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BIOLOGICAL AND ANATOMICAL CHARACTERISTICS OF THE ROSE-SCENTED GERANIUM (*PELARGONIUM GRAVEOLENS*, L'HÉR.) GROWN IN THE SOUTH OF TUNISIA

MAHER BOUKHRIS[1,2], CHEDLYA BEN AHMED[1], IMED MEZGHANI[1], MOHAMED BOUAZIZ[2], MAKKI BOUKHRIS[1*] AND SAMI SAYADI[2]

[1]*Department of Life Sciences, Sfax Faculty of Science, Laboratory of Plant Biology and Ecophysiology, Sfax, Tunisia*
[2]*Laboratory of Environmental Bioprocess, Centre of Biotechnology of Sfax, Tunisia*
[*]*Corresponding author: e-mail: makki.boukhris@yahoo.fr; Tel: (+216) 74 27 64 00; Fax: (+216) 74 27 44 37*

Abstract

Pelargonium graveolens, L'Hér., is among the most cultivated plants in Tunisian gardens as ornamental species. Although it is well known for its exceptional aroma and essential oils benefits, it is still unknown on the scale of its biological and anatomical characteristics. It is in this context that this study lies to account for the characterization of this species. In fact, the anatomical investigations showed that while the stomata density of this species was high particularly on the leaf underside (42.13 st/mm^2), it was much lower (6.36 st/mm^2) in the petiole. Besides, the flowers are grouped into umbelform-cym inflorescence. Furthermore, the observations of the fingerprints of different aerial organs using the light microscope have shown that all parts are covered with two types of hairs or trichomes (glandular and non-glandular). A high density of both hair types was recorded on the different tissues analyzed in this study with an outstanding superiority for the stems. The results of leaf analysis have also shown that chlorophyll a content is higher than the chlorophyll b one.

Introduction

Over several decades, plants have been considered as the principal therapeutic and cosmetic tool for humans (Pokorny, 1991) all over the world. Numerous plans of medicinal and aromatic plants (MAP) extension included in the strategy of aromatic plants development in Tunisia seem to be so important and it is estimated that it could reach 7800 ha in the next few years. A great number of aromatic and medicinal species are currently used industrially (Mithila *et al.,* 2001; Sukhumpinij *et al.,* 2010; Khattak 2012). Among the wild plants, the lavender (Tsuro *et al.,* 2001; Lehrner *et al.,* 2005), the rosemary, the thyme (Mousavizadeh *et al.,* 2011), the myrthe, and the wild mint can be cited. As in the case of crops, the sour orange, the basil (Klimánková *et al.,* 2008), the jasmine, the rose and geranium (Saxena *et al.,* 2008) can be mentioned. In fact, the list of MAP is longer and could involve most of wild plants and many cultivated species (Ali, 2011; Lubbe & Verpoorte, 2011).

The present study deals with rose-scented geranium which was originally known in the South of Africa (Ko *et al.,* 2007; Saxena *et al.,* 2008). After that, it was introduced in several countries including India, China, France, Egypt, Spain, Morocco, Algeria and Tunisia. In the latter, although rose-scented geranium is grown successfully in gardens under different Mediterranean bioclimate (saharian, arid, semi-arid and humid climates) it cannot withstand the frosts for a long period (Rejeb *et al.,* 2006). As a cultivated plant, man intervenes in different ways (irrigation, windbreaks, size). Overall, it is a plant that grows as well under dry states (rained conditions) as under irrigated ones. Nowadays, in Tunisia, the rose-scented geranium is among the most cultivated plants in gardens as an ornamental species (Boukhris *et al.,* 2013). Indeed, in addition to the benefits of its essential oils (Ko *et al.,* 2007), the *Pelargonium* has an exceptional aroma. Per consequent, people are more interested in its cultivation particularly in the industrial and polluted zones as Sfax (Tunisia). Nevertheless, this species has only been known on the level of its industrial and/or medicinal use some decades now (Ram *et al.,* 1997; Virendra *et al.,* 2002; Ram *et al.,* 2003; Saxena *et al.,* 2008). To the best of our knowledge, no information is available about *Pelargonium graveolens* biology. Consequently, it is important to further characterize the rose-scented geranium plant on both botanical and morphological scale. That's why, the main objective of this study is to describe some morphological, physiological and anatomical features of the *Pelargonium graveolens* in such a way as to better recognize this species. Such features consist in the morphological description and botanical classification of the plant, the inflorescence characterization, stomata and hair densities and stomatal conductance and glandular and non-glandular hair categorization.

Material and Methods

Plant material and experimental design: This study was carried out at a botanical garden in Sakiet Ezzit in Sfax, Tunisia (52° 38N, 13° 19E). One-year-old rose-scented geranium plants (*Pelargonium graveolens*, L'Hér.,) were used in 2008-crop year. In this experiment, 15 plants divided into three plots of five plants each were used.The plants used were almost similar and grown under ambient environmental conditions with natural sunlight and temperature. The climate of this region is of an arid type with an annual rainfall and temperature averages of 200 mm water/year and 23°C, respectively. The climatic characteristics of the experimental site were recorded, over three successive crop years (2006-2008), from the National Meteorological Institute in Sfax (Anon., 2009). The results are shown in Fig. 1.

The sandy soil of the experimental orchard (3.0% clay, 9.6% silt, 87.4% sand) was too poor with regard to its nitrogen (0.04%) and organic matter (0.62%) contents. Moreover, it was characterized by 23.57% limestone, an electrical conductivity of 1.13 dS / m and a pH of 8.79. The nutrient status of the analyzed soil was of 23 ppm for phosphorus, 197 ppm for sodium, 172 ppm for potassium, 6200 ppm for calcium and 242 ppm for magnesium contents.

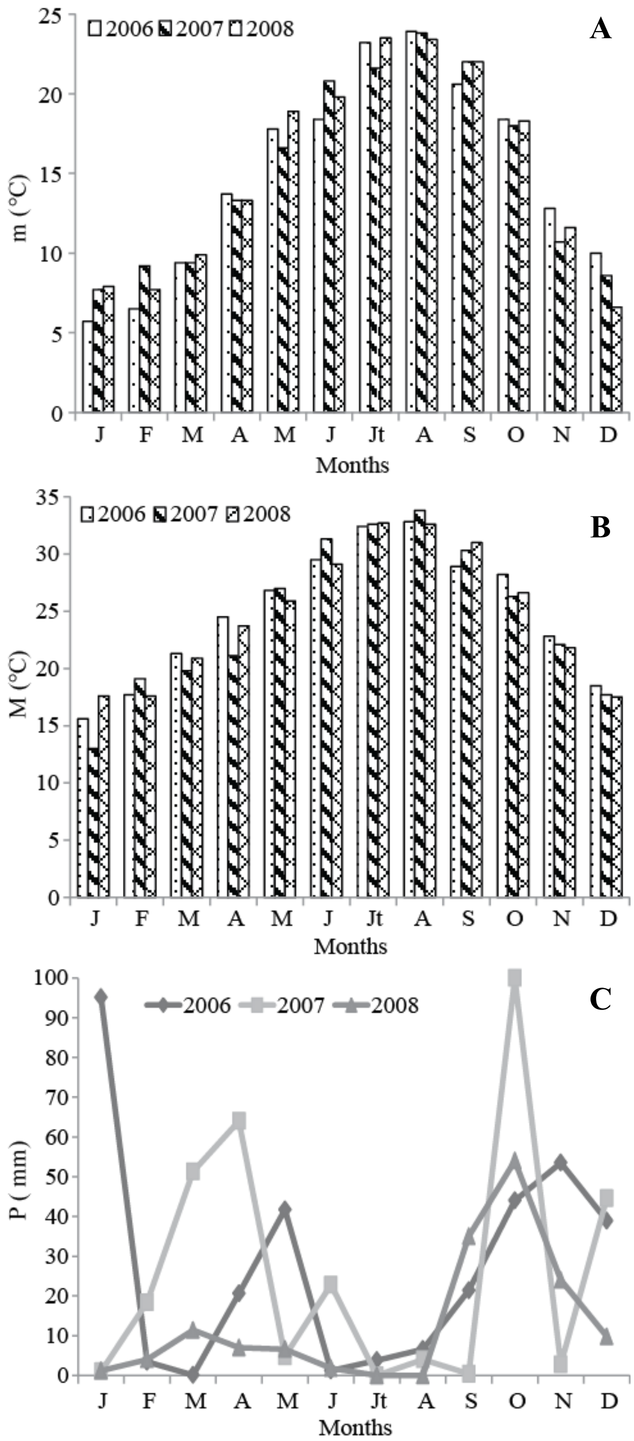

Fig. 1. Monthly variation of maximum (A) and minimum (B) temperatures and precipitation averages (C) of the experimental site over three successive crop years (2006/2008) (NIM., 2009).

The different measurements taken in this research work were realized on samples (stems, leaves, flowers) collected in April 2008 in coincidence with the full flowering period of *Pelargonium graveolens* L'Hér., grown in the south of Tunisia (Sfax).

Leaf area measurement: Leaf area was determined using an area meter (Li-Cor, Lincoln, NE) coupled to a computer. It was measured on 30 leaves collected on the same twigs of the selected plants. These leaves were divided into three groups of 10 leaves each: young leaves (YL), medium leaves (ML) and old leaves (OL).

Anatomical characterization: To characterize some anatomical features for the different plant tissues (blade, stem and petiole) analyzed in this experiment, they were fixed in alcohol 70°. Very thin cross-sections were performed using a razor blade. After that, they were placed in a bleach solution for 15 minutes and then rinsed with distilled water and soaked in a solution of acetic acid 30% for 5 minutes. Next, they were placed in a dye solution (green-carmin) for 3 minutes and rinsed for several times with distilled water. The prepared anatomical sections were placed on a slide with a drop of glycerin and water and observed under an ordinary microscope coupled to a computer.

Stomata and hair densities: The stomata and hair densities were determined, as described by Jones (1998), using the fingerprint of the epidermis obtained by a repeated application of a nail polish layer on the surface of blade, petiole, stem and sepal. Once dried, the varnish layer is removed with adhesive tape and placed on a glass slide. The counting of stomata and hairs was done on a light microscope coupled to the computer using special software.

Stomatal conductance and chlorophyll measurements: The stomatal conductance of the different leaf types used in this experiment (young, medium and old leaves) was measured with a diffusion promoter. The chlorophyll a and chlorophyll b concentrations were determined as described by Bacelar *et al.,* (2006).

Statistical analysis: The statistical analyses were performed using SPSS 11.0 for Windows (Statistical Package for Social Science), and treatment means were compared using student's t-test. At least three replicates were used for each data analysis.

Results and Discussion

Climatic conditions of the region: The Sfax city (south of Tunisia) is characterized by an arid climate of a Mediterranean type. The annual rainfall and temperature averages over a 52-year period are 250mm and 23°C, respectively. During three successive crop years (2006/2008), the monthly variation of temperature showed that the mean varied from 10°C in January to 28°C in August. While the average of temperature of the hottest period (July-August) was registered in 2007 (33.7°C), that of the coldest period (January) was of 5.7°C in 2006 and 7.7°C in 2007/2008 crop years (Fig. 1A and B). Indeed, the 2006 crop year presented a mild winter (the temperature average varied from 5 to 7°C), whereas those of 2007 and 2008 had a warm winter (temperature average superior to 7°C). In general, these conditions are favorable for the geranium rose development as reported by Rejeb *et al.,* (2006) indicating that, for this species, the cold risk could only be presented at a temperature average inferior to 3°C.

In addition, the above mentioned data show that the precipitation average of the region during the three successive crop years is irregular (Fig. 1C). The precipitation was virtually absent during the summer period (June-July-August). In fact, the year with low temperature (2006 crop year) presented the highest precipitation average. The highest amount was registered in autumn-winter period, but it remains less than 100 mm in 2006 crop year, and than 60 mm for 2007 and 2008 crop years. Indeed, these variations are characteristics of the arid climate of the south of Tunisia where the geranium rose could develop perfectly. Nevertheless, as in most cultivated species, the geranium development is dependent not only on the climate conditions but also on the different surrounding environmental conditions as reported by Rouphael *et al.,* (2008). In a study about the influence of irrigation system and nutrient solution concentration on potted geranium production under various conditions of radiation and temperature, the same authors have signaled that the effect of the growing season on the total plant growth is more pronounced than the effects of irrigation system and nutrient solution concentration.

Classification of *Pelargonium graveolens* L'Hér.: The genus *Pelargonium,* whose fruit shape is reminiscent of the stork beak (Fournier, 1961), derives from the Greek "Pelargos" representative of a stork. The genus includes about 300 species (Emberger, 1960). The "*graveolens*" species was described for the first time by Müller (1963) and Boukef (1986), but it was indicated neither in the flora of Tunisia (Alapetite, 1979; Le Floc'h & Boulos, 2008) nor in that of the Mediterranean basin which is interested particularly in spontaneous species (Greüter *et al.,* 1986). Besides, it was not described in "Flore Succincte et Illustrée des Zones Arides et Sahariennes de la Tunisie" (Chaieb & Boukhris, 1998). Nevertheless, two related species that are the *Pelargonium capitatum* and *Pelargonium robertianum* were reported by Rejeb *et al.,* (2006).

Based on the classification of Cronquist (1988) and the new Angiosperm Phylogeny Approach proposed by Spichiger *et al.,* (2002), the botanical classification of this species presented in Table 1 could be adopted. Many French names such as geranium rose, geranium, scented geranium, pelargonium fragrant were cited for this species, but the most used one is that of rose-scented geranium.

Table 1. Botanical classification of *Pelargonium graveolens* L'Hér.

Kingdom	Viridiplantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub-class	Rosidae
Order	Geraniales
Family	Geraniaceae
Genus	*Pelargonium*
Species	*graveolens*
Tunisian name	Attirchia

Morphological description of the species

The port: The rose-scented geranium, used in this experiment, is a compiled and branched shrub forming clumps of 1 to 2m of both height and width. It is also characterized by its rapid growth particularly in width, but height growth is slowed down if it reaches 2 meters. According to Vincent (1995a), the *Pelargonium graveolens* has a typical branching acrotone. In this development type, the terminal bud and the buds near to the apex grow before those placed at the bottom of the shoot. By studying the architectural characteristics of this species, Vincent (1995b) has noted that the development of an axillary shoot is closely dependent on the presence of subtending leaves and that the flowering occurs at the terminal position. According to Gorenflot & Foucault (1997), the notion of a port in a plant is dynamic during its life cycle, because several changes can occur and change the silhouette.

Leaf morphology: On the same branch, leaves exist at different stages of development, size and thickness. In the rose-scented geranium, we could distinguish juvenile, medium and mature leaves (Fig. 2). The arrangement of leaves along the stem is often alternating. The distance between two successive nodes varies from 1 to 8 cm, and at each leaf axil, a bud is found. The leaf consists of a blade, a petiole and two stipules. The indentations dividing the leaf blade reach the deep, but do not meet the midrib. In addition, the blade is cordate at the base, and was covered with hairs visible to the naked eye. They are more important on the underside than on the upper one.

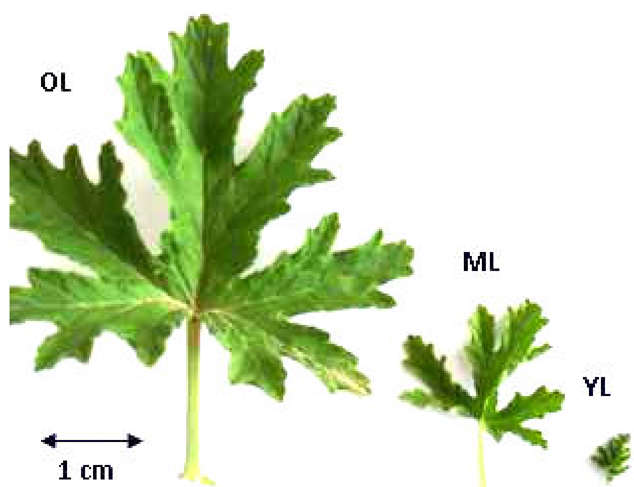

Fig. 2. Leaf morphology of *Pelargonium graveolens* L'Hér.(OL : Old leaf ; ML : Medium leaf ; YL : Young leaf).

The petiole length varies from 0.5 to 9 cm. It is also covered with numerous hairs. Stipules, which are leaf appendages on either side at the base of the petiole are slender and divided into 2 parts. The leaf area average varies from 21.08 to 24.23 cm^2 for mature leaves and from 3.83 to 4.68 cm^2 for middle leaves; while those juvenile do not exceed 1 cm^2. The differences between leaf areas of these leaves are statistically significant (p<0.05), which result from the leaf growth rate depending on the leaf age. Similarly, the leaf thickness is dependent on the leaf age. The average is of 0.41, 0.25 and 0.19 mm for mature, middle and juvenile leaves, respectively (Table 2).

Table 2. Leaf Area, leaf thickness, Stomatal conductance (Gs), chlorophyll a (ch a) content, chlorophyll b (ch b) content and ch a/ch b ratio of young (YL), medium (ML) and old (OL) leaves of *Pelargonium graveolens* L'Hér.

	YL	ML	OL
Leaf area (cm^2)	0.78 ± 0.28^{a}	4.23 ± 0.43^{b}	24.08 ± 3.13^{c}
Leak thickness (mm)	0.19 ± 0.03^{a}	0.25 ± 0.05^{b}	0.41 ± 0.06^{c}
Gs (mmol $m^{-2}s^{-1}$)	393.30 ± 31.30^{a}	210.5 ± 22.39^{b}	87.00 ± 11.24^{c}
Ch a (mg/g)	8.65 ± 0.43^{a}	10.02 ± 0.81^{b}	13.06 ± 0.80^{c}
Ch b (mg/g)	2.71 ± 0.18^{a}	3.09 ± 0.34^{b}	3.50 ± 0.61^{b}
Ch a/ Ch b	3.19 ± 0.03^{a}	3.23 ± 0.22^{a}	3.73 ± 0.40^{b}

Values are given as means ± S.D for 30 leaves in each group. Bars with different letters differ significantly (p<0.05)

Table 3. Stomata density, length and diameter and glandular and non glandular hairs densities of upper side, under side of blade, petiole and stem of *Pelargonium graveolens* L'Hér.

	Upper side	Under side	Petiole	Stem
Stomata density (mm^2)	8.78 ± 1.15^{a}	42.13 ± 2.95^{b}	6.36 ± 0.83^{c}	4.23 ± 0.46^{d}
Stomata length (µm)	39 ± 8^{a}	55 ± 15^{b}	45.21 ± 7.31^{a}	39 ± 8.53^{a}
Stomata large (µm)	32 ± 6.12^{a}	20.3 ± 5.84^{b}	24.91 ± 5^{b}	22.13 ± 4.61^{b}
Glandular hairs density (mm^2)	48.78 ± 4.21^{a}	81.93 ± 5.32^{b}	52.94 ± 6.66^{a}	87.95 ± 9.13^{c}
Non glandular hairs density (mm^2)	77.29 ± 9.43^{a}	100.83 ± 12.7^{b}	82.29 ± 8.64^{a}	129.37 ± 19.15^{c}

Values are given as means ± S.D for 30 organs in each group. Bars with different letters differ significantly (p<0.05)

The microscopic observation of footprints of leaf epidermis shows the presence of elliptical stomata with an irregular repartition (Fig. 3). These stomata constitute an ostiole delimited with two stomata cells surrounded by two guard cells. The results representing the stomata density per unit area for each type of the plant organelles are shown in Table 3.

The analyzed results show that the stomata density for the upperside (8.78 st/mm^2) is lower than that of the under one (42.13 st/mm^2) with statistically significant differences between them. In the petiole, the density is lower (6.36 st/mm^2). In addition, the length of the stomata on the underside (55 µm) is larger than that of the upper surface (Table 3), which could explain the success of photosynthesis in the underside of the leaf. Indeed, it is accepted that a high number of stomata provides a good freshener sheet leading to a decrease in tissue temperature, which promotes photosynthesis. Likewise, Niu *et al.,* (2005) have reported that a low stomata density (as in the case of the upper side of blade and petiole in our case study) influences more sweat and CO_2 assimilation because the stomatal resistance to vapor diffusion is relatively larger than the diffusion of CO_2 diffusion. These features could be adopted by the rose-scented geranium to better develop under the arid climate conditions characterizing the south of Tunisia.

The flowers: The flowers are grouped into umbelform-cym inflorescence. Indeed, the inflorescence stalk is of a variable length (6-8 cm) (Fig. 4B). The involucres constitute small bracts and numerous flowers (9-11) (Fig. 4A). Each flower is borne by a short pedicel. For the 40 inflorescences analysed in this study, the average of floral pedicel length is 0.59 cm (Fig. 5). Concerning the meristem of the inflorescence, it has a definite embryology. It builds periodical bracts and flower buds whose development takes place from the bottom. These buds are not of the same age, and are transformed after a few days in flowers. Inside the same inflorescence, the oldest flowers are placed in the centre and the youngest ones are developed in the outside.

During the study year (2008), the flowering buds of this species appeared late in winter (15th February), and the flowering process advanced perfectly during the second week of March. It was staggered over time and finished approximately at the end of May. It seems that the flowering process is correlated with ambient temperature. Indeed, as it was suggested by Amirtage *et al.,* (1983), the flowering process, particularly, in the case of geranium, is dependent not only on the quantum flux density, but also on the air temperature through their effects on net photosynthesis. On the other hand, the coincidence of flower bud organogenesis initiating with the increase in air temperature under the arid climate in the south of Tunisia justifies the correlation between these parameters. Nevertheless, this effect seems to be cultivar-dependent. Indeed, in *Pelargonium hortorum*, Torkel Welander (1983) has shown that the vegetative development is definitely affected by temperature, whereas generative development (number of inflorescences per plant) is only slightly influenced.

In the geranium rose of the present case study, the flower is zygomorphic (Symmetry with respect to a single plane) and is hypogenous (the protective caps are inserted below the ovary). This species presents cyclic pentamer flowers. Furthermore, the presence of both protective and fertile pieces (Fig. 6A) can be noticed. Inside the same species, the flower morphology seems to be cultivar-dependent. In fact, in *Pelargonium rapaceum*, the flowers are pink or yellow and most of them have red stripes on the lower part of the upper petals (Sukhumpinij *et al.,* 2010). The same authors have confirmed that the flower shape of *P. rapaceum* resembles to that of legume flowers.

The protective parts (perianth): The perianth constitutes of external and protective parts called sepals which form the calyx (Fig. 6B) and petals which form the corolla (Fig. 6C).

The calyx: it consists of five free oval sepals at different sizes (two large and three small). They are attached to the pedicel. While the underside was hairy (covered with hairs, Fig. 5B), the upper one is not covered with hairs.

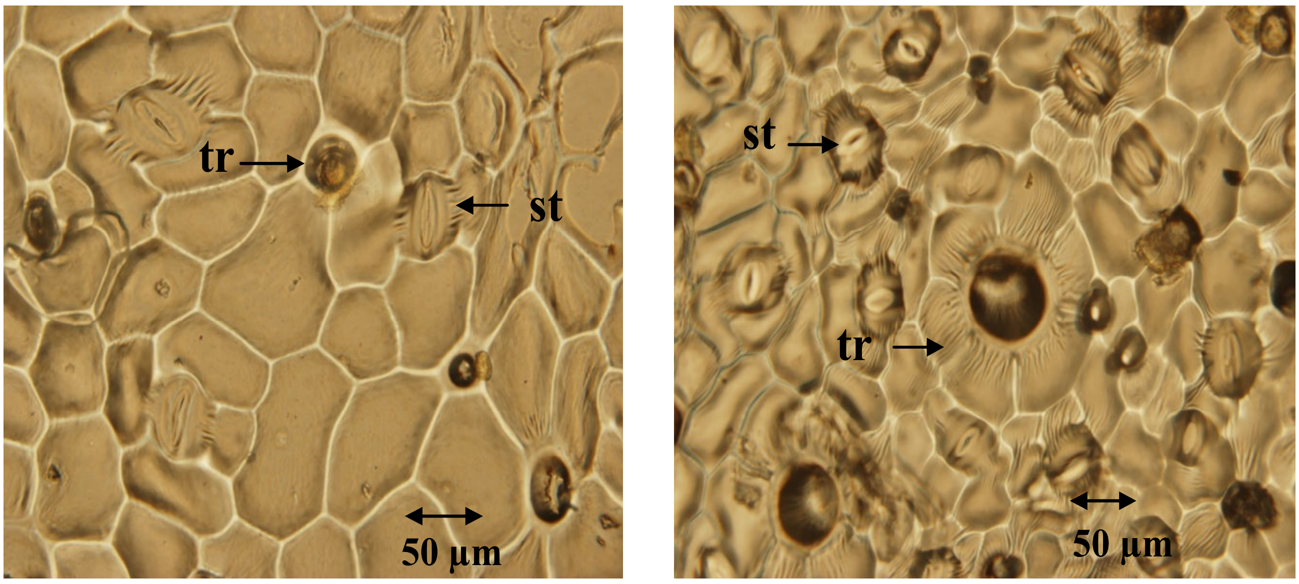

Fig. 3. Microscopic observation of footprints of upper side (A) and under side (B) of leaf epidermis of *Pelargonium graveolens* L'Hér. (st: stomata, tr: trichome.).

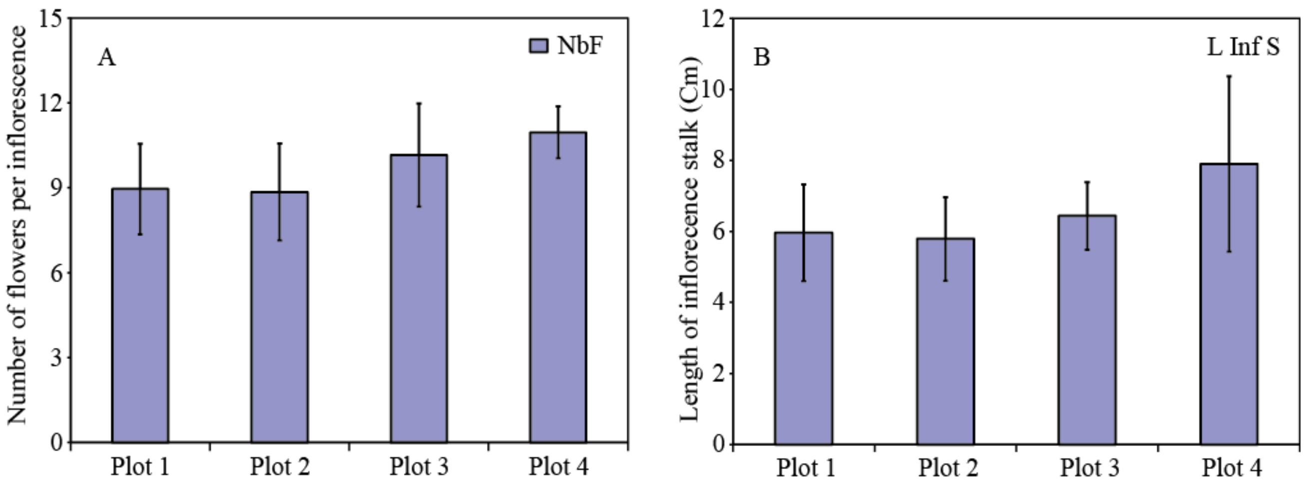

Fig. 4. Average of flower number per inflorescence (A) and inflorescence stalk length (Cm) (B) in the different plots of inflorescences (each one of 20) collected on 9 April 2008. Values represent the mean of 20 measurements ± SE.

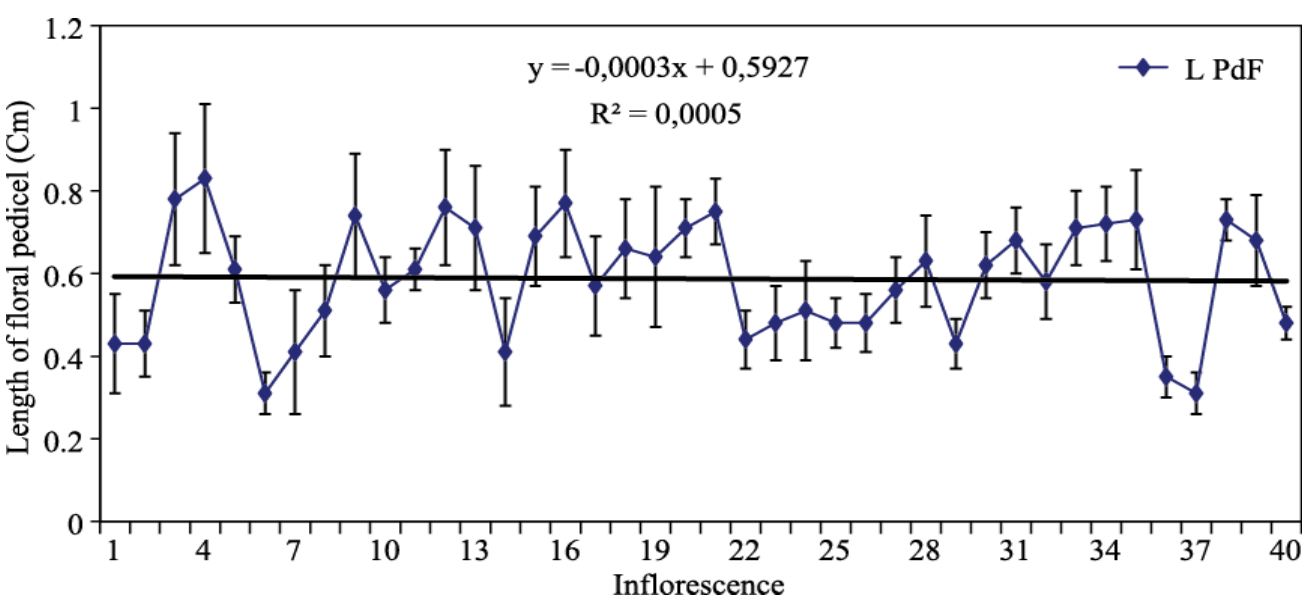

Fig. 5. Mean floral pedicel length (L PdF, Cm) of the different flowers from 40 inflorescences collected on 9 April 2008.

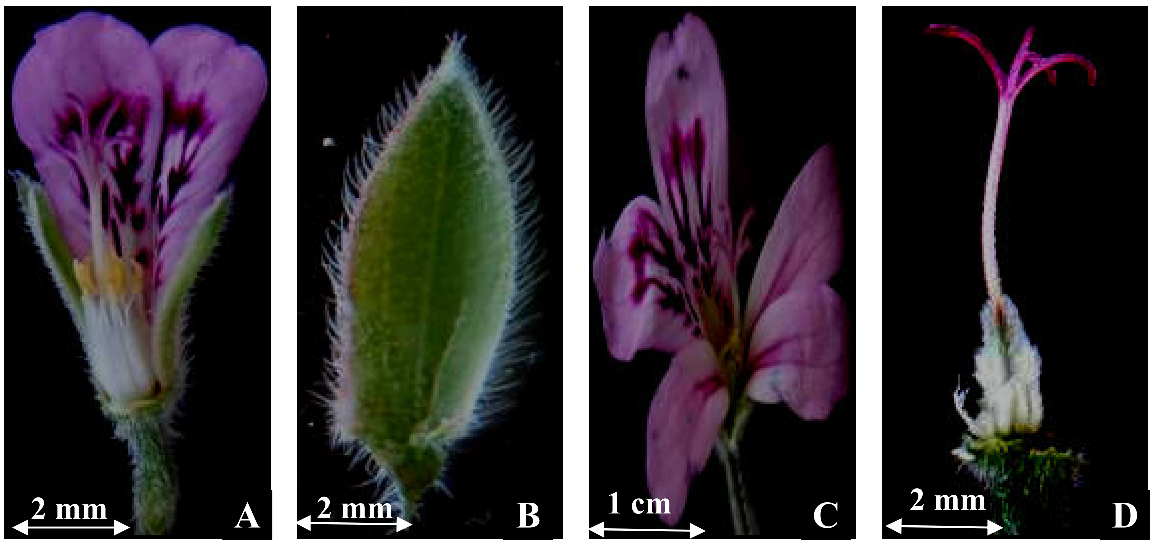

Fig. 6. The principal components of the inflorescence of *P. graveolens*. A: a young flower in which we have deleted 3 petals and 2 sepals to observe clearly the different flowring species. B: a sepal covered with hairs on the under side; C: a flower with the five petals. These petals are rose-purple, and two among them have purple veins; D: a flower from which we have deleted the perianth and the androecium to observe clearly the ovay which is superior and too hairy with a long style and 5 bent stigmas.

The corolla: it consists of five free oval petals (two are large and three are small,). The flower is characterized by free petals and sepals. These petals are rose-purple. The two upper petals are different from the others by their size and coloration. They are oval, tall and have purple veins (Fig. 6C). In comparison to other cultivars of the *Pelargonium* (Amirtage *et al.,* 1983; Torkel Welander, 1983; Sukhumpinij *et al.,* 2010), it appears that each cultivar had particular characteristics. Such patterns imply the importance of the characterization of such interesting plants, as the aromatic and medicinal ones, as well on the scale of their industrial use as on the scale of their biological and anatomical attributes which seem to be highly cultivar-dependent.

Reproductive parts: The flower is bisexual. It is formed of male (androecium) and female (gynoecium) organs.

The androecium: it consists of 10 fused stamens arranged in two whorls. The five stamens existing in the interior of the flower, which re called staminodes, are short and sterile. The stamens nets are welded at the base. The anthers are clearly visible only in the bud floral.

The gynoecium (pistil): The five styles forming the pistil are fused into a long hair style which is transformed into a mature feathery edge. The 5 stigmas are red-colored, separate and wiry. As for the ovary, it is too hairy and free and superior (above the sepals, Fig. 6D) and contains five carpels. Each lodge has an ovule and the placentation is generally axile.

The floral formulate and diagram: The floral formulate (FF) of the rose-scented geranium (*Pelargonium graveolens*, L'Hér.) developed in the south of Tunisia and used in this investigation could be described as:

FF: 5S + 5P+ (5+5) Si + 5C; where: S: sepal; P: petal; Si: stamens and C: carpel.

Taking into account the floral formulate, the floral diagram of this species is as described in Fig. 7.

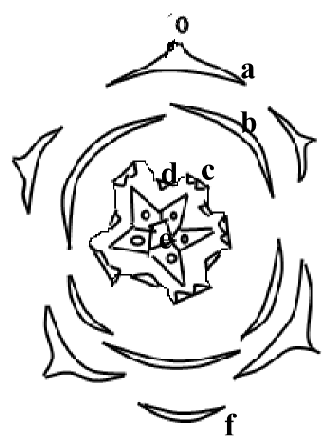

Fig. 7. Floral Diagram of *Pelargonium graveolens* L'Hér. (a: sepal; b: petal; c: stamens; d: staminode; e: gynoecium with axile placentation; f: bract).

Anatomical characterization

The anatomical characterization was carried out on the blade, the petiole and the stem.

The blade: As it is shown in Fig. 8, the upper epidermis has a medium thickness (50 µm) and is covered with a thin cuticle (22 µm). The lower epidermis was covered with two types of hairs: elongated non-glandular and secretory glandular hairs. The supporting tissues are represented by the angular collenchyma located inside the conductive vessels.

The petiole: Above the epidermis (65 µm) exists a thin cuticle (30 µm). As in the case of the blade, it is covered with detectors and glandular hairs (Fig. 9A). The supporting tissues have an important development: a tangential collenchyma exists in the epidermis. The sclerenchyma is located at the periphery of the petiole, as a continuous sleeve, and inside the centre of the vascular bundles which ensures the rigidity of the tissue.

The stems: The young stems are green and covered with hairs visible to the naked eye, whereas the seniors are brown and woody. The epidermis and the cuticle of the stem were thicker than those of the blade (80 and 50 µm, respectively). The epidermis is also covered with non-glandular and glandular hairs. The sclerenchyma formed a continuous layer around the vascular bundles that have a secondary structure (Fig. 9B).

The hairs: In the *Pelargonium graveolens* L'Hér., used in this experiment, the different aerial organs (leaves, stems and flowers) are covered with two types of hairs (glandular hairs containing essences and non-glandular hairs). As reported by Ko *et al.,* (2007), the hairs (also called trichomes) are quite common on *Pelargonium* leaves, stems and flowers with glandular and non-glandular bodies occurring as well on the abaxial as on the adaxial surfaces of a leaf. In this preliminary study, our observations are focused on fingerprints using the light microscope coupled to a computer with specific software.

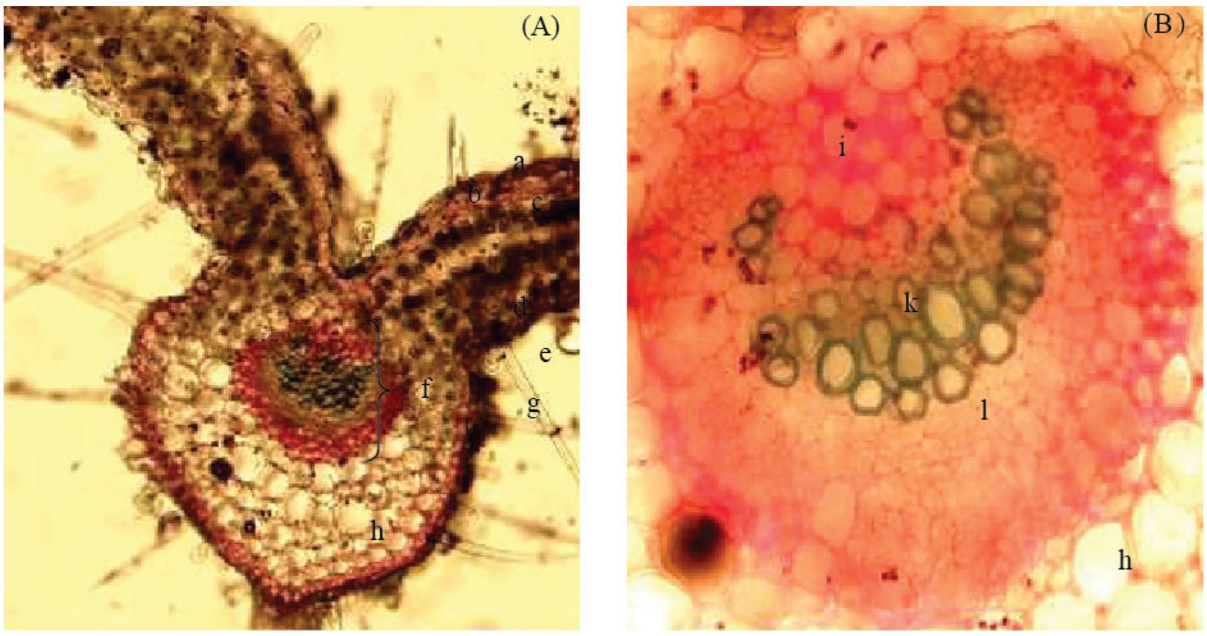

Fig. 8. Microscopic observations of the blade (A, view 10 x) and the central leaf vein (B, view 100 x) of *Pelargonium graveolens* L'Hér. (a: cuticule, b: upper epidermis, c: palisade parenchyma, d: lower epidermis, e: glandular hairs, f: vascular bundle, g: non glandular hairs, h: spongy parenchyma, i: collenchyma, k: xylem, l: phloem).

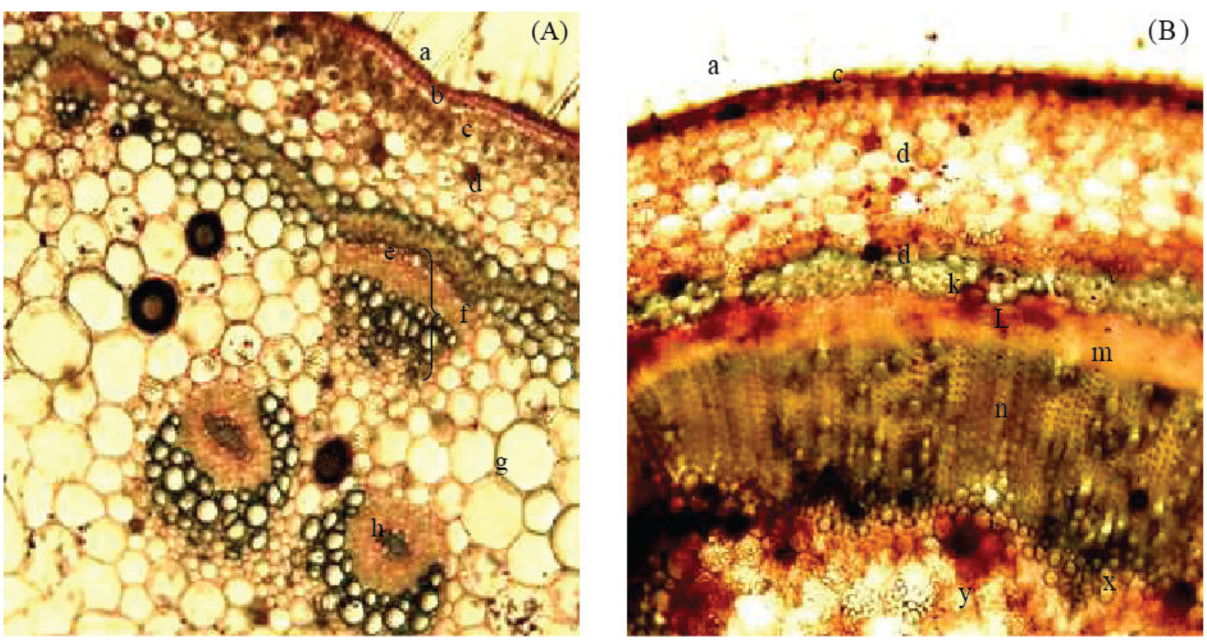

Fig. 9. Microscopic observation of the petiol (A, view 10 x) and stem (B, view 40 x) of *Pelargonium graveolens* L'Hér. (a: tector hairs, b: cuticule, c: epidermis, d: cortical parenchyma, e: sclerenchyma ring, f: vascular bundle, g: spongy parenchyma, h: sclerenchyma, k: crushed primary phloem, L: secondary phloem, m: cambium, n: secondary xylem, x: primary xylem, y: marrow).

Table 4. Length and largeness of glandular and non-glandular hairs of blade, petiole, stem and sepal of *Pelargonium graveolens* L'Hér.

	Blade	Petiole	Stem	Sepal
Glandular hairs length (µm)	196 ± 49 [a]	76 ± 15 [b]	145 ± 34 [c]	96 ± 28 [b]
Glandular hairs large (µm)	83 ± 15 [a]	54 ± 9 [b]	76 ± 13 [a]	46 ± 11 [b]
Non glandular hairs length (µm)	664 ± 131 [a]	225 ± 74 [b]	1070 ± 199 [c]	691 ± 182 [a]
Non glandular hairs large (µm)	61 ± 19 [a]	43 ± 17 [b]	79 ± 19 [c]	68 ± 14 [a]

Values are given as means ± S.D for 30 organs in each group. Bars with different letters differ significantly ($p<0.05$)

Non-glandular hairs: The non-glandular hairs are present on the blade, the petiole, the sepal and especially on the stem of *Pelargonium graveolens*. They are unicellular and bicellular structures attached to the epidermis. The non glandular hairs' densities on the higher face of the blade and the petiole were of 77.29 and 82.29 hairs/mm^2, respectively. This density was higher on the lower face (100.83 hairs/mm^2) and particularly on the stem reaching 129.37 hairs/mm^2 (Table 3). Statistically, the differences between these densities for the different organs were significant. In fact, these values are comparable to those recorded in *Pelargonium fragrans* by Ko *et al.,* (2007). Actually, the values recorded in the case of *P. fragrans* were of 68 and 97 trichomes per mm^2 on the adaxial and abaxial leaf surfaces, respectively. On the other hand, these results imply that the hair densities are cultivar-dependent. Such pattern allows us to suggest that such characteristics could be determined by the genetic characteristics of each cultivar inside the same species.

Concerning the non glandular hair length, the highest one was recorded on the stem (1070 µm). However, those of the blade and the sepal did not exceed 700 µm. On the petiole, the hair length was around 225 µm (Table 4). For the hair large, the values registered in the case of the different analyzed organs were of 79, 43, 68 and 61µm, for the stem, the petiole, the sepal and the blade, respectively (Table 4).

On the physiological scale, these hairs are known by their important role in reducing the water loss, and thus improving the resistance of the plants to arid environments.

Glandular hairs: In contrast to the non glandular hairs, the glandular ones are pluricellular structures. Nonetheless, they are also present as well on the blade, the petiole, the stem as on the sepal, but at different densities. The structures present external cells empty of essential oils. When the cuticle is destructed, the essential oils are dispersed into the atmosphere, which is a phenomenon that plays an important role in the attraction of "fertile insects".

The hairs disposition is too variable depending on the organ (Khokhar *et al.,* 2012). As in the case of non glandular hairs, the glandular hairs density was higher on the lower face of the blade than on the higher one, but lower than that of the stem (Table 3). The same pattern was recorded in *P. fragrans* by Ko *et al.,* (2007) who, more to the point, signaled the existence of two types of capitates glandular hairs (Type I and type II) with different densities according to the trichome development stage. Kaya *et al.,* (2007) indicated the identification of two types of glandular trichomes (peltate and capitate trichomes) in *Nepeta congesta*, but at different densities for the different analyzed aerial organs (stem, leaves and flowers). The preliminary results of the characterization of the different hair types in the *P. graveolens* grown in the south of Tunisia showed the existence of four types of glandular hairs (peltate, capitate type I, capitate type II and capitate type III) as registered in *Salvia smyrnea* by Baran *et al.,* (2010). Conversely, the highest glandular hair length was registered on the blade (196 µm) and on the stem (145 µm). Those recorded on the petiole and the sepals were of 76 and 96 µm, respectively (Table 4).

Physiological characteristics

Stomatal conductance: The stomatal conductance is considered as an indicator of both hydration status and transpiration rate level of the plant. As it is directly related to gas exchanges, its variation is generally determined by the stomta opening and closure phenomenon (Ben Ahmed *et al.*, 2007). The measurements of stomatal conductance for the different leaves used in this experiment showed that the highest value was recorded in young leaves and that this parameter was negatively correlated with leaf age. In fact, the older the leaf was, the lower the stomatal conductance was (Table 2). Indeed, the stomatal conductance was of 393, 210 and 87 mmol m^{-2} s^{-1} for young, medium and old leaves, respectively. These differences could be explained by the fact that the older the leaf was, the lower the hydration status was. As a consequence, leaves with low water status would close their stomata in order to avoid water loss and maintain its hydration status at appropriate levels, particularly under the contrasting climatic conditions characterizing the south of Tunisia.

Leaf contents in chlorophyll: The results of leaf analysis (Table 2) showed that in all leaf types used in this experiment (young, medium and old), chlorophyll a content was higher than the chlorophyll b one. Furthermore, young leaves showed the lowest content in chlorophyll a (8.6 mg/g M.S); however the old ones have the highest level (13.06 mg/g M.S). For the chlorophyll b, these contents were almost similar and the differences between the leaves were not significant. Indeed, these contents were of 2.7, 3.1 and 3.5 mg/g M.S in young, medium and old leaves, respectively. On the other hand, the chl a/chl b ratio was higher in old leaves (3.7) than in the medium (3.2) and young (3.1) ones (Table 2). Nevertheless, the differences between them were not significant.

Conclusion

The results described in the present study for morphological and anatomical characterization is the first report for *Pelargonium graveolens* L'Hér., which is extended in the south of Tunisia. Besides, our structural investigations have shown that this species had a high density of stomata particularly on the underside of leaf. Similarly, a high density of both glandular and non glandular hairs was recorded on the different tissues analyzed in this study with a remarkable superiority for the stems. The same results have also proven, in comparison to previous findings, that the morphology and anatomy characteristics of this species are cultivar-dependent.

Several reports reinforce the idea that the hairs are responsible for the essential oils secretion. Therefore, a specific characterization of the different hair types in the *Pelargonium graveolens* L'Hér., as well as the essential oils accumulated, and more detailed anatomical and ultrastructural studies are in progress. Furthermore, as this species is well developed in the polluted area of Sfax, Tunisia, an investigation on the different physiological, biochemical and anatomical mechanisms adopted by this cultivar, in particular, to resist to such harsh environmental conditions is intended to be conducted in the future.

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