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Leaf Micromorphology of Some *Viola* spp. from Iran

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Introduction

The *Violaceae* Batsch family with 23 genera and approximately 825-900 species is generally distributed across the tropical and subtropical regions of the world (Munzinger & Ballard, 2003; Watson & Dallwitz, 1992-97). The *Viola* genus is the largest in the *Violaceae* family, including 526-600 species that grow in most regions of the world (Ballard et al., 1999). According to Flora of Iran, only the genus Viola with 19 species is distributed in Iran. *Viola* species in Iran are placed in two sections (*Viola* section with 14 species and *Melanium* section with 5 species). *V. ignobilis*, *V. suavis*, *V. odorata*, and *V. hirta* belong to *Viola* section and two other species belong to *Melanium* section (Khatamsaz, 1991). Morphology has always been the most essential criterion to make comparisons between the species, and initial identifications have thus been performed based on morphology. Results of Qorbani et al., (2022) suggested a remarkable diversity in *viola* populations (Ghorbani et al., 2022). Stomata are among the leaf traits investigated in this regard (Miskin et al., 1972). Researchers believe that number of stoma and density per unit area varies across the genera, species, and varieties and thus present an area for further research (Dennert, 1884). The results of leaf micromorphology studies have shown that epidermal cells, stomata, trichomes, and epicuticular waxes on the surface of the leaf epidermis are useful diagnostic and

classification features (Esmaeili et al., 2019; Song et al., 2020; Patel et al., 2020). In the investigation of leaf morphology and micromorphology of *Anemone* L. and *Pulsatilla* Mill species. Traits such as length, general shape, epidermal pattern, and leaf shape have the most diversity and are useful in separating species and varieties (Shojaee et al., 2020). Because so far no serious study has been reported on the investigation of the micromorphological characteristics of the leaves of these species by scanning electron microscopy (SEM). Therefore, in the present study, for the first time, the micromorphological characteristics of the leaves of 6 species of violets from different parts of Iran were investigated by scanning electron microscope (SEM).

Material and Methods

Leaf samples of the six *viola* species were collected from their habitats in the four provinces of Golestan, Mazandaran, East Azarbaijan, and Ilam in April 2022 and were then dried. Samples were sent over to the herbarium of the East Azerbaijan Agricultural and Natural Resources Research and Training Center (AZH) and Tabriz University Faculty of Medical Sciences Herbarium (TBZFPH) to be identified and acquire herbarium vouchers. The examined species included *V. ignobilis, V. modesta, V. occulta, V. suavis*, *V. odorata* and *V. hirta*. Small sections of the leaves were separated and placed in a sputter coater for 10-15 minutes prior to performing electron microscope imaging. A thin layer of gold was then applied to the samples following the Physical Vapor Deposition (PVD) method. Leaf stoma structure was then studied using various electron microscope magnitudes (Smith et al., 1989). Imaging was performed using a FESEM electron microscope (MIRA III model). Leaf traits including aperture and trichome dimensions and density were measured using Image J v.1.52, and data obtained on leaf micro-morphology underwent statistical analysis in SPSS v.26. WARD cluster analysis was implemented to determine the similarities between the species. The terminology applied in the leaf surface micro-morphology study was built on the research of Metcalfe & Chalk, 1950.

Result and Discussion

Results of electron microscope imaging revealed the largest aperture dimension to have been observed in the *V. occulta* species, while the smallest aperture belonged to the *V. ignobilis* species. The highest and lowest aperture densities were observed in *V. hirta* and *V. occulta* species, respectively. The apertures in *V. ignobilis, V. modesta, V. occulta,* and *V. suavis* species were scaly and granular, whereas the *V. odorata* had smooth apertures and *V. hirta* revealed to have wrinkled apertures. Results on the studied species' apertures suggested that they had prominent outer and overlapping inner edges in *V. suavis, V. odorata,* and *V. hirta* species, while the apertures in *V. ignobilis, V. modesta,* and *V. occulta* were prominent in outer and raised in inner edges. Aperture openings were jagged in *V. ignobilis, V. occulta,* and *V. odorata,* interrupted in *V. modesta* and *V. suavis,* and smooth in *V. hirta* species. Stomata were ovoid in all the studied species. Stomatal

edges and protective cells other than apertures were covered in was in all the studied species. Granular grains, wax layers, and small wax planes were found the most in *V. modesta* and *V. ignobilis* species and the least in *V. occulta* and *V. hirta* species. Microscopic analysis results revealed that the trichomes were recumbent in *V. odorata* and *V. occulta*, semi-erect in *V. odorata* and *V. occulta*, and erect in *V. ignobilis* and *V. modesta* species. The trichomes were observed in three shapes including conical (*V. ignobilis*, *V. modesta*, *V. odorata*, and *V. suavis*), triangular (*V. occulta*), and articulate (*V. hirta*). Moreover, the trichomes were found in three states including straight (*V. ignobilis* and *V. occulta*), wavy (*V. suavis*, *V. modesta*, and *V. odorata*), and curved (*V. hirta*). The longest and shortest trichomes were found in *V. hirta* and *V. occulta*. In terms of the wax distribution pattern, microscopic analysis results revealed that the waxes were inclined to be placed on trichomes in the form of granular grains of various sizes across the species. The largest and smallest granular grains were observed in *V. odorata* (2.73 to 5.71µm) and *V. hirta* (0.8 to 2.53 µm), respectively. The largest and smallest number of granular grains diameters were also found in *V. modesta* and *V. occulta* species, respectively.

The dendrogram drawn based on micro-morphological leaf traits included two main clusters, one including *V. suavis*, *V. odorata*, *V. ignobilis*, *V. modesta*, and *V. occulta*, and the other containing the *V. hirta* specie. The six species were thus distinguished based on leaf micro-morphological traits. The highest resemblance in terms of traits was observed between *V. ignobilis* and *V. modesta*, and *V. suavis* and *V. odorata* species. *V. hirta* was also revealed to be the most distinct species in terms of micro-morphological traits.

According to the results of the present research, in *V. ignobilis*, trichomes are mostly located on the veins. In the species *V. suavis*, the trichomes are short, and the species *V. occulta* has few trichomes, which Khatamsaz (1991) mentioned in the flora of Iran. According to the dendrogram diagram, the most similarity in terms of leaf micromorphological traits was observed between the two species *V. modesta* and *V. ignobilis*, as well as the species *V. odorata* and *V. suavis*, this result is consistent with the results of microscopic image analysis. The two species *V. modesta* and *V. ignobilis* are similar in terms of trichome shape (long cone), having short trichomes, the number of trichome cells (unicellular), and the type of leaf surface ornamentals. Also, the two species *V. odorata* and *V. suavis* are different from each other in terms of trichome shape (long cone), trichome dimensions (long trichomes), trichome state (waviness), trichome density, number of trichome cells (unicellularity) and the type of leaf surface ornamentals, are similar.

Conclusions

The *V. hirta* species is significantly different from the other 5 species in terms of the dimensions, density and type of stoma (stoma with folded edges) and especially the dimensions, density and shape of the trichomes (uncinate and multicellularity). And according to the dendrogram diagram,

this species is in a separate cluster. These traits in this species are useful in identifying and distinguishing the species. But in 5 other species (*V. ignobilis*, *V. modesta*, *V. occulta*, *V. suavis*, *V. odorata*) despite the differences in terms of density, size and shape of stoma and trichome, the species can be separated to some extent. And considering that these traits are affected by environmental conditions, they do not have taxonomic value. It is suggested that pollen micromorphology and molecular markers be investigated and studied in these species.

Keywords: Stomata, Taxonomy, Trichome, Violet