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Morphological Study of *Viola canescens* Wall. Ex, Roxb. A Medicinal Herb from North-Western Himalaya

Rakesh Kumar and Vikas Sharma

Department of Botany, SBBS University Jalandhar, Punjab -144030, India

***Corresponding Author Email id:** saghotrark@gmail.com

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Abstract: *Viola canescens* Wall. Ex., Roxb., also known as Himalayan white violet, belongs to the Violaceae family. It is widespread in Pakistan, India, Nepal, China, and Bhutan, with a specific presence in Himachal Pradesh, Uttarakhand, Uttar Pradesh, Arunachal Pradesh, Jammu and Kashmir, Meghalaya, Sikkim, and West Bengal in India. This study commenced with morphological analysis, followed by ultra-structural and genetic characterization using SSR markers. *V. canescens* populations were surveyed in the Northwestern Himalayan regions of Uttarakhand, Jammu and Kashmir, and Himachal Pradesh. Qualitative traits aligned with prior records, but variations in quantitative traits were noted. This morphological exploration serves as a foundation for future research endeavors and aids in the selection of plant populations for cultivation, offering valuable insights for ongoing and prospective studies.

Keywords: *V. canescens*, Medicinal plant, Qualitative characters, Quantitative characters

Introduction

The Violaceae family consists of about 22 genera and 900 species distributed all around the world, and the members of the family show great diversity in growth forms, morphology, floral structure, fruit, and seed morphology (Neha *et al.,* 2017). It is commonly found in India, Pakistan, Nepal, China, and Bhutan (Muhammad *et al.,* 2012), is restricted to mountainous areas, and occurs mostly in tropical and temperate zones (Singh *et al.,* 2005). In India, it is found in Himachal Pradesh, Uttarakhand, Uttar Pradesh, Arunachal Pradesh, Jammu and Kashmir, Meghalaya, Sikkim, and West Bengal (Sharma and Bhardwaj, 2016; Chandra *et al.,* 2015). In Himachal Pradesh, it is reported from the Pangi Valley of Chamba District, Chamba, Solan, Kangra, Sirmaur, Mandi, Kullu, and Shimla. (Rana *et al*., 2014; Chauhan and Bhardwaj, 1984; Rani *et al.,* 2011; Choudhary and Lee, 2012; Thakur *et al*., 2012; Kumar *et al.,* 2013; Masood *et al*., 2014; Kumar and Sharma, 2016). In Uttarakhand, it is reported from the Garhwal region of the Himalaya, the Nagdev reserve forest of Pauri Garhwal, the Nainital catchment area, and Nanda Devi National Park (Agnihotri *et al*., 2012; Dua *et al*., 2011; Rana *et al*., 2010; Rana and Samant, 2009; Shah *et al*., 2014; Suyal *et al*., 2010). It is commonly known as Banfsha or Vanaksha in Jammu and Kashmir; Ratmundi, Gugluphul, and Banfsaha in Himachal Pradesh; and Vanafsha or Banfasha and Kauru in Uttarakhand (Masood *et al*., 2014; Rana *et al*., 2010). It is the most popular herb used in Ayurveda, Unani, Siddha, and homoeopathy and has historically been used to treat a variety of common illnesses, including colds, coughs, and sore throats, as well as several serious conditions, including renal issues, cancer, jaundice, and immune boosters and blood purifiers (Rana *et al.,* 2010; Kumar *et al.,* 2013; Mann *et al*., 2016; Hamayun *et al*., 2006; Abbasi *et al*., 2010; Hussain and Bano,

2011; Kumar *et al*., 2013). Various chemicals are found in plants, which include methyl salicylate, alkaloid violin, quercitrin, saponins, and glucosides (Rana *et al*., 2010). It is being used unscientifically for therapeutic purposes without using any protective measures due to its strong medicinal potential. As seen in the Swat valley in Ajad Kashmir and the Malam Jabba valley in Swat Pakistan, this plant is quickly vanishing from its natural environment due to large-scale, indiscriminate collection from the wild (Hamayun *et al.*, 2006). However, due to unscientific exploitation and habitat loss, their distribution and occurrence have decreased remarkably in the North-Western Himalayas. Hence, characterization of their existing germplasm at the morphological and molecular level and conservation of their diverse germplasm are urgently required. Further, characterization should be carried out at the phenotypic, genetic, and phytochemical levels to identify the best chemotypes and genetic stocks for future utilization and improvement.

Materials and methods

Study area : A plant of *V. canescens* grows in different locations of the Himalayas. Therefore, the study was conducted in the following regions: Himachal Pradesh, Jammu and Kashmir, and Uttarakhand.

Plant survey and collection: The field surveys were carried out in different regions of the North Western Himalaya of the Indian Himalayan region, representing two districts, Kullu and Mandi, of the state of Himachal Pradesh; Pauri district of Uttarakhand; and Kathua district of Jammu and Kashmir. Prashar Lake, Shikhari Devi, and Chowki in Mandi district; Solang Valley and Jalori Pass of Kullu district in Himachal Pradesh; Nagdev Reserve Forest of Pauri district in Uttarakhand; Bani Banhore, Sukrala, Koti, Bagan, Katli, Machhedi, Thal, Malhar, and Badnota of district Kathua in Jammu and Kashmir. Plant samples and parts of plants were captured through photographs and collected during surveys at different intervals of a growing season. The plants were properly packed in paper envelopes, brought to the lab, and analyzed for their variations from plant to plant.

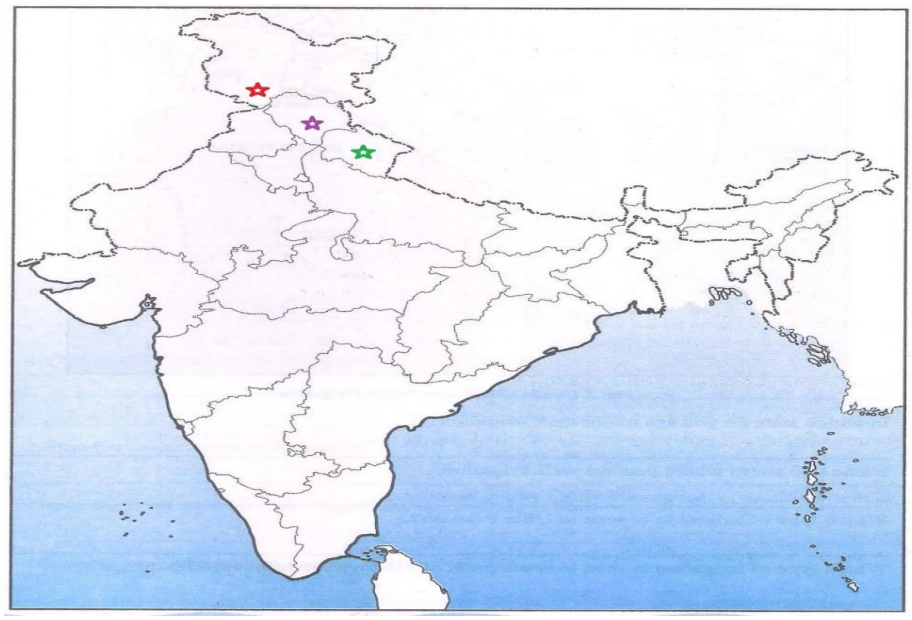

Figure 1. Map of India shows study regions.

Morphological analysis: Morphological analysis includes both qualitative and quantitative traits. Qualitative characters include habitat, habit, external shape, stem, root, leaf characters, and flower characters: attachment of flowers, bracts and bracteoles, presence of reproductive organs, number of floral parts, arrangement of floral organs, color, calyx, corolla, androecium, gynecium, fruit, seed, etc. Quantitative characters include plant length, main stem length, primary root length, number of leaves per plant, leaf width, leaf length, petiole length, lamina length, pedicel length, and number of flowers per plant, etc.

Results and Discussions

During the survey, samples of *V. canescens* were collected from different study sites. Data observations were made on the spot where the plant was growing, while others were observed in the lab. During observation, we studied both the qualitative and quantitative morphological traits of plants (Table 1 and Fig. 3).

Qualitative traits showed that plants mostly grow in moist and shady places. It is a perennial herb with a prostate, erect, and subglabrous external shape. Stem is short or missing. Roots are branched, cylindrical, and rhizomatous. Leaves are green in color, simple, canescent, ovate-cordate, petiolate, base auriculate, reticulate venation, and apex acute. Stipules were free, lanceolate, two in number, hairy, reddish at the base, and acuminate at the apex. Flowers are hermaphrodite, pedicellate, completely zygomorphic, bracteolate, and white or purple in color. On the lower edge of the petal, there were dark violet steaks. Sepals are green, hairy, acute, lanceolate, and polysepalous (5 in number). Petals are 5 in number, sub-equal, oval, polypetalous, and have slender lateral petals. Stamen are 5 in number, hypogynous, and anthers are locued and in ring form. Spurs are short and compressed. Stigma beaked, ovary superior, 3 capillaries. The fruit of the plant is a round capsule with many seeds. During the study, it was observed that there were no major qualitative differences observed between the studied populations of plants and with earlier records (Table 1).

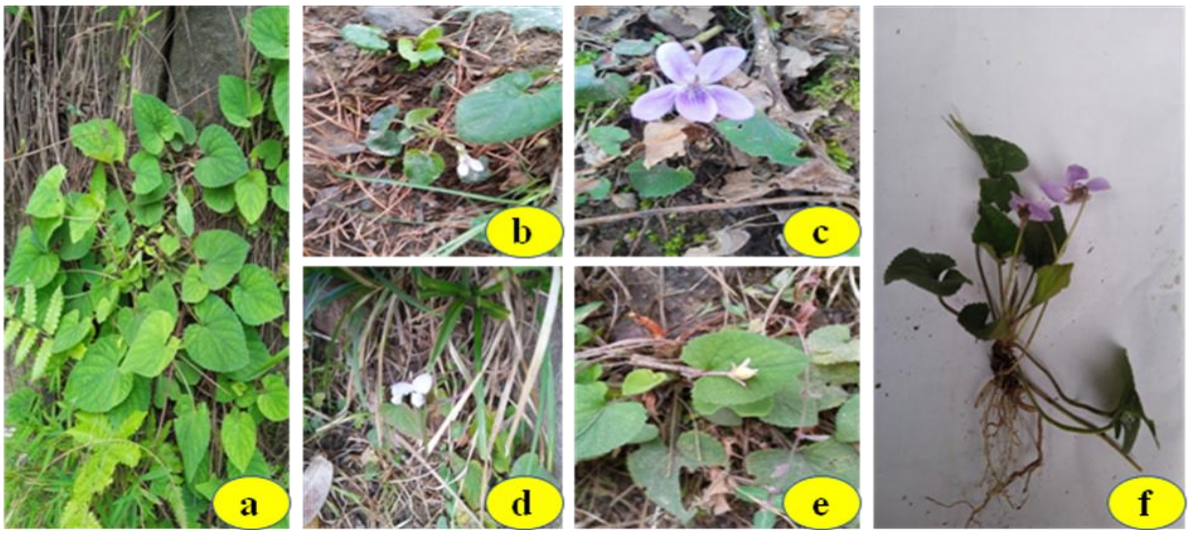

Figure 2. Photographs of *V. canescens* captured from different locations of northwestern Himalayan regions. **a)** Nagdev forests of Pauri (Uttarakhand), **b)** Plant with flower, photograph captured at Prashar (Himachal Pradesh), **c)** Jalori pass (Himachal Pradesh), **d)** Bani (J&K), **e)** Plant with mature fruits, photograph captured at Machhedi (J&K), **f)** Whole plant, photograph captured at Thal (J&K).

Table 1. Qualitative characters of *V. canescens* collected from different locations

S. No	Characters	Qualitative characters
1.	Habitat	Plant mostly grows in moist and shady places
2.	Habit	Perennial herb
3.	External shape	Prostate, erect, and sub glabrous
4.	Stem	Stem is short, or missing
5.	Roots	Branched, cylindrical, and rhizomatous
6.	Leaves	Green in color, simple, canescent, ovate-cordate, petiolate, base auriculate, reticulate venation, apex acute. Stipules were free, lanceolate, two in number, hairy, reddish at the base, and acuminate at the apex.
7.	Flower	Hermaphrodite, pedicellate, completely zygomorphic, bracteolate, and of a white or purple color. On the lower petal, there were dark violet steaks.
8.	Calyx	Five sepals that are hairy, acute, lanceolate, and polysepalous
9.	Corolla	Petals are 5 in number, sub equal, oval, polypetalous, with slender lateral petals
10.	Androecium	Stamen 5, hypogynous, anthers are locued and in ring form. Spur are short, compressed.
11.	Gynoecium	Stigma beaked, Ovary superior, 3 capillary
12.	Fruit	The fruit of plant is round, and capsule
13	Seeds	Tri-valved with many seeds

Quantitative traits such as plant length (PL), main stem length (MSL), primary root length (PRL), number of leaves per plant (NLP), leaf length (LL), leaf width (LW), petiole length (PL), lamina length (LaL), pedicel length (PeL), and number of flowers per plant (NFP) were included in the analysis (Fig. 3).

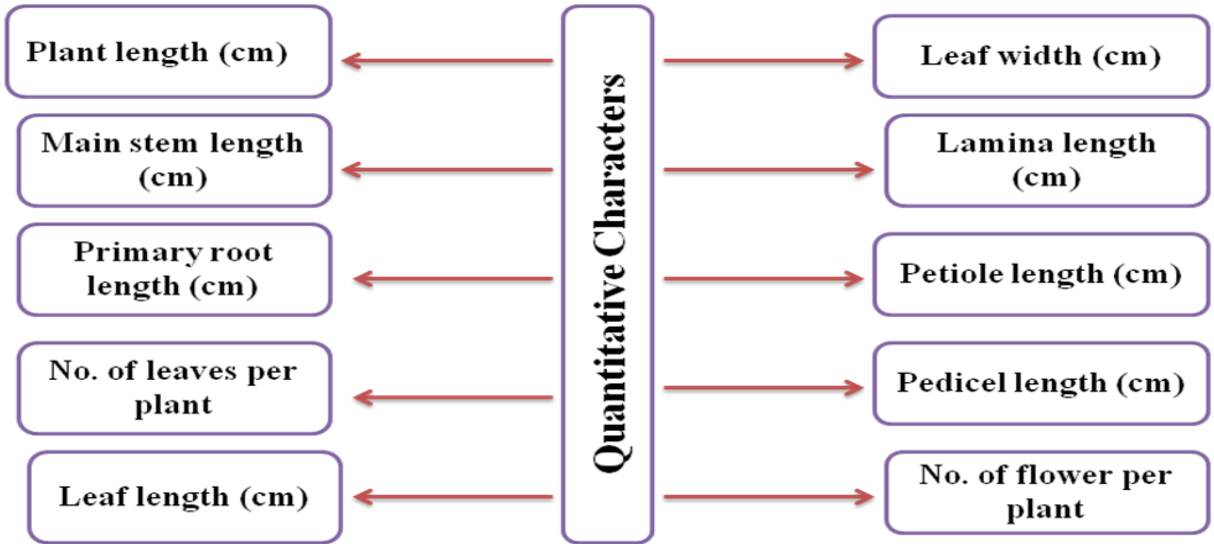

Figure 3. Various quantitative characters were used to study *V. canescens*

For the analysis of quantitative characters, we have taken four plants from each of the eight selected sites, such as Nagdev Reserve Forest (Uttarakhand), Prashar, Solang Valley, and Jalori Pass (Himachal Pradesh), Thal, Machhedi, Banhore, and Bani (Jammu &

Kashmir). Further results of the study based on plant length show that the maximum length was observed as (13.83) at Jalori Pass and the lowest was observed as (9.33) at Nagdev Pauri. Apart from this, the observations made at different study strands showed that the maximum value for stem length was recorded as (0.50) at Jalori Pass and the lowest was (0.20) at Nagdev Pauri. For the primary root length of the plant, it was observed that the Prashar shows the highest root length (7.95), and the lowest values were observed as (3.68) at Nagdev Reserve Forest.

During this study, it was also observed that in terms of the number of leaves per plant, the maximum number of leaves was recorded at 10.25 at Solang Valley (Himachal Pradesh) and the minimum was 4.50 at Thall and Machhedi. The leaf length of the plant shows that the Jalori pass was recorded for maximum leaf length (3.40), and the minimum was observed as (2.70) at Nagdev Pauri. Similarly, in the case of leaf width, the highest value was recorded as (3.38) at Jalori Pass, while the lowest value was recorded as (2.48) at Nagdev Pauri. The maximum length of lamina was observed at Jalori Pass (4.13) and the lowest at Prashar (2.90). The petiole length of the sample plant was also observed at all the study sites, where the maximum value was recorded at Jalori Pass (8.35) and the minimum value was observed at Nagdev (4.88). Observations were also made on the pedicel length of the plant, and the highest value was recorded at 7.48 at Banhore, while the lowest value was observed at 4.80 at Machhedi. In the case of the number of flowers per plant, the highest average value was observed as 1.75 at all sites except Banhore and Pauri, where the lowest value was observed as 1.50. (Table 2)

Table 2. Quantitative characters of *V. canescens* collected from different locations

Locations	**Quantitative characters**									
	PL	**MSL**	**PRL**	**NLP**	**LL**	**LW**	**LaL**	**PtL**	**PdL**	**NFP**
Pauri	9.33	0.20	3.68	7.50	2.70	2.48	3.00	4.88	5.90	1.50
Prashar	9.53	0.43	7.95	7.75	2.93	2.98	2.90	5.83	5.15	1.75
Solang Valley	10.68	0.33	4.48	10.25	3.15	3.03	3.38	6.43	6.25	1.75
Jalori Pass	13.83	0.50	6.30	4.75	3.40	3.38	4.13	8.35	6.35	1.75
Machhedi	12.58	0.30	6.40	4.50	3.30	2.73	4.00	6.73	4.80	1.75
Thal	13.23	0.43	6.53	4.50	3.18	2.88	3.88	7.03	7.03	1.75
Banhore	12.38	0.30	6.38	4.75	3.08	3.00	3.80	6.40	7.48	1.50
Bani	12.65	0.28	5.95	5.50	2.85	2.60	3.48	5.93	6.33	1.75

Therefore, similar results were obtained from the literature reviewed, which shows that phenotypic variations occur either due to different geographical conditions, altitudinal variations, reproductive isolations, or demographic factors (Shinwari *et al*., 2014; Fritts, 2015; Dar *et al*., 2018). Similar types of studies on genetic diversity have also been conducted in various plants like chestnut (Serdar and Kurt, 2011); *Enset ventricosum* (Haile, 2014); *andrographic paniculate* (Disha and Tirkey, 2016); *Ficus carica* (Çalişkan *et al*., 2012); and *Pharsalus lunatus* (Purwanti *et al*., 2019). All these studies also reveal that such types of differences are due to different environmental conditions affecting the quantitative characters, which are controlled by major and minor genes in plants and easily affected by the changed environment. Therefore, the present study supports that all

qualitative variations observed during the study in different populations of *V. canescens* may be due to differences in climatic or environmental conditions, which cannot be used for the identification of plants.

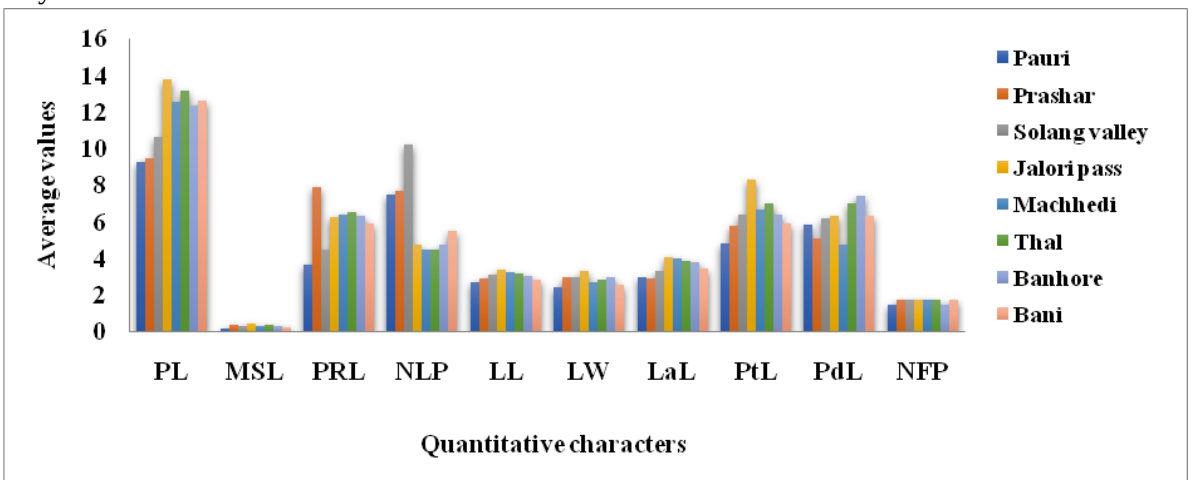

Figure 4. Average values of quantitative characters of *V. canescens* collected from different location

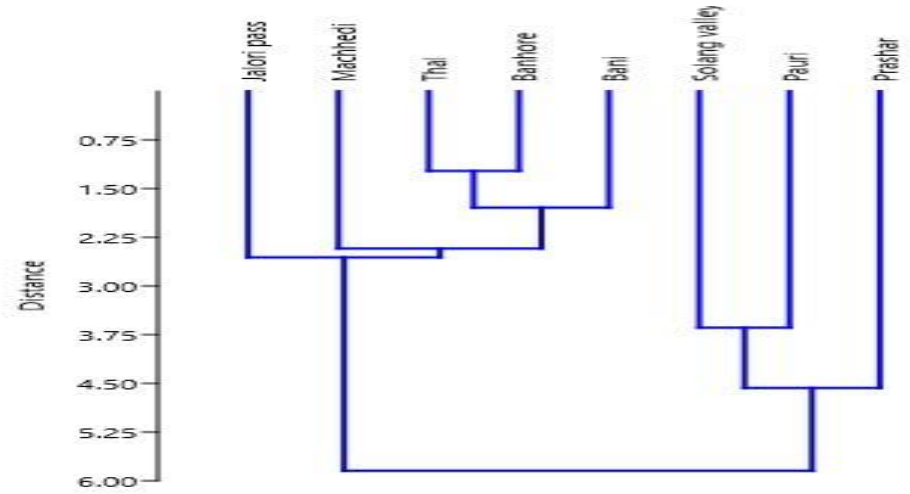

Figure 5. Dendrogram constructed based on the quantitative characters *of V. canescens* collected from different locations

Conclusions

V. canescens, a important medicinal herb in traditional healthcare. A comprehensive study evaluating both qualitative and quantitative characteristics to gauge morphological diversity. While qualitative traits remained consistent, quantitative variations were noted, attributed to diverse climatic factors. The valuable data obtained aids future initiatives in the conservation, management, and enhancement of *V. canescens*. Furthermore, this information facilitates the correlation of various secondary metabolites within the species. Morphological evaluation of the plant is pivotal for planning future research, identifying potential morphotypes, and potentially discovering new species, emphasizing the significance of this study in guiding comprehensive exploration and understanding of *V. canescens.*

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