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Gentiana tubiflora (Wall. ex G.Don) Griseb.: Observation on flower colour variation from Sikkim Himalaya

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Abstract
Flower colour variation is common among flowering plants, encompassing different traits linked to both floral and vegetative characteristics. The colour of the flowers plays a crucial role for the attracting of the pollinators, and the differences in flower colour across populations correspond to variations in pollinator communities. Typically, the flowers of *Gentiana tubiflora* (Wall. ex G.Don) Griseb. bears dark blue flowers, however, we have discovered two populations bearing white-flowers of this species within the same locality in the Sikkim Himalayan region. Few reports suggest that the selective pressures on flower colour, genetic mutations of floral pigments like anthocyanins or the roles of pollinators contribute to flower colour variation in many angiosperms. These factors may also account for the colour variations as found in different populations of *G. tubiflora*.

Keywords: Flower colour variation, *Gentiana tubiflora*, Genetic mutation, Sikkim Himalaya.

INTRODUCTION

Gentiana L., the largest genus of the family Gentianaceae, is mainly characterized by well-developed usually symmetrical plicae in between the corolla lobes. The genus is represented by about 365 species worldwide and largely distributed in the temperate and alpine regions of Asia, Europe, America, North-west Africa, eastern Australia (Ho and Pringle, 1995; Ho and Liu, 2001; Mabberley, 2017). In India, the genus is corresponded to about 72 species (Jayanthi, 2022) majority of which are distributed in the Himalayan region, specifically in the Eastern Himalaya.

Gentiana tubiflora (Wall. ex G.Don) Griseb., one of the commonly occuring members of *Gentiana*, is distributed throughout the Indian Himalayan Region (IHR) covering the states like Sikkim, Himachal Pradesh, Jammu & Kashmir and Uttarakhand (Jayanthi, 2022; Maity and Saha, 2024). The species is a perennial, stoloniferous, 4–5 cm tall herb mostly with suberect, simple or little branched stems. Leaves are more or less fleshy and crowded at base of stem like a rosette. Flower is solitary at each branch tip, usually dark blue, unspotted, tubular or narrowly funnel-shaped.

Notably, during a plant expedition in 2015, we came across two white-flowered populations of *G. tubiflora* in the Sikkim Himalaya. Each of these populations consists of 3–4 individuals within the same locality. The white-flowered individuals bear identical characteristics with the blue-flowered individuals except the flower colour. These populations are contiguous to the typical dark blue-flowered populations and the physiography (altitude) and climate (temperature, rainfall) are identical for both the populations bearing dark blue flowers and those bearing white flowers (Fig. 1).

In this study, our primary objectives are to document the flower colour variation in *G. tubiflora* and to explore any correlations with environmental factors. Additionally, we also aim to investigate the potential taxonomic/ecological/economic (horticultural) significance of this variant.

MATERIALS & METHODS

Extensive field surveys were carried out in 2015–2016 in the alpine zone of the Sikkim Himalaya. The plant specimens of both white-flowered and blue-flowered populations were studied in the fields, photographed and collected for further studies. The specimens were then indentified with the help of relevant literature (Clarke, 1883; Garg, 1987; Ho and Pringle, 1995; Aitken, 1999; Ho and Liu, 2001; *et al.*, 2018; Maity and Saha, 2024). The specimens were processed for herbarium following standard methodology and deposited at Calcutta University Herbarium (CUH).

RESULTS AND DISCUSSION

Gentiana tubiflora is therefore found in two morph-forms as of flower colour. Most of the populations of this species develop blue flowers, while a few populations are with white flowers (Fig. 1). Detail morphological study of both the blue-flowered form and white-flowered form reveals that there is no difference exists in plant characteristic features except the flower colour. Flower colour may vary due to natural selection related to abiotic factors (Arista *et al.*, 2013). Notably, in this case, abiotic factors like temperature, altitude, rainfall may not be responsible for the flower colour variation among the populations of *G. tubiflora*, because these specimens/ or rather populations are growing in the same locality where blue-flowered populations are abundant.

Variation in flower colour is largely influenced by the presence of the floral pigment anthocyanin (Warren and Mackenzie, 2001; Rausher, 2008; Veiga *et al.*, 2015). It accounts for blue, red, purple colours produced through the flavonoid biosynthetic pathway that produces chemical compounds. These chemical compounds are not only responsible for flower colour variation but also for protection of plants against heat stress and UV radiation damage (Rausher, 2008; Veiga *et al.*, 2015). Through the epidermis-specific gene expressions of flavonoid metabolism, floral pigments like anthocyanin dissolves in vacuole sap of epidermal cells of petals. Mutation in the anthocyanin gene results variation in flower colour (Morimoto *et al.*, 2020). Thus, there is a possibility that the flower colour variation in *G. tubiflora* may also be linked to the spontaneous mutation in anthocyanin producing genes, however, further study is needed to ascertain the fact.

Pollinators are mostly attracted by bright-coloured flowers than that of white flowers. Pollinators are reported to be responsible for exerting selective pressures on flower colour (Veiga *et al.*, 2015). Selective pressures by herbivores may also account for flower colour variation because different types of enzymes are involved in the production of pigments and probably that affect the synthesis of secondary compounds against herbivore damage (Armbruster, 2002; Irwin *et al.*, 2003; Strauss *et al.*, 2004; Strauss and Whittall, 2006; Lev-Yadun and Gould, 2009; Veiga *et al.*, 2015).

Charlesworth (2006) reported that the formation of white flowers in *G. leucomelaena* is a reproductively viable strategy that simultaneously conserves energy and reduces costs of nutrient (e.g. in flower pigments like anthocyanin) especially in cold condition when pollinator density is low. We predicted that a similar phenomenon may occur in case of *G. tubiflora* species.

Colour variation in flowers also found in other families like Malvaceae, Solanaceae, Verbenaceae, etc. (Mohan Ram and Mathur, 1984; Narbona *et al*., 2021). During the flower's life span of *Brunfelsia pauciflora* (Solanaceae) the petal colour changes from dark purple to lilac then to pure white (Narbona *et al*., 2021). In *Lantana camara* (Verbenaceae) flowers undergo colour change before fertilization and post fertilization (Mohan Ram and Mathur, 1984). Flower colouration is occurred in *L. camara* by masking of carotenoids by differential amounts of anthocyanins after post fertilization as a result pollinator energy is conserved due to this colour change (Maheshwari *et al*., 2014).

Specialized bee flowers are often blue and blue is also preferred by more specialized Diptera (Bombyliidae) (Knoll, 1921; Kevan, 1978). Nocturnal flowers are frequently white or pale, light colours being better visible in the darkness (Baker, 1961).

At the cytological level three types of pigments may be distinguished (i) they may be in the vacuoles, dissolved in the cell sap (vacuolar fluid), (ii) they may be in the cytoplasm as crystals or dissolved in oil, (iii) they may (rarely) be in the cell walls as in *Leonotis* (Lamiaceae) (Guitler, 1934; Harborne, 1967; Scogin, 1983).

Type (i) made up of flavonoid, containing anthocyanins (blue, violet, red); anthoxanthins (yellowish, white, ultraviolet), anthochlor (Chalcones and aurones) (yellow and ultraviolet) e.g. in Asteraceae, Scrophulariaceae, etc.

CONCLUSION

The flower colour variation in *G. tubiflora* from dark blue to white is unrelated to variation in altitude, temperature, rainfall, nutrients and radiation as the individuals of both the populations are from the same locality in Sikkim Himalaya. Natural selection exerted by biotic agents, genetic drift or founder effects can also contribute to the changes in flower coloration. Synthesis of the secondary compounds against herbivore damage may affect involvement of enzyme in pigment production resulting colour variation (Lev-Yadun and Gould, 2009; Veiga *et al.,* 2015). The variation in flower colour in *G. tubiflora* is probably also driven by variation in other factors, such as genetic mutation in floral pigment (anthocyanin) or by biotic selective pressures by pollinators or herbivores (Veiga *et al.,* 2015; Sobral *et al.,* 2015). Flower colour variation in *G. tubiflora* is thus probably a local adaptation and pollinators, herbivores and flower colour pigments may play a pivotal role in this adaptation.

This flower colour variation in *G. tubiflora* may be utilized in horticulture for its both types of flowers as dark blue and white. Its cultivation for commercial purposes is another important consideration. Therefore, conducting an extensive survey in other populations of *G. tubiflora* in the future is crucial for taxonomic characterization and exploring new possibilities in taxonomic research and that will open up new prospective in the field.

Fig. 1. *Gentiana tubiflora*: **A**. Habitat; **B**. Dark blue flowers; **C**. White flowers (inset open flower)

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