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Impact of Habitat Variability on Phenotypic Attributes of Hypericum perforatum L. along an Elevational Gradient in Kashmir Himalaya

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Abstract:

A number of environmental factors such as mean temperature, precipitation, soil characteristics etc. changes with elevational gradient and thereby, affect the morphological pattern of a plant species. Phenotypic attributes of a particular species varies along different altitudes in order to adapt and also to overcome these changeable and stressful conditions. The present study was undertaken to assess the distribution pattern and impact of habitat variability along an elevational gradient on morphology of an important medicinal plant *Hypericum perforatum* growing in Kashmir Himalaya. *Hypericum perforatum*, member of family Hypericaceae, is a perennial herb and distributed in North Western Himalaya. The species exhibited distinct variability and a peculiar trend in morphological traits in response to different environmental conditions along an elevational gradient.

Keywords: Distribution, Elevational gradient, *Hypericum perforatum*, Hypericaceae, Kashmir Himalaya, Morphological traits

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1. Introduction

Hypericum perforatum L. commonly known as St. John's wort, is an elite medicinal plant of Kashmir Himalaya, which has been used by ancient herbalists (locally called as Hakims) since 2000 years ago [1]. *H. perforatum* depicts remarkable variation in its morphological traits, breeding system and ploidy level [2]. *H. perforatum* is commonly habitual in pastures and meadows and depicts the association with diversity of herbs and shrubs. There are more than 450 species under the genus *Hypericum* which behave as wides in both temperate as well as in tropical mountainous regions of the World [3]. *H. perforatum* is a treasure of potent and novel medicinal drugs and has been widely used for the treatment of various ailments such as burns, skin injuries, neuralgia, and depression [4]. *H. perforatum* holds promise of numerous compounds such as hypericin, pseudohypericin, flavonoids, oligomeric procyanidines and hyperforin [5,6].

In Kashmir, *H. perforatum* is fairly common on dry hilly slopes and waste places. For the completion of its life cycle, the plant species requires mild warm temperature and prolonged growing season [7]. It is less vigorous and produces limited fruit set as compared to the plants at lower altitude [8]. Taxonomically it is a perennial herb, erect, height up to 0.3-1.2m. The tallness and profuse branched pattern depends upon the genetic makeup and environmental parameters of a particular site (Phenotype = Genotype × Environment). The plant bears rhizomatous root system where the shallow roots produce vegetative buds to develop new crowns. The stem is suffrutescent relatively smooth to touch that appear to have a rust colour (Fig. 4B), and multiple stem arise from a crown. Stems are woody at the base, profusely and multi branched, leaves are simple 1.5-3 cm long and 1.5 to 5 mm wide**.** Leaves are yellow green in colour and display obvious translucent dots when held up to the luminosity, giving them a 'perforated' appearance. The transparent dots are occasionally interspersed with a few black dots on the lower surface [9]. In phenological cycle, the stage from May to September is an extensive flowering period of *H.perforatum*. The particular arrangement of stamens in bundles (fascicles), is a characteristic feature of the whole genus *Hypericum* [10]. The flowers develop in clusters and are 1-2 cm in diameter (Fig. 4C). The fruit is a numerous seeded 3- celled dehiscent capsule 5 to 10 mm long. Seeds are small 1 mm long, round; darkly coloured, netted with coarse grooves, possesses resinous smell like turpentine. The reproductive behaviour of *H. perforatum* is facultative apomictic and the pollen mother cell (PMC) undergoes a normal meiosis to produce viable pollens [11,12]. There is an evidence for both self-pollination and pollination by a diversity of insects (entomophily) [13].There is variability in seed set formation along elevational gradient, range from about 15,000 to 34,000 seeds per plant on average [14]. Variation in seed production may result due to influence of various environmental factors, seasonal fluctuations, and competition for resources or herbivory.

The present study was carried out to understand the variation in phenotypic characteristics along an altitudinal gradient. This study aimed at developing strategies for cultivation and sustainable use of wild populations and to find the environments that are most favorable and productive for the growth of *H. perforatum.*

2. Material and Methods

The material for the present study was a medicinally important plant *H. Perforatum*. During the present study robust methods and approaches were carried out in the field, laboratory and in natural habitats to understand the phenotypic variability and reproductive strategies of the species. The data on various aspects of the phenotypic variability and seed germination were recorded during 2015 to 2016.

3. Survey, collection and documentation

An extensive field survey of different habitats of Kashmir Himalaya was accomplished to recognize specific areas across different geological conditions covering a wide range of habitats. To study the phenotypic variability of *H. perforatum* along different elevational gradient, a total of four sites (populations) were selected. The location of selected sites are shown in a map (Fig. 1). Present studies were carried out at four different selected sites of Kashmir Himalaya from March to November. Geographical origins along with altitude, latitude and longitude of four select study sites are listed in Table 1. All plant samples were collected at flowering stage. The propagules and young seedlings of the species were also transplanted in the Kashmir University Botanical garden (KUBG).

Table 1: Salient features of the selected sites

Study Sites	Location	Altitude (m)	Latitude	Longitude	Habitat
01.	Kangan, Ganderbal	1835	34^0 14'47.5776''N	$74^0$47'17.28''E	Open slopes and pastures
02.	Pahalgam, Anantnag	2350	$34^0$02'36.00''N	$75^0$14'24.00''E	Open Slopes and disturbed sites
03.	Drang, Tangmarg	2235	34'03'32.30''N	$74^0$25'4.57''E	Sunny Open Slope with rock cervices
04.	*KUBG	1595	34'07'8.24''N	$74^0$50'19.1''E	Open field

***KUBG - Kashmir University Botanical Garden.**

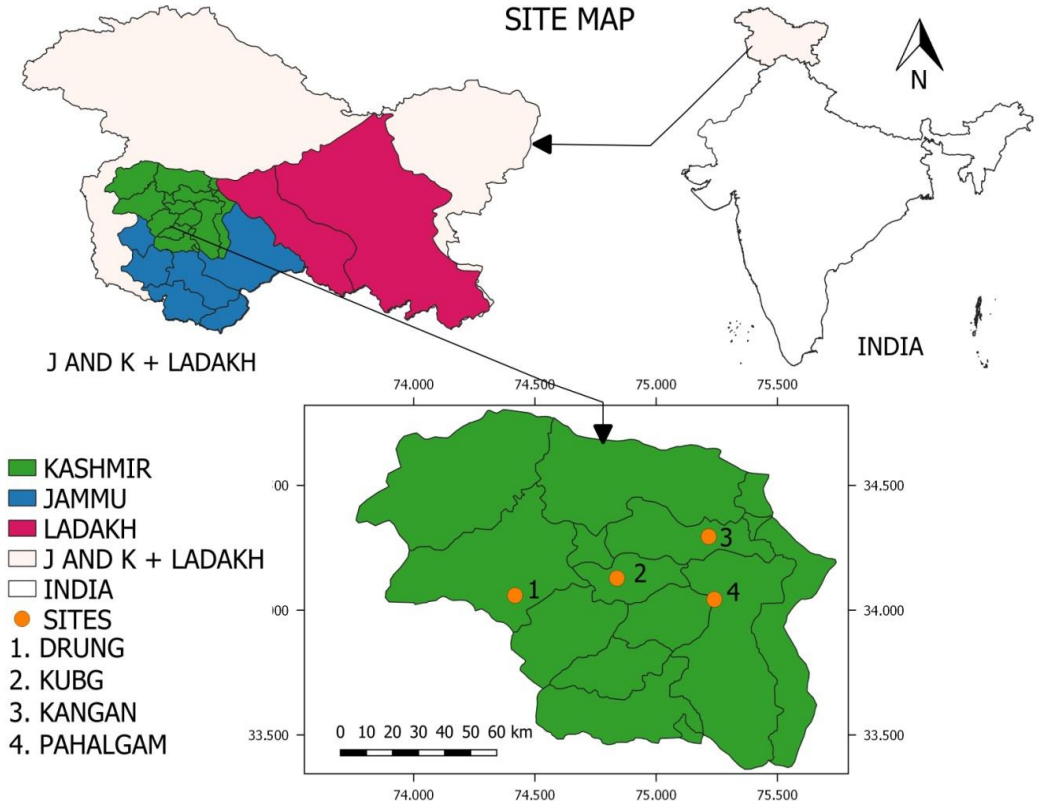

Fig. 1: Study sites of *Hypericum perforatum*

4. Species morphology and phenotypic variability

Field trips were organized to the select sites fortnightly keeping the standard procedures into consideration. All the morphological traits, habitat, associated species and sample collections were recorded at the maturity (flowering stage) of the plant. Herbarium specimens were prepared by following standard herbarium procedures and the plant species were identified by consulting stock of relevant literature and herbarium studies at KASH (Kashmir University Herbarium). Photographs were taken with the aid of Samsung camera having resolution of 16pxl. The morphological traits that were analysed for phenotypic variability are plant height, number of leaves, mean leaf length, mean leaf breadth, rhizome and root length, inflorescence dimensions etc. The density of glands was observed under microscope. Simple statistics (i.e. mean and Standard Deviation) were used in order to compare all morphological traits.

5. Results and Discussion

With increasing altitude plant life is challenged by a range of environmental stresses and as such plants have to cope up with these stress conditions by altering their morphological and biochemical attributes [15]. The variation in morphological characters of plants with changing altitude indicates plastic and evolutionary changes in these traits which

Table 2: Phenotypic Variability of *Hypericum perforatum* across different study sites

S.No.	Phenotypic character	Population	Range of Variation	Mean ± SD
01.	Plant height (cm)	Drang, Tangmarg	25-40	32.5±4.57
		Kangan, Ganderbal	19-37	27.16±6.31
		Pahalgam,Anantnag	18-30	24.50±4.42
		KUBG	23-52	36.63±9.07
02.	No. of leaves per plant	Drang, Tangmarg	97-130	110.16±11.85
		Kangan, Ganderbal	87-120	107.33±11.42
		Pahalgam,Anantnag	98-131	116±10.31
		KUBG	89-149	132.33±20.92
03.	Root length (cm)	Drang, Tangmarg	8-14	10.58±2.70
		Kangan, Ganderbal	7.9-12	10.40±1.62
		Pahalgam,Anantnag	7.9-13	10.33±1.83
		KUBG	7-16	10.05±3.09
04.	Inflorescence length (cm)	Drang, Tangmarg	79-150	123.66±25.32
		Kangan, Ganderbal	100-121	93.22±10.31
		Pahalgam,Anantnag	79-102	92.16±10.41
		KUBG	87-118	97.66±10.07
05.	Leaf length per plant (cm)	Drang, Tangmarg	1-1.7	1.23±0.02
		Kangan, Ganderbal	1.5-2.0	1.43±0.01
		Pahalgam,Anantnag	1.2-1.3	1.12±0.01
		KUBG	1.6-2.4	1.59±0.09
06.	Stem diameter (cm)	Drang, Tangmarg	4-8	8.23±1.0
		Kangan, Ganderbal	6-9	8.41±1.01
		Pahalgam,Anantnag	4-9	8.11±1.01
		KUBG	5-11	8.89±1.23
07.	Dark gland density on leaves	Drang, Tangmarg	31-70	50.00±13.28
		Kangan, Ganderbal	42-83	52.32±13.30
		Pahalgam,Anantnag	37-98	52.76±13.27
		KUBG	26-69	49.67±12.32
08.	Light gland density on leaves	Drang, Tangmarg	108-416	226.5±97.87
		Kangan, Ganderbal	200-395	201.7±69.66
		Pahalgam,Anantnag	200-350	200.31±77.23
		KUBG	100-200	157.75±39.67

would influence population performance across altitudinally or climatically variable conditions [16]. These morphological variations across altitude not just give explicit botanical identity to a species but can also uncover fascinating highlights helpful in understanding the scope of morphological variations present across different ecological zones.

During the present study variability in phenotypic traits of *H. perforatum* were analyzed for different populations along an elevational gradient and revealed the extensive variability in morphological attributes along the altitudinal gradient.The present investigation revealed that *H. perforatum* grows mostly in open grassy slopes along with shrubberies, in disturbed soil along the roadside and on the rocky slopes where enough soil cover is available. It grows along a varied range of altitude from 1000-4000 m above sea level. In Kangan (Ganderbal), it grows along the sloppy area within the shrubs of *Indigofera* sp. In Drang, the plant species thrives well in the rocky areas where abundant soil is available. In Pahalgam, it grows along roadside and disturbed open slopes within shrubberies. *H. perforatum* shows freely multiple branching pattern arising from base, that typically range from 18 to 40

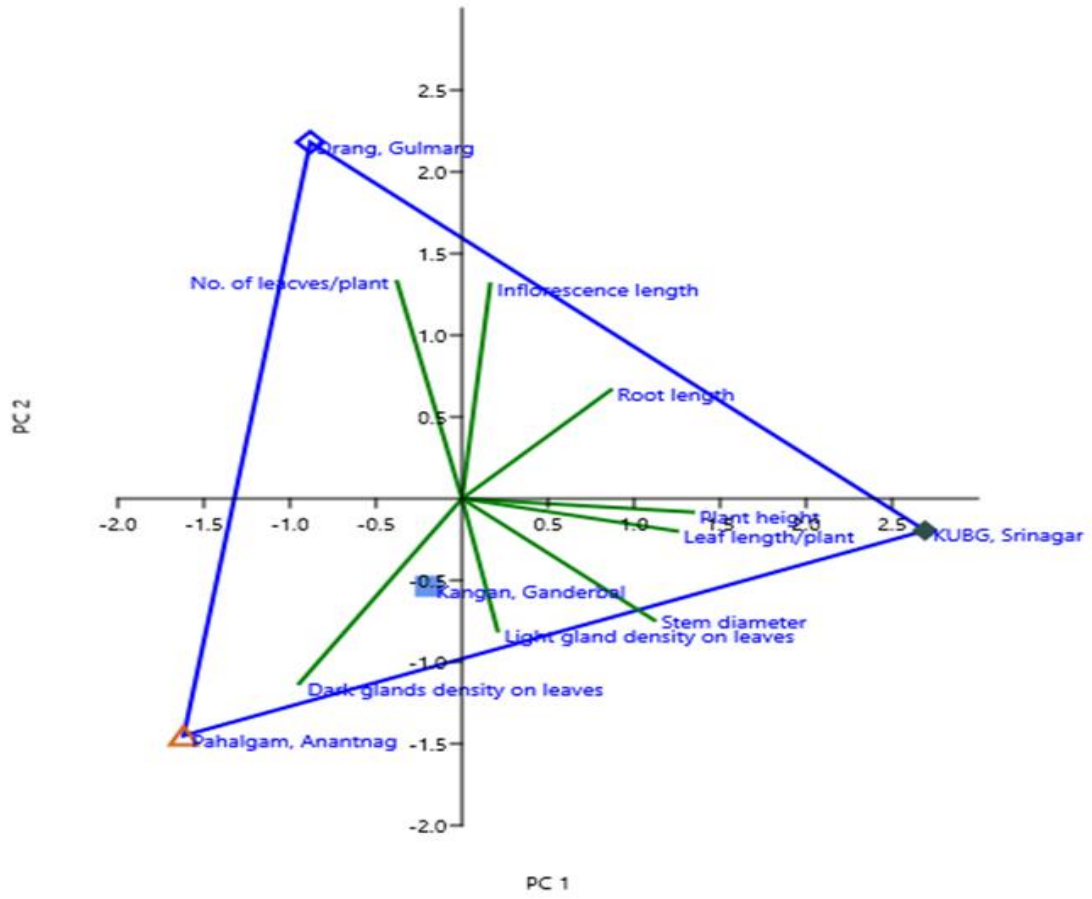

Fig. 2: Principle Component Analysis (PCA) of morphological traits of *Hypericum perforatum*

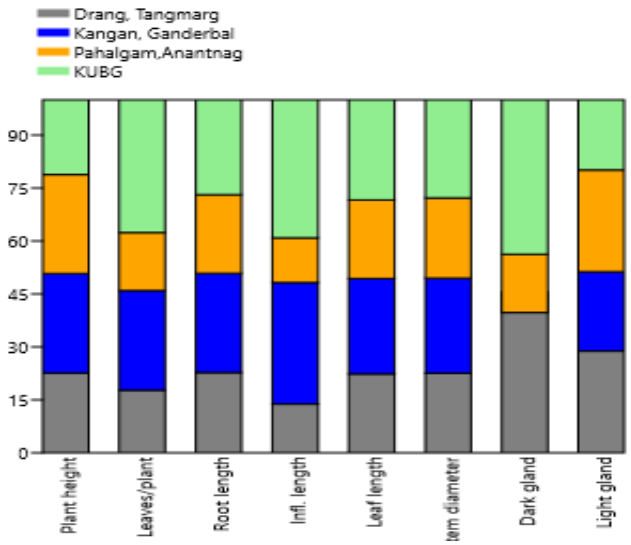

Fig. 3: Stacked bar chart of morphological traits of *Hypericum perforatum*

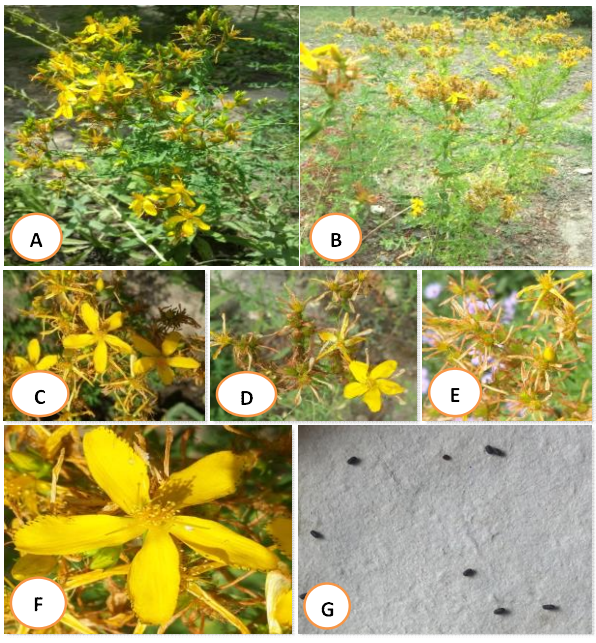

Fig. 4. *Hypericum perforatum* L. A – B. Habit; C – D. Inflorescence; E – Fruits; F – Flower; G – Seeds

cm in height. The stems and branches are profusely covered by oblong, smooth-margined leaves that are 1 to 2 cm long and 0.3-1.0 cm wide. The leaves harbour numerous minute translucent spots or pores (perforation) that indicates the species epithet of the plant species. It produces bunches of five-petaled yellow flowers that are typically 1.0-2.0 cm wide and the whole inflorescence stretches between 79-121 cm. The dges of the petals are usually netted with black dots. Flowers produce a blood-red pigment upon crushing. By late summer, capsules get formed that bears numerous tiny, dark-brown seeds. Dehiscence of capsule disperses seeds in poor soils,

and is commonly found in meadows, fields, waste areas, roadsides, and abandoned land. The plant exhibits a significant variation in its morphological (phenotypic) traits under different set of environmental conditions along an altitudinal gradient. The data collected from the sites is simplified in terms of range of variation, mean and standard deviation as shown in the Table 2. In this study we used the PAST software (Version 3) to get scatter plot (PCA) and Stacked bar chart for different morphological traits given in Figs. 2 & 3.

The plants of *H. perforatum* were also evaluated in terms of dark and light gland density on the leaves. The population at Pahalgam possessed the greatest dark glands density on the leaves i.e. 52.76±13.27 and the least dark gland density on the leaves were seen in KUBG (49.67±12.32). It can also be seen from the data that more the mean plant height; more is the number of leaves per plant because of the increased number of branching pattern e.g., average plant height at Drang is 32.5±4.57 cm with mean number of leaves more i.e. 110.16±11.85 as compared to Kangan having mean plant height 27.16±6.31cm and 107.33±11.42 mean no of leaves per plant. It can also be inferred from the data that mean root length is positively correlated with altitude with highest root length at Drang (10.58±2.70cm) and lowest at KUBG with 10.05±3.09cm. Altitude has a profound effect on inflorescence length with population of Drang having highest inflorescence length (123.66±25.32cm) and the population at Kangan (Ganderbal) with least inflorescence.

5. Conclusion

The present study revealed that *Hypericum perforatum* grown at different altitudes exhibited differences in plant architecture, biochemistry and reproduction. The results depicted a wide range of suitable habitats for the growth of *H. perforatum*.The species exhibited significant phenotypic variability across different populations along an altitudinal gradient. It can be inferred from the present study that variation in environmental conditions proved to have an enormous impact on the growth dynamics and development of *H. perforatum*. It can be suggested from the present study that the plants growing at **low altitudes** were comparatively much more diverse and vigorous in respect of the various morphological features.

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Conflict of interest

The authors declared that they have no conflict of interest.

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