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Leaf Micromorphology and Pollen Grain Characteristics of *Nicotiana glauca*. R.C. Graham and *Nicotiana tabacum*. L.(Solanaceae, Nicotianoideae)

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Abstract

The micromorphological variation of the leaf and pollen grain characteristics in two species of Nicotiana was examined using a light microscope (LM) and scanning electron microscopy (SEM). These species are Nicotiana glauca R.C. Graham (wild species) and Nicotiana tabacum L. (cultivated species). The leaves are simple. The other leaf micromorphological features presented variation. The leaf architectural characteristics were also observed. Leaf venation is pinnate brochidodromous. The epidermal cell shape, on the abaxial leaf surface, is polygonal to irregular in Nicotiana glauca but it is so adaxially in all the two species the cells are irregular on both the epidermal layers. The anticlinal walls are either straight or sinuous. The leaves are amphistomatic. The stomata are commonly anomocytic to anisocytic. Foliar trichomes are glandular and non-glandular. Some of the foliar micromorphological characters as viewed under SEM were beneficial in species identification. The pollen shape is prolate with aperture tricolpate without opercula, the colpi shallow sunken with their ends acute. The exine sculpture is reticulate with granulate or granulate-microperforated to fine granulate and irregularly reticulate to perforate.

Keywords: Leaf micromorphology- Pollen grains - Light microscope (LM); Scanning electron microscopy (SEM)- Nicotiana Solanaceae -Nicotianoideae.

Introduction

The Solanaceae is one the largest and economically most important families of angiosperms, including important food, spice, and drug plants [1]. There has been much change in recent years regarding generic circumscription in the family estimated it to contain 83 genera and 2671 species, but the most recent estimate is that the family includes more than 3000 species [1]. Approximately half the species in the family are included in the widespread, morphologically diverse, and economically important genus *Solanum*. The family is nearly cosmopolitan in distribution, found throughout tropical and temperate regions, but with a concentration of diversity in Australia and Latin America [2]. Solanaceae in Egypt represent that 30 species, two subspecies, and five varieties belonging to eight genera as constituting the wild [3]. The largest genus is *Solanum* comprising nine species, two subspecies, and three varieties. The Libyan flora comprises 24 species, and two varieties belonging to ten genera namely; *Datura*, *Hyoscyamus*, *Lycium*, *Nicotiana*, *Physalis*, *Solanum*, *Capsicum*, *Lycopersicum*, *Petunia* and *Withania*. The largest genus is *Solanum* with 5 species, and two varieties while the smallest genera are *Withania*, *Lycopersicum* and *Physalis* with one species each [4].

Solanaceae is well known for possessing a diverse range of alkaloids. One of the most important groups of these compounds is called the tropane alkaloid; including Scopolamine, Atropine, and Hyoscyamine. These are the key alkaloids of this family. The presence of these alkaloids makes many genera of this family medicinally important viz., *Datura*, *Hyoscyamus*, *Solanum*, and *Withania*. In addition, Solanaceae is regarded as a source of many species beneficial to human health, diet, beauty and ornamental use [5-9]. Species of the family used as food include *Solanum tuberosum*, *Lycopersicon esculentum*, *Solanum melongena*, etc., while *Cestrum diurnum*, *Petunia hybrida* and many others are grown as garden ornamentals. The Solanaceae, Scrophulariaceae, Nolanaceae, and some other families allocated under order Tubiflorae of the subclass Metachlamydeae (Sympetalae) [10]. The genus *Nicotiana* with 66 species, mostly found in the tropical and subtropical countries, especially North and South America, the Pacific, Australia, and Southwest Africa [4]. The main objectives of this study were designed to complete taxonomical data by using micromorphology characters of leaf and pollen grains in both identification and circumscription of the studied species.

Materials and Methods

In the present work, two species of Solanaceae were investigated for their leaf morphology and pollen characteristics by using (LM) and (SEM). These species include: wild plant from the Egyptian flora (*Nicotiana glauca* R.C. Graham) and locally cultivated species as an economic crop (*Nicotiana tabacum* L.).

A. Leaf Morphological Characteristics

The general leaf macro-morphological features were recorded. The petiole length was recorded depending on the average of five measurements from the third internodes. For LM leaf epidermal study, freshly collected leaves were used. The laminas of three leaves, taken from the third internodes, of three different plants for each species, were collected. The adaxial and abaxial epidermal peels were obtained from the fresh leaves after being washed in water, painted with fingernail polish on both the upper and lower surfaces, and allowed to dry. After drying, each epidermal peel was affixed on a clean slide and observed under a light microscope [11]. At least three slides were made from each epidermal layer for each species. In addition, freehand sections were cut from the fresh leaves to investigate the trichomes types.

Photographs illustrating the epidermal features were taken from the slides using an OPTECH-light microscope equipped with a USB 2.0 digital camera. The leaf venation was investigated after the fresh leaves for each species, one at a time, have been immersed, for one hour, in 20% Sodium hypochlorite solution for clearing at room temperature, washed several times in distilled water, transferred into 50% alcohol. The stained leaves were dried and pressed between filter papers till drawing using a stereomicroscope. The terminology of Manual Leaf Architecture was adopted for the description of the leaf venation type of the examined species [12].

For (SEM) leaf epidermal study, the prepared herbarium specimens were utilized. For each examined species one-centimeter square piece, representing the adaxial epidermis and the abaxial one, were cut from the standard median portion of the lamina near the mid-rib. Such pieces were directly mounted on stubs without any treatment, coated with a thin layer of gold, examined, and photographed using JEOL JSM-6510 LV scanning electron microscope at Faculty of Agriculture, Mansoura University, Egypt.

B. Pollen Morphology

For (LM) palynological investigation, the pollen grains were obtained from the prepared herbarium specimens (unopened flower), autolyzed according to [13]. The pollen grains were examined and photographed using the above-mentioned light microscope. The pollen measurements (µm) are based on 20 pollen grains. For (SEM) palynological examination, un autolyzed pollen grains were directly discharged from the mature flower buds on stubs covered with double tape, coated with a thin layer of gold, and examined and photographed using the fore-mentioned scanning microscope at the Faculty of Agriculture, Mansoura University, Mansoura, Egypt. Description of the pollen grains' shape- classes and the relation between the Polar Axis (P) and Equatorial Axis (E) of the grains were adopted [14].

Results

Leaf micromorphological characteristics of *Nicotiana glauca* Graham. (Plate 1)

Elliptical to lanceolate or ovate, acute, exstipulate, and alternate leaves. Petiole length 2.1(1.8-2.5) cm. Lamina simple, ovate, scarcely hairy, with cuneate base, acute apex, and entire margin. Lamina 1° vein category pinnate and 2° vein category weak brochidodromous (plate A). The epidermal cells under (LM) being irregular with straight to sinuous to U-shaped anticlinal on the adaxial surface (plate B), but polygonal to irregular with straight to U-shaped anticlinal walls on the abaxial surface (plate C). Stomata anomocytic on both the epidermal layers (plate C). Trichomes are non-glandular on both surfaces and being unicellular and bicellular (plates D and E) respectively. The epidermal cells under (SEM) being irregularly reticulate, with channeled and straight- sinuous anticlinal walls on the adaxial surface (plate F); but irregularity reticulate with numerous stomata, with channeled and straight- sinuous anticlinal walls (plate G).

Leaf micromorphological characteristics of *Nicotiana tabacum* L. (Plate 2)

Ovate to elliptical or lanceolate, acuminate, glaucous exstipulate, and alternate leaves. Petiole length 1.5 (1-2.5) cm. Lamina simple, obovate, scarcely hairy, with cuneate base, obtuse apex, and entire margin. Lamina 1° vein category pinnate and 2° vein category weak brochidodromous (plate A). The epidermal cells under (LM) being irregular with intensely sinuous to V- and U-shaped anticlinal walls on both the adaxial and abaxial surfaces (plates B and C). Stomata of the anomocytic to anisocytic type on the adaxial epidermis (plate B) but paracytic and anisocytic on the abaxial one (plate C). Trichomes are non-glandular

and glandular together on both surfaces (plates D and E). The non-glandular trichomes are unicellular and multicellular uniseriate. The glandular trichomes with a multicellular uniseriate stalk (plate E). The epidermal cells under (SEM) being incomplete irregularly reticulate with the anticlinal walls raised and straight–sinuous on both leaf surfaces (plates F and G) respectively.

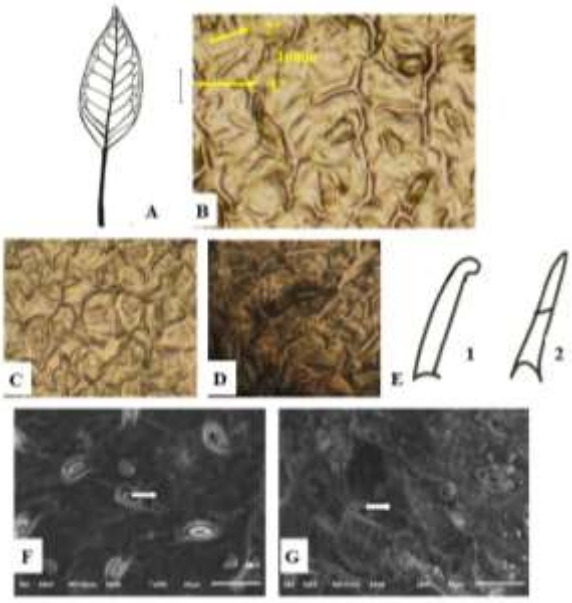

Plate 1: (A - G). Leaf micromorphological characteristics of *Nicotiana glauca* A. Leaf general morphology; B- G. Leaf epidermal morphology under (LM and SEM): B. Adaxial leaf surface, x= 660. **C**. Abaxial leaf surface x = 534**.** D, E. Trichomes types detected on the leaf: 1. Unicellular, 2. Bicellular. F. Adaxial leaf surface under SEM with dispersed stomata (White-arrowed); G. Abaxial leaf surface under SEM with dispersed stomata (White-arrowed).

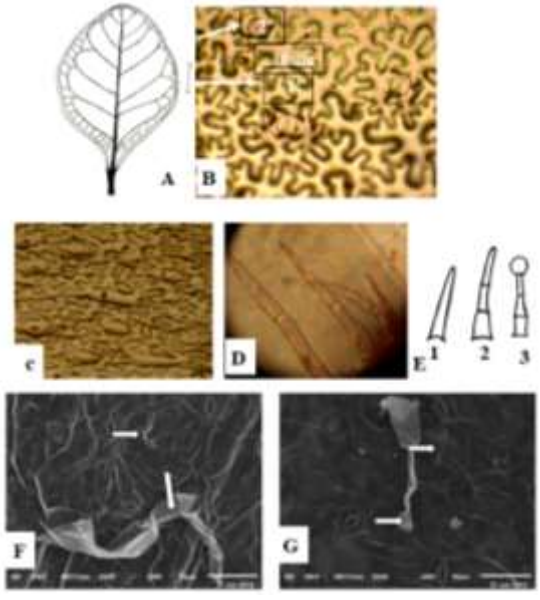

Plate 2: (A- G). Leaf micromorphological characteristics of *Nicotiana tabacum*. A. Leaf general morphology; B- G. Leaf epidermal morphology under (LM and SEM): B. Adaxial leaf surface, x=840; C. Abaxial leaf surface, x= 660; D & E. Trichomes types as detected on the leaf: 1. Unicellular, 2. Multicellular uniseriate, 3. Glandular with multicellular uniseriate stalk; F. Adaxial leaf surface under SEM with dispersed stomata and whip-like trichomes (White-arrowed); G. Abaxial leaf surface under SEM with dispersed stomata and whip-like glandular trichomes (White-arrowed).

Pollen Morphology

Nicotiana glauca Graham. (Plate 3): Isopolar, ellipsoidshapeunder (LM) in equatorial view and triangular in polar view. The polar axis length is23.6±3.4 µm while the equatorial diameter is16.4±2.6 µm. The pollen shape is prolate tricolpate without opercula (plates A, B, and C) respectively. Under (SEM) the colpi shallow sunken with their ends acute, exine sculpturing fine granulate (plate D and E) respectively**.**

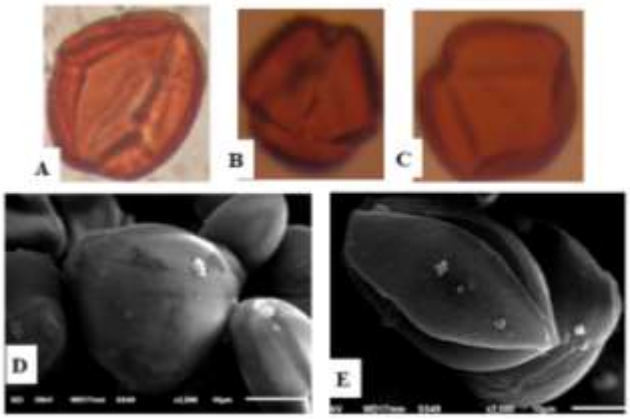

Plate 3: Pollen grains morphology of ***Nicotiana glauca*** L.D and E: equtorial view respectively.

Nicotiana tabacum L. (Plate 4): Isopolar, ellipsoid shape under (LM) in equatorial view and rounded-triangular in polar view. The polar axis length is 23.5±2.4 µm while the equatorial diameter is17.6±2.3 µm. The pollen shape is prolate, tricolpate without opercula (plates A, B, and C) respectively. Under (SEM) the colpi sunken with their apices acute and the exine sculpturing irregularly reticulate to perforate (plates D and E) respectively.

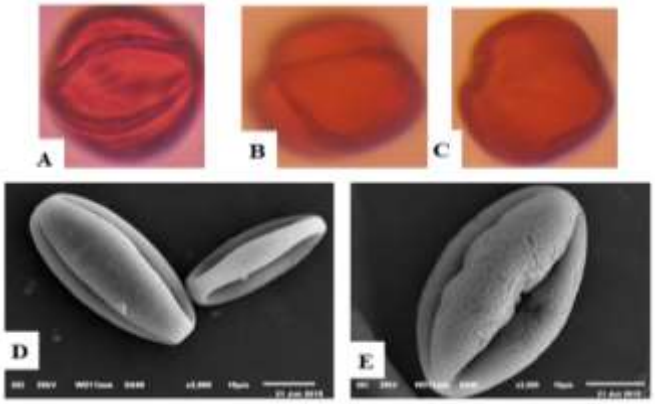

Plate 4: Pollen grains morphology of *Nicotiana tabacum* L.D and E: equtorial view respectively.

Discussion

The family Solanaceae is one of the most important families of flowering plants economically, floristically, ethnobotanically, and scientifically [6]. Micromorphology of leaf, shape, characteristics of leaf epidermis, and distribution of stomata were examined in the present study and interpret that structural characteristic have been shown in other species belonging to this family and are assumed to be associated with arid habitats [15]. The other important factor which is assumed to enhance the photosynthetic efficiency was the presence of well-developed systems of intercellular spaces which might be involved in facilitating rapid gas exchange.

Contents of the crystals, possibly produced by Nicotiana glauca leaves contribute to the toxic effects of secondary metabolites extracted from this plant [16]. It is, therefore, assumed that these secondary metabolites might serve as a defense mechanism. In this regard, the anatomical and morphological characteristics could be used as the additional tool in the classification of this species with the genus Nicotiana [17].

The reason for such good adaptation of the species Nicotiana glauca in new habitats might be its known capacity of self-pollination with probably successful self-fertilization (8). Moreover, it is known that Nicotiana glauca produces a large number of seeds dispersed mostly by wind, has early reproductive maturity, a rapid rate of growth, is grazing- and drought-resistant, and tolerant to a wide range of environmental conditions [18].

Trichomes are non-glandular on both surfaces and being unicellular and bicellular of Nicotiana glauca while non-glandular and glandular on both surfaces, the non-glandular trichomes are unicellular and multicellular uniseriate, the glandular trichomes with a multicellular uniseriate stalk of Nicotiana tabacum that congruent with the study of [19], that show unique bicellular to multicellular stalk glandular trichomes of Nicotiana tabacum, a mono-species genus in Nigeria are reported. They are believed to be responsible for the unique smell that emanates from the Nicotiana tabacum. The genus Nicotiana is the only genus in the family with multicellular stalk glandular trichomes. Leaf glandular trichomes of the non-model plant species Nicotiana tabacum represent a biologically active and stress-responsive tissue that contributes to plant defense response against biotic and abiotic stress and also influences leaf aroma and smoke flavor.

The stomata type can be singled out as being the most significant in relation to the taxonomic separation of the taxa. The studied taxa showed only anomocytic type on both the epidermal layers of Nicotiana glauca, while anomocytic to anisocytic type on the adaxial epidermis and only paracytic, anisocytosis type on the abaxial of Nicotiana tabacum that congruent with various studies of Solanaceae. Six types of stomata were examined on the abaxial and adaxial side of leaves *viz; anomocytic anisocytic, anamotetracytic, tetracytic, isocyanic*, and paracytic. Anomocytic type of stomata was represented in *Solanum villosum, S. Lycopersicum, S. pseudo-capsicum, S. melangena, S. tuberosum, Capsicum annuum, C. frutiscense, Hyocyamus niger, Cestrum nocturnum*, and D. *stramonium. Anisocytic type of stomata* was examined in *Solanum surratense, Solanum nigrum, Datura innoxia, Petunia hybrida*, and *Atropa accuminata. Stomata*l characteristics such as the type and index are among the anatomical parameter used in plant *taxonomy [20]*.

An old study that dealt with Solanaceae explained that mature stomata are anomocytic, anisocytic, paracytic and diacytic. The development of anomocytic stomata is haplocheilic or perigenous, while that of the other types is syndetocheilic or mesogenous. Abnormal stomata with a single guard cell, unequal guard cells, aborted guard cells and arrested development have been observed. Contiguous stomata are also common. The abnormal stoma with a single guard cell is formed due to disintegration of one of the guard cell before or after pore formation [21].

The pollen morphology of the family is quite heterogeneous [14]. However, tricolpate grains are universally present. Pollen grains are usually radially symmetrical, isopolar, prolate-spheroidal to sub-prolate, or prolate rarely oblate spheroidal. Usually tricolpate rarely 4-corporate, colpal membrane finely-coarsely granulated or sub-psilate. That is incongruent with our results in many characters. The significance of pollen morphology in Plant Systematics has been stressed by various researchers. The data from pollen grains are known to be useful at all levels of the taxonomic hierarchy (generic, subgeneric, inter-specific, and intra specific levels), and can often be helpful in suggesting a relationship [22]. On the most recent molecular phylogenetic studies of the family, the taxonomic synopsis of the Solanaceae divided into the following subfamilies Cestroideae, Goetzeoideae, Nicotianoideae, Petunioideae, Schizanthoideae, Schwenckioideae, and Solanoidea. The studied species belong to the subfamily Nicotianoideae which includes two tribes, Nicotianeae (Nicotiana only) and Anthocercideae [6].

In the present study, SEM-based pollen characters are found to be useful in the identification and discrimination of taxonomically related genera and species, it's clear in exine sculpture vary between two species, fine granulate and irregularly reticulate to perforate we can easily discriminate. Tricolpate pollens are observed in all the species except in Datura metal. Datura metal is observed to have only acolpate and monocolpate types of pollen, which indicates that it is a primitive dicotyledon [26], also it has a characteristic thick wall that delimits it from the other species studied. Nicotiana tabacum is the only species of the family studied with tricolpate pollen grains. It can thus be affirmed that the tricolpate type of pollen grains found in Nicotiana tabacum only, is a mark of recent evolutionary development in the species [19].

The pollen grains appear as Acolpate pollen grain type, tricolpate, tricolpate which is known as the phenomenon of polymorphism [13]. With respect of Nicotiana plumbaginifolia Pollen grains is 3-colporate, 3-zonocolporate. Shape: Prolate-spheroidal. Aperture: Ectoaperture-colpus not sunken long margin irregular, end acute Endoaperture: circular. Exine: Sexine thinner than nexine. Ornamentation: Tectum rugulate-fossulate, densely punctuate more or less psilate towards the apocolpial region. Outline: Equatorial view elliptic, polar view trilobed, 3-lobed [7]. A number of colpi on pollen grains have been a useful tool in tracing evolutionary relationships among the species of a genus. The advanced dicotyledon has more colpi than the primitive ones, with either a colpus (monocolpate) or none at all exculpate [13,23]. Acolpate pollen grains are observed in Capsicum frutescens, Datura metal, Nicotiana tabacum, and Solanum gilo

while monocolpate is observed in Capsicum chinense, Datura metal, Solanum gilo, and Solanum Indicum [7]. In addition, the studied species have pollen grains that differ in size, shape, equatorial view, polar view, aperture features, and exine ornamentation, confirming that Solanaceae is a eurypalynous family [24]. In this regard, the micromorphological characteristics of leaf and pollen grains morphology could be used as the additional tool in the classification of these species with the genus Nicotiana.

Conclusion

The present work was showed the micromorphological of leaf and pollen grain characteristics. Leaf venation, epidermal cell shape on both the epidermal layers, stomata type, foliar trichomes glandular and non-glandular, pollen shape, aperture type, and exine sculpture are significant characteristics beneficial between studied species.

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