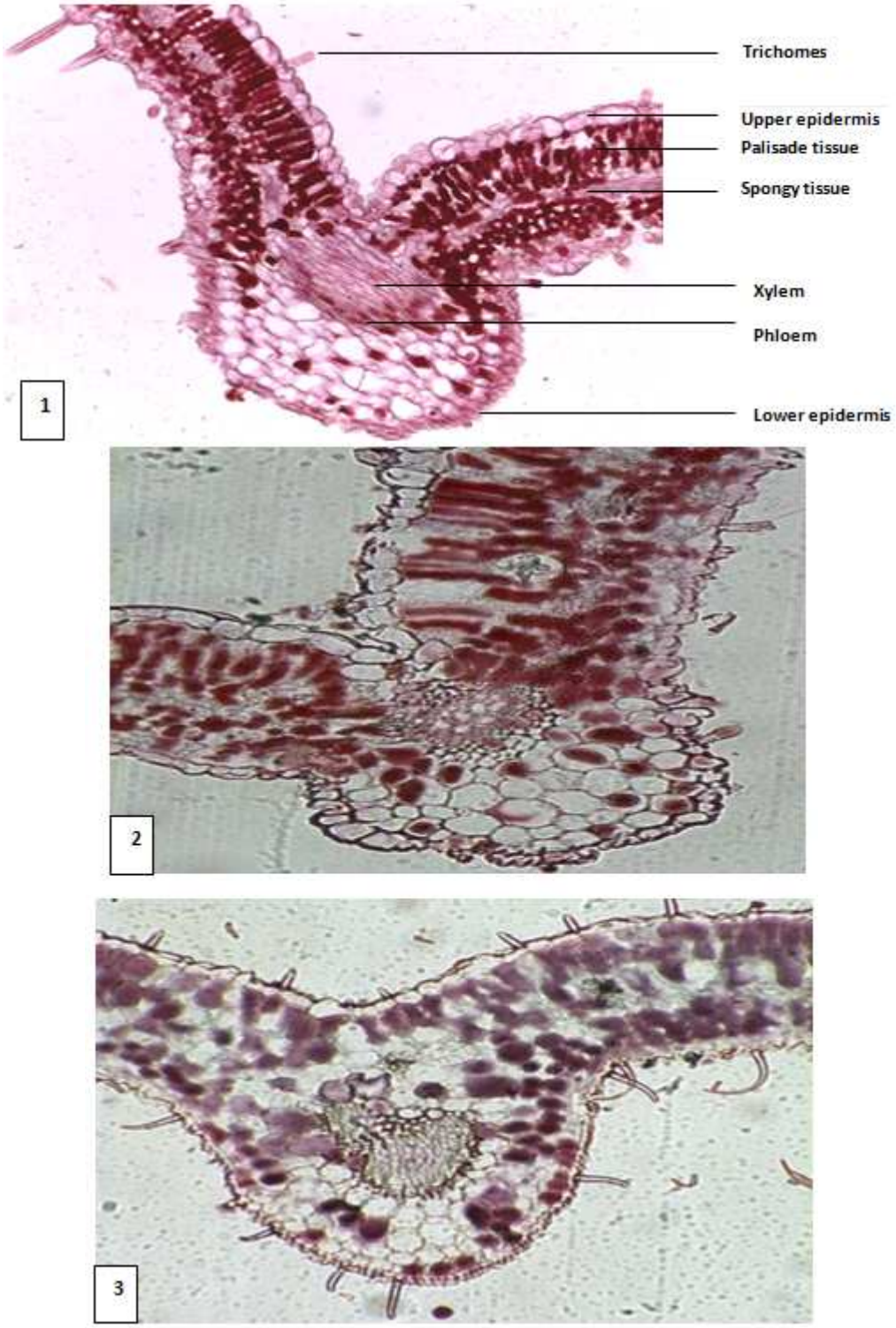
In all studied species the epidermis consists of a single layer of rectangular cells covered with a thick cuticle layer. The non glandular and glandular hairs are present in the epidermis. The cortex consists of 1-2 layers of collenchyma underlying the epidermis and the inner layers of the cortex comprised thin walled compact parenchyma cells. A ring of fiber above vascular bundles consists of 2-3 layers.

The vascular bundles are arranged in a ring and separated from one another by the ground tissue. The number of vascular collateral bundles differ from 8-15. The pith occupies a large portion of the section and consists of parenchyma cells.

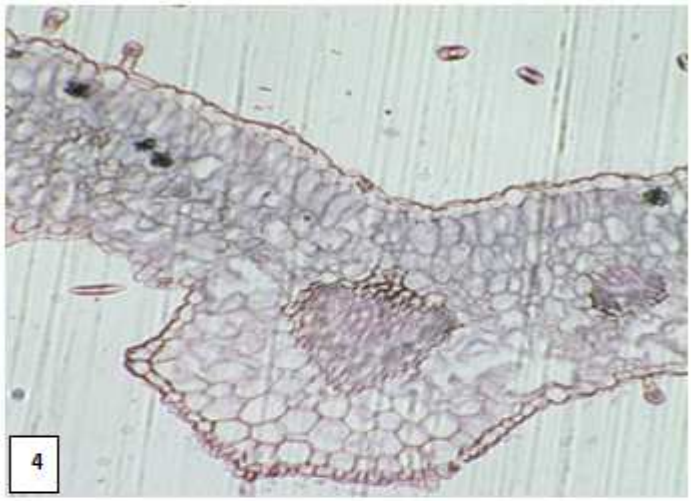
Lyubov *et al.* [11] stated that the characteristic feature of petioles of *Geranium sanguineum* is a presence of a single simple multicellular trichomes. The simple unicellular trichomes are present only on petioles of *G. sibiricum* and *G. sanguineum*, the latter has a great number of them. The petioles of *G. macrorrhizum* are larger in diameter. This causes an increase in the number of layers of collenchyma, and the thickness of the epidermis, performing the mechanical function. The number of vascular bundles in all investigated representatives of *G. macrorrhizum* twice as much compared to the other studied species. In *G. robertianum* are observed, sometimes, 3 and 4 vascular bundles, collenchyma is very poorly marked, indentifying with the significantly thicker epidermis.

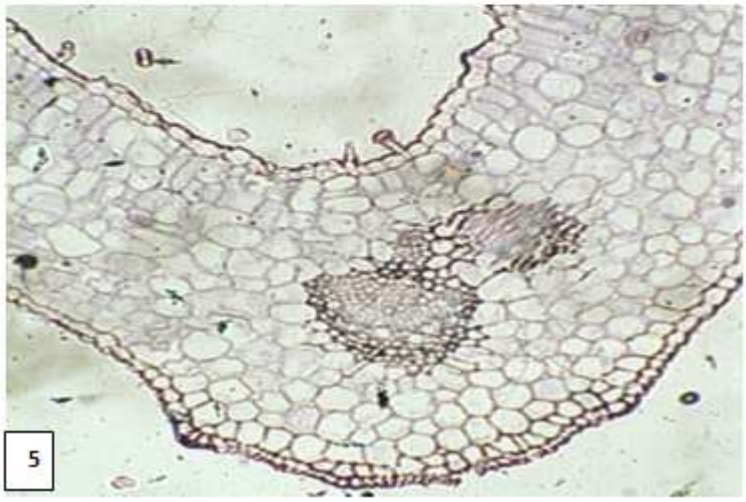
Table (6): Anatomical measurements (µ) and counts of different tissues of leaf lamina and petiole of six studied *Pelargonium* species

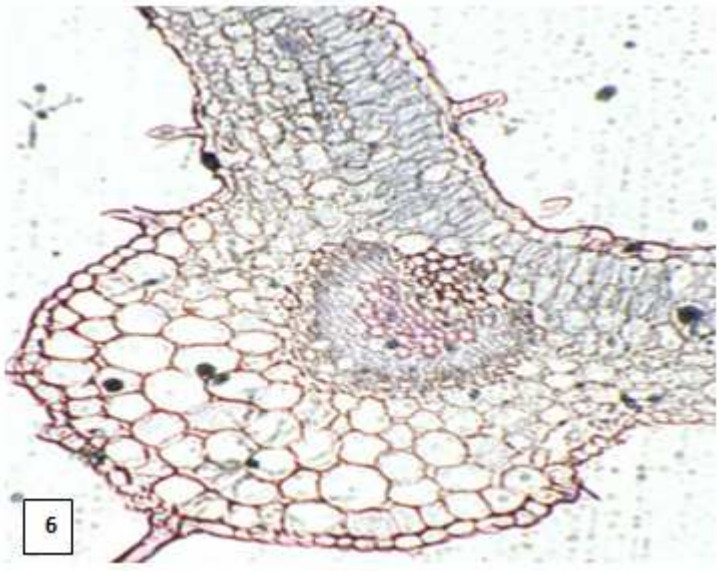
Characters	*p. graveolens*	*P. radula*	*P. fragrance*	*P. peltatum*	*P. zonale*	*P. grandiflorum*
a-Lamina						
Upper epidermis	47	55	45	53	35	35
Palisade tissues thickness	170	232	150	235	230	205
Spongy tissues thickness	190	310	300	315	390	297
Vascular bundle thickness	310	370	250	400	300	550
Xylem thickness	170	185	95	170	100	100
Phloem thickness	95	98	70	115	85	125
Fiber thickness	65	120	80	118	50	160
Main vein thickness	795	920	960	1010	980	1340
Lower epidermis	35	30	25	30	30	30
Lamina thickness	430	705	520	605	628	518
b- Petiole						
Epidermis thickness	25	20	12	30	17	15
Collenchyma thickness	95	75	20	70	70	30
Parenchyma thickness	230	280	160	220	210	330
Fibers thickness	95	85	45	50	90	55
Vascular bundle number	8	9	7	13	13	16
Xylem thickness	210	200	110	210	280	240
Phloem thickness	510	380	100	125	100	90
Pith diameter	2120	2720	960	1710	2570	2860
Cross section diameter	4240	4625	1930	2930	3720	3860



1. ***P.graveolens,*** **2-** ***P. radula*** **and 3-** ***P. fragrance.***

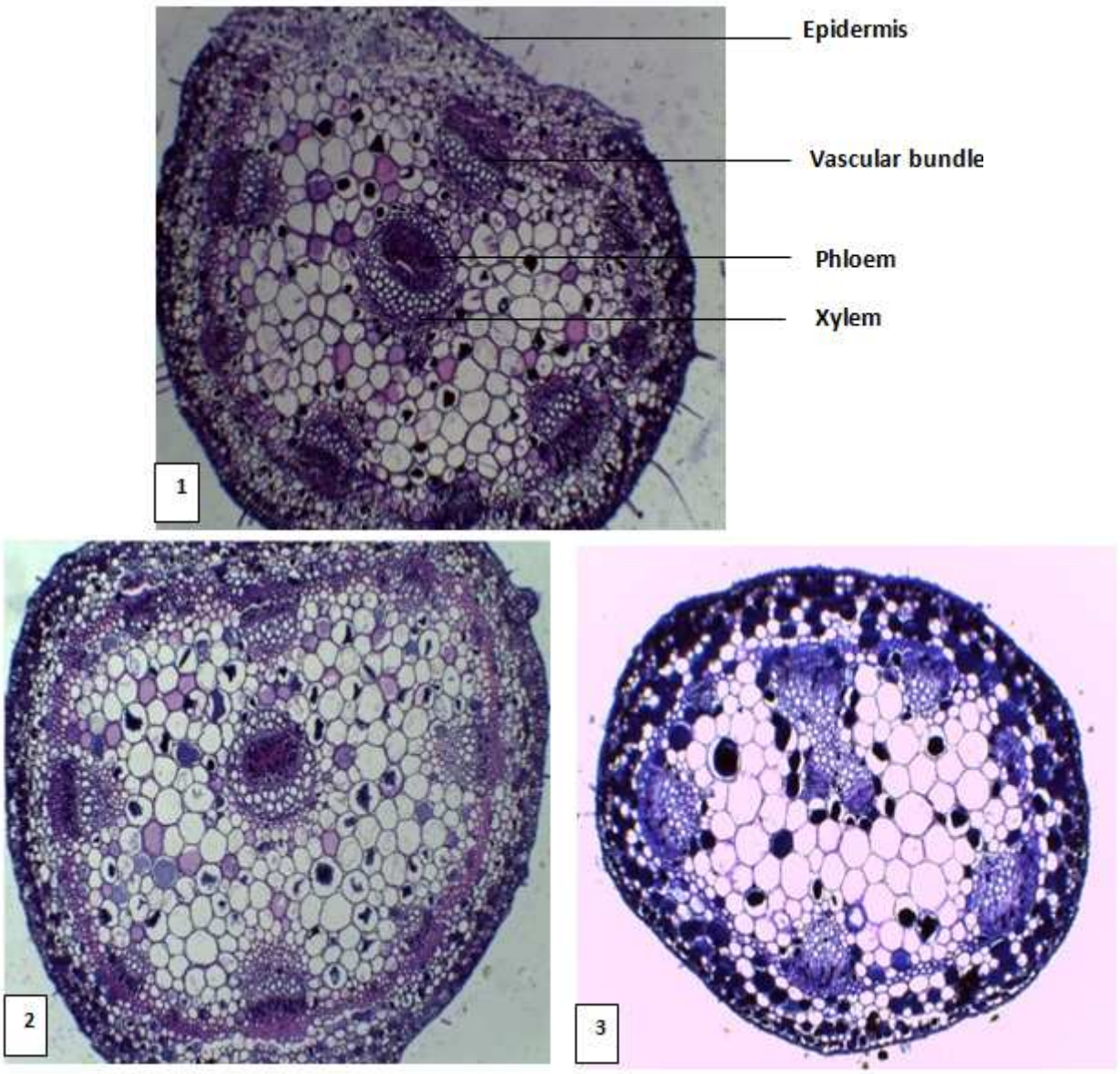




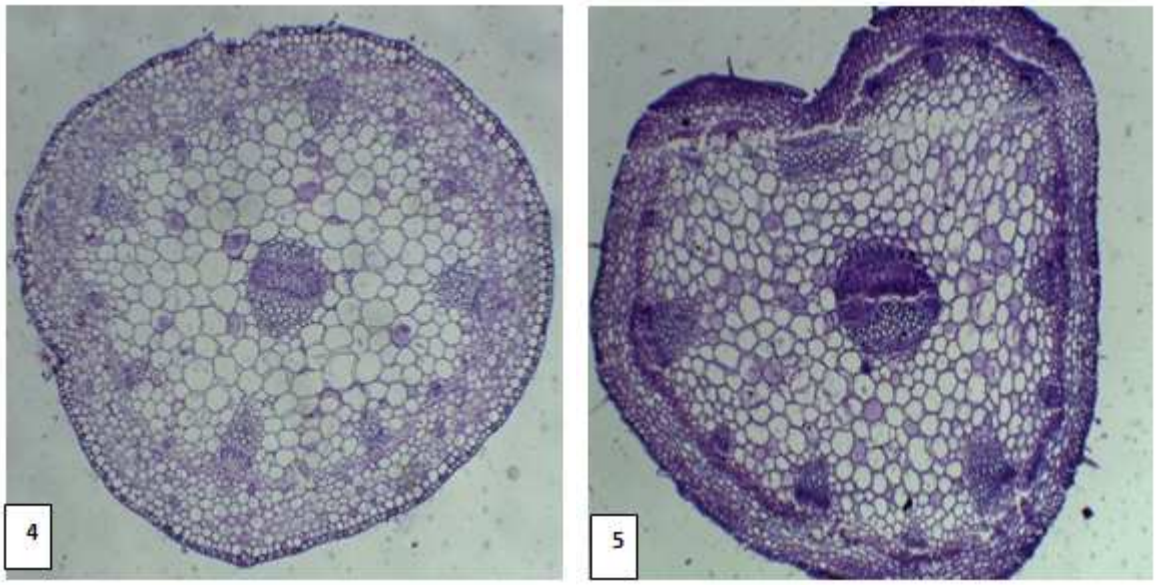


4- *P.peltatum*, 5- *P. zonale* and 6- *P. grandiflorum*

Fig. (5): Transverse sections of the middle part of the leaf lamina of studied *Pelargonium* species



1. P.graveolens,* 2- *P. radula* and 3- *P. fragrance.



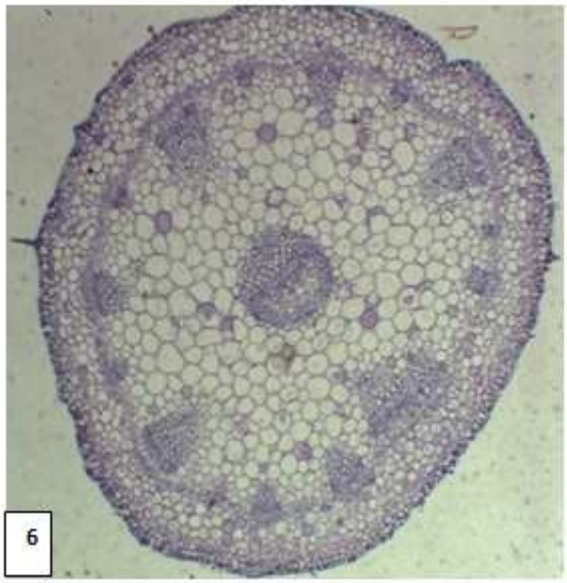


Fig. (6): Transverse sections of leaf petiole of studied *Pelargonium* species
4- *P.peltatum*, 5- *P. zonale* and 6- *P. grandiflorum*.

Botanical key:
The following key was proposed to distinguish and identify the studied species. The skeleton of these key is based on the macro and micro morphological characters of the leaf.

A. Leaf praemorsum shape in outline, dull and pale green, leaf sectioned old spice, glaucous and tiny texture.
AA. Leaf pinnatifidum lobed in shape, green, leaf sectioned lemon, hard and rigid texture. Rugose upper and lower surfaces of epidermis… …..*p. radula*
AAA. Leaf palmatum
b. Leaf sectioned rose
c. Leaf dull grayish green, have brownish zonal marking, cordatus base. Rugose- favulariate upper and lower surfaces of epidermis……..……..……...*p. grandiflorum*
cc. Leaf green, hastatus base. Reticulate upper surface of epidermis. Rugose lower surface of epidermis…*P. graveolens*
bb. Leaf sectioned strawberry, semi succulent, trailing. Rugose upper surface of epidermis. Indistinct lower surface of epidermis
…….……*p. peltatum*
bbb. Leaf sectioned camphor, succulent. Rugose upper surface of epidermis. Favulariate lower surface of epidermis……*p. zonale*

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