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RESEARCH ARTICLE

Impacts of habitat variability on the phenotypic traits of *Aconitum violaceum* Jacq. ex Stapf. at different altitudes and environmental conditions in the Ladakh Himalaya, India

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Abstract

Aconitum violaceum Jacq. ex Stapf is endemic to the Northern Himalayan regions of India, Pakistan and Nepal. To adapt and endure the rigorous stressful situations, phenotypic features of a specific plant species vary across different climatic conditions and elevations. The aim of this study was to reveal the impact of altitude and environmental conditions on the morphological attributes of a threatened species *A. violaceum*. Plants at high elevations (Maan-man, 4010 m asl) were short; whereas the plants grown at ecological conditions of population-I (Khawous, 3220 m asl) showed better growth and development in all morphological attributes. It has been noticed that there is a considerable reduction in the plant height, rhizome, leaf dimension, flower number and leaf number at higher elevations. Regression analysis and Pearson's correlation coefficient demonstrated a positive relationship between plant height and all other morphological attributes. Principal component analysis (PCA) revealed that the habitat, ecological conditions and elevations of population-I (Khawous) and Population-II (Numsuru) were proved to be relatively better for the luxuriant growth and development of an endemic plant species *A. violaceum*. From the observations, it can be inferred that *A. violaceum* grows successfully at an elevation ranges between 3000 to 3500 m asl. This study provides a comprehensive account of phenotypic variability concerning environmental conditions and suggested a suitable environment for sustainable cultivation of this important medicinal plant species in the Ladakh Himalaya.

Keywords

Distribution, habitat variability, morphology, phenotypic variability, regression

Introduction

Phenotypic plasticity is the potential of a genotype to manifest alternative morphological, behavioural and physiological attributes in response to environmental conditions (1, 2). To thrive under adverse environmental conditions, plants can either adjust to the local environmental circumstances genetically or respond to environmental variation through phenotypic plasticity (3, 4). Phenotypic plasticity can play an essential role in evolution by altering or modifying the developmental pathways in plant bodies, thus leading to the creation of phenotypic diversity in nature (1). Variations in general growth forms of resource accumulating organs such as roots, tubers, rhizomes, leaves and flowers of a plant are essential to adjust the available resources (5). Plants acclimatize themselves under acute environmental conditions either by manifesting phenotypic malleability or by adapting genetically (6). Phenotypic flexibility is crucial for plants as they are stationary and must live in a constantly challenging environment (6, 7). Phenotypic variability is believed to exist more regularly at higher elevations, enabling alpine plants to adjust to the temporally fluctuating environment (2, 8). Although; plasticity is beneficial for the plant to survive under extreme climatic conditions; somehow it induces negative impacts on the fitness of plant species (9). With increasing elevations, plants must adapt to altering environmental conditions (10). Plants regulate important physiological processes such as gaseous exchange, photosynthetic rates and vegetative traits in response to climate fluctuations. Reducing plant height with respect to elevation disparity is the most noticeable change in plant morphology (10, 11).

Aconitum is a large genus of family Ranunculaceae with over 300 species distributed in the temperate areas of the Northern Hemisphere; such as Asia, Central Europe and North America (12). *A. violaceum* Jacq. ex Stapf is an erect biennial herb attaining maximum height of 30 cm tall (13, 14), endemic to the Northern Himalayas (15). In India, *A. violaceum* is randomly distributed in the alpine and sub-alpine regions of Jammu and Kashmir, Himachal Pradesh, Uttarakhand and Ladakh Union Territory. *A. violaceum* is known as Aconite, violet monkshood in English; Atis, Bishmool and Mohand in Kashmir region and Bova-nagpo, Jhimba, Yangtso in Ladakh region (16).

A. violaceum is a highly useful medicinal plant, mostly harvested for its tuberous root, which are rich sources of diterpenoid alkaloids (aconitine, indaconitine etc) (17, 18), quercitin, acylated flavonol glycosides, flavonoids, kaempferol, anthraquinones, saponins, benzoic-acid, sparteine, tannins, fatty acids and polysaccharide (19 -21). It also has a wide range of biological activities including antioxidant, antimicrobial, anti-inflammatory, antiviral, anti-malarial, anti-proliferative, analgesic and antipyretic properties (19-21). Ethno-medicinally, it is used to cure boils, cough, fever, asthma, gastric trouble, sciatic pain, intestinal worms (22-24). The species are grown luxuriantly along the edges of *Rhododendron*, *Quercus* and *Abies* forests, glacial riverine, moist rocky areas, open grassy alpine slopes, stabilized alpine meadows, moist semi-shaded slopes (15).

Materials and Methods

In the present study, a dynamic technique was used to understand the phenotypic variability under different environmental and altitudinal conditions. The survey was conducted during May 2019 to October 2021.

Exploration, collection and documentation

Various habitats in Ladakh Himalaya (India) from Zojila to Zanskar in the West; Leh to Nubra in the East, were explored extensively and assessed to choose a specific study sites across the region. To investigate the phenotypic variability in *A. violaceum* along the altitudinal gradient and ecological conditions, 3 sites (populations) have been selected based on their accessibility, habitat structure and specific environmental conditions, along with 1 control site at Kashmir University Botanical garden (KUBG). Given due cognizance to the threat status, vigor mature flowering plants of *A. violaceum* was collected quite judiciously from different study sites. The specimens were identified and deposited in the Kashmir University Herbarium (KASH) under voucher number 3739-KASH. 10-15 individuals of the species were randomly selected from each study site and were marked with tags. The tagged individuals were monitored on regular basis from May 2019 to October 2021. Vegetation type, habitat, geo-coordinates, altitudes along with associated species of the selected sites were recorded. The vegetative propagules (rhizome and seeds) of the plant were judiciously collected and transplanted to KUBG (control site) to study the morphological variations.

Species morphology and phenotypic variability

The randomly selected individuals in the populations were observed continuously at regular intervals of 5-10 days. Variations in morphological traits like plant height, rhizome, petiole, leaf, pedicel dimensions and floral characters such as the dimension of flower whorls, follicles and seeds were recorded.

Statistical analysis

LSD and Tukey's test (one-way ANOVA) were done using IBM-SPSS software (version 23) to compare the significant level and the average ± standard error were computed using Microsoft Excel (office-10). Linear regression and Pearson's correlation coefficient analysis were performed to examine the correlation between various morphological attributes across different environmental conditions and elevations to find an ideal habitat for the better growth and development of this species. Principal component analysis (PCA) was carried out to assess the phenotypic traits in relation to different habitat conditions. Origin-Pro 2019b 64 software was used to analyze the PCA and R- software (version 4.1.2; 64 bit) was used to determine the correlation. To assess the variability in phenotypic features, data was collected for 10-15 plants from each population. The photographs were taken using a Canon DSLR (1300D), an Olympus (EPl-5) camera and a 1-plus 9-pro mobile camera.

Results and Discussion

Distribution

Aconitum violaceum is locally known as Nagpo- boma/ Bona-nagpo, Jhimba, Yangtso. In the Ladakh Union Territory, the species are randomly distributed in the alpine and sub-alpine regions of Drass (Matayeen, Prandrass, Maan-Man, Laabar), Suru Valley (Khawous, Numsuru, Pursa, Kochik, Tangole, Achampore, Choskore, Panikhar) Sapi, Nubra (Chalunka, Turtuk) on the way to Pangong lake, Changthnag and Zanskar region at an altitude of 3100 to 4080 m asl. The distribution pattern and selected area are depicted in the map (Fig.1). In Ladakh it is strictly distributed along the banks of continuous water flow irrigation channels, moist damp slopes under the shade of *Salix* species

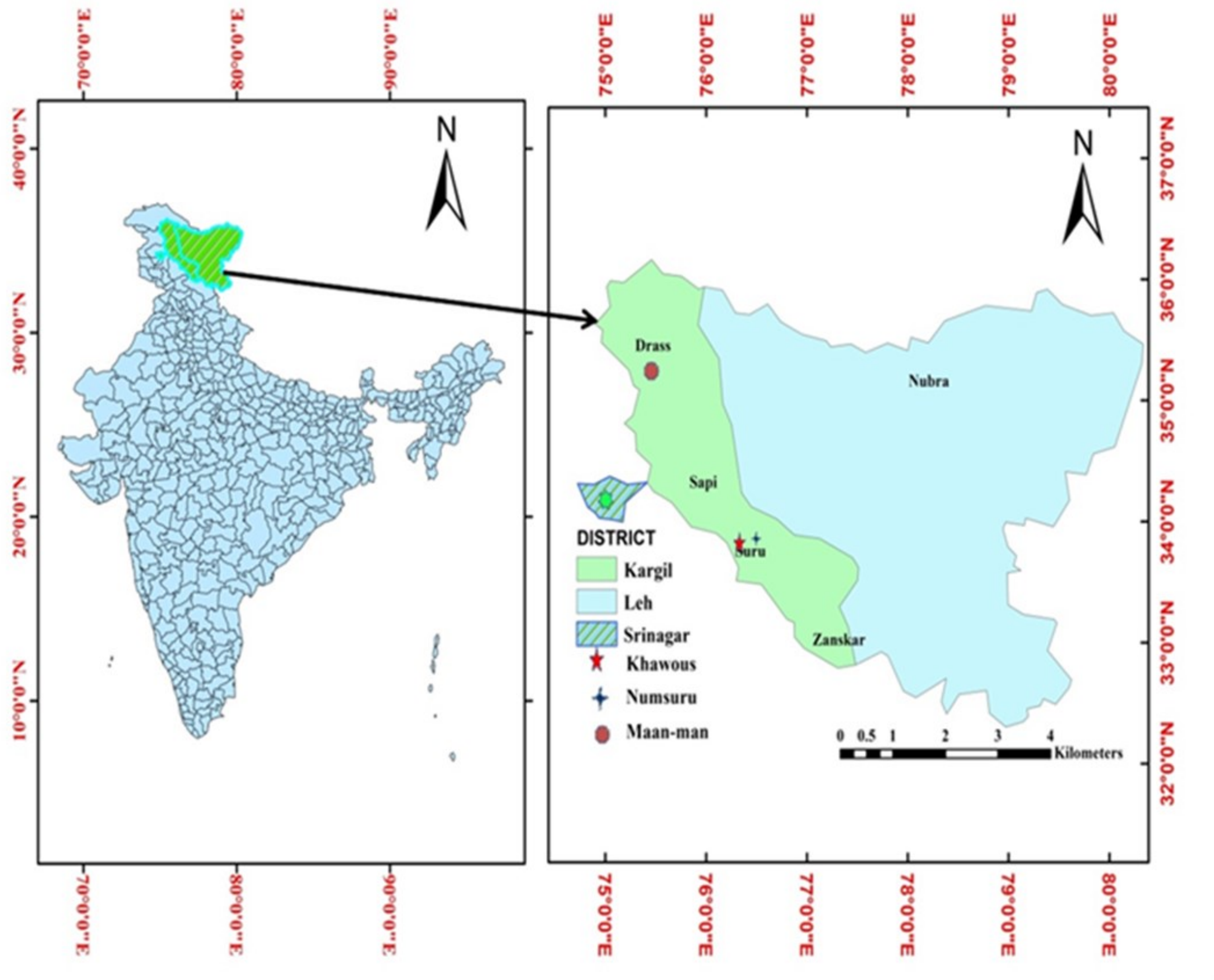


Fig. 1. Distribution pattern of *Aconitum violaceum* Jacq. ex Stapf in Ladakh Himalaya, India.

and *Hippophea rhamnoides,* alpine wet meadows, banks of alpine stabilized sloped and bank of alpine streams, strongly associated with species like *Pedicularis rhinanthoides, Delphinium cashmerianum, Aconitum heterophyllum, Rumex acetosa, Gentianopsis vvedenskyi, Gentianopsis paludosa, Parnassia cabulica, Bistorta vivipara, Carum carvi, Trifolium repens, Ranunculus brotherusii, Geranium pretense, Gentiana carniata, Taraxacum officinale, Anaphalis* spp. etc (Table 1).

Species morphology and phenotypic variability

The morphological characteristics of *A. violaceum* are enumerated in (Table 2), (Fig. 2. A-F). The phenotypic characters of *A. violaceum* showed significant variation at different altitudes and climatic conditions. This phenotypic variation was observed in 4 selected sites. Viz, Khawous (3220 m asl), Numsuru (3350 m asl), Maan-man, Drass (4020 m asl) and Kashmir University Botanical Garden (KUBG) Srinagar (1595 m asl) as a control site. The morphological

Table 1. Habitat characteristics, threat factor and geo-coordinates of the selected study sites.

Sl. No	Locality	Geo- coordinate		Elevation (m ASL)	Vegetation/Habitat	Threat factor
		Latitude	Longitude			
01	Khawous	N34°15.905	E75°96.040	3220	Oasitic; Moist damp places, along the bank of irrigation canals.	Pre-harvesting pressure, habitat fragmentation, over exploitation.
02	Numsuru	N34°12.705	E75°96.720	3340	Damp places, along the bank of irrigation canals, under the shade of *Salix* trees.	Pre-harvesting pressure, habitat fragmentation, over exploitation, prolonged seed dormancy.
03	Maan-man, Drass)	N34°38.592	E75°84.305	4010	Alpine grasslands, moist slopes, bank of streams	Snow slides, prolonged seed dormancy, pre-seed maturation frozen temperature, landslides, overgrazing.
04	KUBG	N34°75.717	E74°50.151	1595	Open field	Nil

Table 2. Morphological attributes of *Aconitum violaceum*

Habit	Biennial erect herb
Root	Paired tuberous root, slightly sweetish in taste.
Stem	Simple, erect up to 50 cm high, glabrous below, hairy above.
Leaves	Orbicular-cordate or reinform, deeply bipinnatisect and 5-partite, upper leaves much reduced or withered at the time of flowering. Lower leaves have long slender petioles; upper leaves has reduced petiole.
Bracts	Similar to upper leaves; but much reduced.
Bracteoles	linear to filiform.
Inflorescence	Short, lax or dense raceme or corymb or reduced to a solitary flower.
Sepals	Sepals violet, rarely blue or yellowish-green with blue veins, variegated, pubescent.
Petals	Nectaries, hairy with truncate recurved lips and hood is gibbous dorsally.
Androecium	Stamens numerous, 4-8 mm long, hairy in the upper part, winged in the lower part and these wings end in tiny teeth.
Gynoecium	Carpel 5, oblong, densely covered with soft, spreading hairs,
Flowering	20^{th} of July to ending August
Fruit	Bunch of 5 follicle, densely hairy
Seed	3-sided, obpyramidal
Senescence	Started from 10^{th} of September

Fig. 2. Morphological attributes of *A.violaceum*. A. Habit; B. Habitat. C. Inflorescence; D. Stamens; E. Ripened follicle; F. Seeds. Scale bar represents 0.5 cm.

traits that were assessed for phenotypic variability are; mean plant height, mean rhizome length, mean rhizome width, mean petiole length, mean leaf length, mean leaf width, mean pedicel length, flower length, flower width, dimension of individual floral whorls (Sepals, petals, stamen and carpels), mean follicle length, mean seed length and mean seed width (Table 3 and 4). The populations of selected sites showed significant variations for studied phenotypic traits along with elevations and environmental conditions. The mean plant height ranges from 29.69±2.91 to 66.4±2.41 cm, old rhizome length 2.74±0.16 to 3.66±0.21 cm, new rhizome length 2.99±0.16 to 4.52±0.29 cm, old rhizome width 0.53±0.04 to 0.70±0.05 cm, new rhizome width 0.88±0.06 to 1.22±0.11 cm, old rhizome girth 1.86± 0.15 to 2.98±0.17 cm, new rhizome girth 2.97±0.09 to 4.34±0.16 cm, basal petiole length 6.90±0.64 to 13.2±1.04 cm, upper petiole length 0.87±0.10 to 2.63±0.29 cm, basal leaf length 2.81±0.20 to 5.53±0.16 cm, basal leaf width 4.56±0.29 to 9.06±0.25 cm, apical leaf length 1.15±0.08 to 1.65±0.19 cm, apical leaf width 0.75±0.07 to 1.14±0.16 cm, pedicel length 3.27±0.34 to 3.93±0.33 cm. Besides this; the floral trait also showed variability with respect to altitude. The average floral length varies from 1.99±0.08 to 2.31±0.10 cm, floral width 2.53±0.10 to 2.91±0.08 cm; petal length along with nectaries 1.35±0.07 to 1.70±0.02 cm; stamen length 0.54±0.21 to 0.61±0.03 cm; carpel length 0.46±0.01 to 0.6±0.02 cm, follicle length 1.18±0.06 to 1.50±0.07 cm and seed length varies from 1.41±0.91 to

Table 3. Phenotypic variability in morphological attributes of *A. violaceum* at different altitudes and environmental conditions

Morphological Characters			Population (m asl)			
	Unit (cm)	**Code**	**Khawous-I (3220) N = 15**	**Numsuru-II (3340) 15**	**Man-Maan-Drass-III (4010) 15**	**KUBG-IV (1595) Control site 10**
	Plant Height (cm)	PH	*56.75±3.82^{c}	40.55±2.91^{b}	29.69±2.91^{a}	66.4±2.41^{c}
	Old rhizome length (cm)	ORL	3.44±0.21^{b}	2.95±0.15^{a,b}	2.74±0.16^{a}	3.66±0.21^{c}
	New rhizome Length (cm)	NRL	4.52±0.29^{c}	3.79±0.17^{b}	2.99±0.16^{a}	4.41±0.30^{c}
	Old rhizome width(cm)	ORW	0.70±0.05^{a}	0.58±0.04^{a}	0.53±0.04^{a}	0.61±0.03^{a}
Rhizome	New rhizome width (cm)	NRW	1.17±0.05^{b}	0.96±0.05^{a}	0.88±0.06^{a}	1.18±0.11^{b}
	Old rhizome girth (mm)	ORG	2.88±0.14^{b}	2.21±0.16^{a}	1.86± 0.15^{a}	2.98±0.17^{b}
	New rhizome girth (mm)	NRG	4.34±0.16^{c}	3.62±0.15^{b}	2.97±0.09^{a}	4.11±0.23^{c}
	Fresh rhizome yield/ plant (g)	FRY	3.94±0.33^{b}	2.76±0.26^{a}	2.39±0.14^{a}	3.41±0.36^{b}
Petiole	Basal petiole length (cm)	BPL	12.23±0.94^{b}	10.18±1.11^{a,b}	6.90±0.64^{a}	13.2±1.04^{b}
	Upper petiole length (cm)	UPL	1.42±0.22^{b}	1.74±0.28^{a,b}	0.87±0.10^{a}	2.63±0.29^{c}
	Basal leaf length (cm)	BLL	5.53±0.16^{c}	3.26±0.17^{a}	2.81±0.20^{a}	4.52±0.46^{b}
	Basal leaf width (cm)	BLW	9.06±0.25^{b}	5.36±0.38^{b}	4.56±0.29^{a}	8.08±0.91^{b}
Leaves	Middle leaf length (cm)	MLL	3.25±0.15^{b}	2.72±0.12^{a}	2.51±0.09^{a}	3.38±0.27^{b}
	Middle leaf width (cm)	MLW	5.79±0.27^{b}	4.15±0.22^{a}	4.07±0.22^{a}	5.54±0.59^{b}
	Apical leaf length (cm)	ALL	1.37±0.05^{b}	1.28±0.07^{a}	1.15±0.08^{a}	1.65±0.19^{b}
	Apical leaf width (cm)	ALW	0.90±0.08^{b}	0.83±0.09^{a}	0.75±0.07^{a}	1.14±0.16^{b}
Pedicel	pedicel length (cm)	PL	3.51±0.37^{a}	3.35±0.36^{a}	3.27±0.34^{a}	3.93±0.33^{a}

Different superscript letters (a, b and c) indicates that means are significantly different among different populations. (Tukey test: P ≤ 0.05)
*Mean± Standard Error; N=Sample size

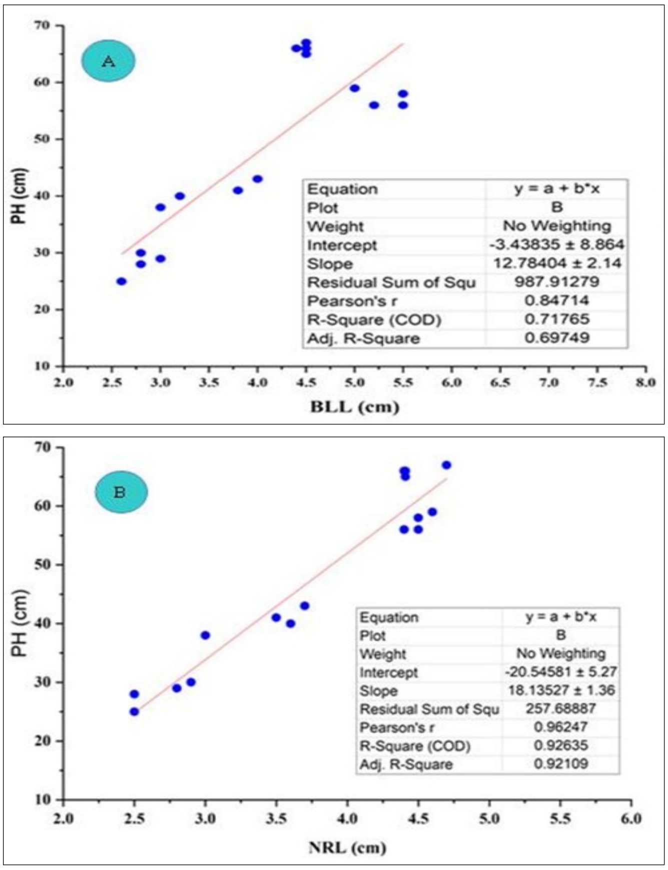
Table 4. Phenotypic variability in floral attributes of *A. violaceum* at different altitudes and environmental condition

Floral Characters			Population (m asl)		
	Unit (cm)	**Code**	**Khawous-I (3220) N =15**	**Goma-Numsuru-II (3340) 15**	**Man-Maan-Drass (4010) 15**
Flower	Flower length	FL	2.31±0.10^{b}	2.28±0.08^{a}	1.99±0.08^{a}
	Flower width	FW	2.91±0.08^{b}	2.84±0.06^{b}	2.53±0.10^{a}
	Posterior sepal length	PSL	1.65±0.08^{b}	1.50±0.08^{a,b}	1.29±0.05^{a}
	Posterior sepal width	PSW	1.07±0.10^{a}	1.09±0.09^{a}	0.9±0.03^{a}
	Lateral sepal length	LSL	1.5±0.06^{a}	1.38±0.07^{a}	1.36±0.10^{a}
Sepal	Lateral sepal width	LSW	1.0±0.04^{a}	0.978±0.06^{a}	0.952±0.77^{a}
	Inner sepal length	ISL	1.03±0.03^{a}	0.982±0.03^{a}	0.971±0.44^{a}
	Inner sepal width	ISW	0.51±0.05^{a}	0.426±0.02^{a}	0.383±0.38^{a}
Petal with nectaries	Petal length	PtL	1.70±0.02^{a}	1.37±0.16^{a}	1.35±0.07^{a}
Stamen	Filament length	FltL	0.61±0.03^{a}	0.59±0.02^{a}	0.54±0.21^{a}
Carpel	Carpel length	CL	0.6±0.02^{b}	0.51±0.01^{a}	0.46±0.01^{a}
Fruit	Follicle length	FoL	1.50±0.07^{b}	1.27±0.05^{a}	1.18±0.06^{a}
Seed	Seed length (mm)	SL	1.90±0.04^{b}	1.78±0.05^{b}	1.41±0.91^{a}
	Seed width (mm)	SW	0.95±0.05^{b}	0.89±0.02^{a,b}	0.79±0.03^{a}

Different superscript letters (a, b and c) indicates that means are significantly different among different populations. (Tukey test: P≤ 0.05)
Mean± Standard Error; N=Sample size

1.90±0.04 cm. The floral traits of control site (KUBG) are not mentioned here because it provided the same result as population-II. Most of the plants grown at the control site (KUBG) dried before reaching to flowering stage, as the environment was not suitable for its establishment. Most of the quantitative morphological traits showed a negative correlation with respect to altitude (i.e., they displayed a steady decline as altitude increased).

The morphological characteristics analyzed vary significantly across the selected populations. The different populations showed considerable morphological variation along with the altitude. Compared to all the populations; Population-I (Khawous 3220 m) exhibits the highest value for nearly all the morphological traits. The population established at the control site (KUBG) showed the highest value in terms of plant height and leaf dimension; however, they lost vigor as the summer temperature gradually increased. The remaining established individual plantlets developed flowers and fruits similar to the population-II, whereas the population-III (Maan-man, Drass) exhibited the lowest values for all the phenotypic characters. There was a positive correlation between plant height and other quantitative characters (i.e., individual plants with large height have larger growth forms for other phenotypic traits such as rhizome, leaf dimension, leaf number and flower number). There was a positive correlation between plant height and new rhizome length (r^2= 0.92635), plant height and basal leaf length (r^2= 0.71765), plant height and basal leaf width (r^2= 0.90183), plant height and number of leaves (r^2= 0.41227), plant height and number of flowers (r^2= 0.02309) as shown in (Fig. 3. A-F). Pearson's correlation coefficient also showed a positive correlation for almost all quantitative phenotypic traits (rhizome dimension, leaf dimension, leaf number, inflorescence length, flower number etc) (Fig. 4). Principle Component Analysis (PCA) revealed that there is a gradual decrease in the size of morphological traits with the increase in altitude and vice versa (Fig. 5). The population growing at high elevation (Maan-man, Drass) showed a decrease in size of all phenotypic traits as compared to other populations, thus sepa-



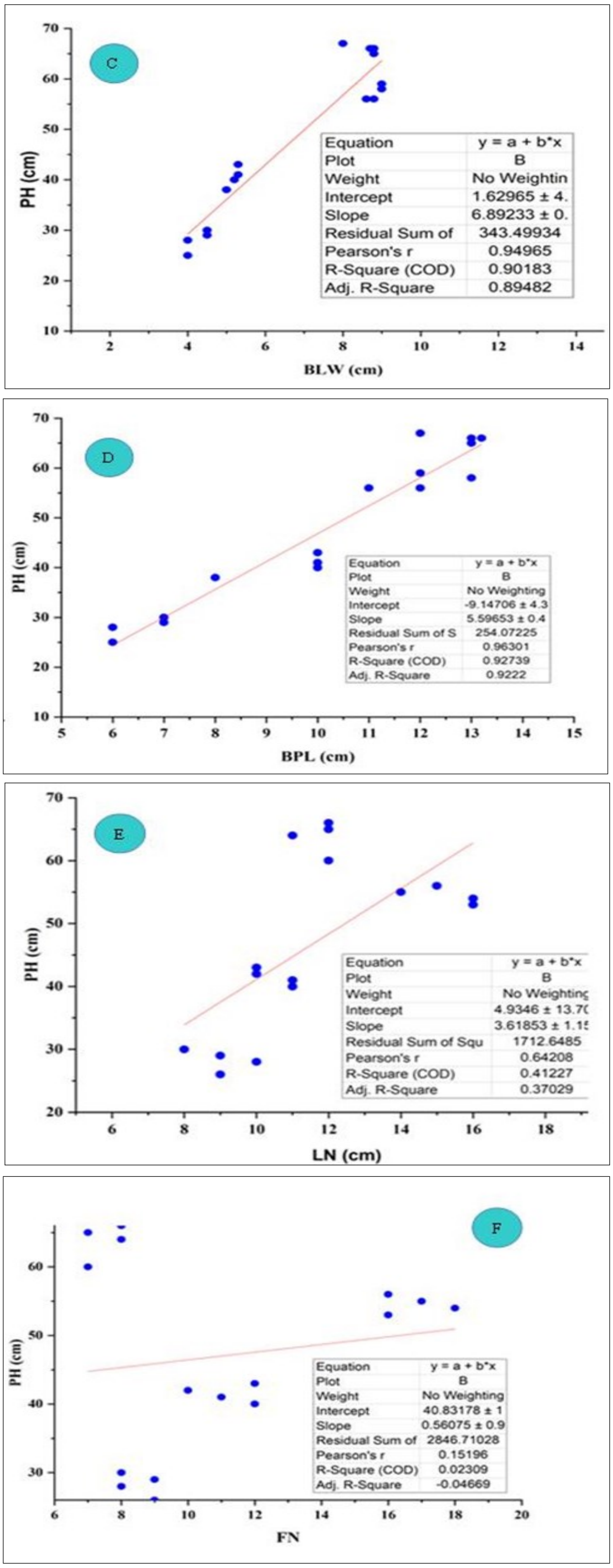


Fig. 3. Graph (**A–F**). Regression analyses between several morphological characters of *A. violaceum*

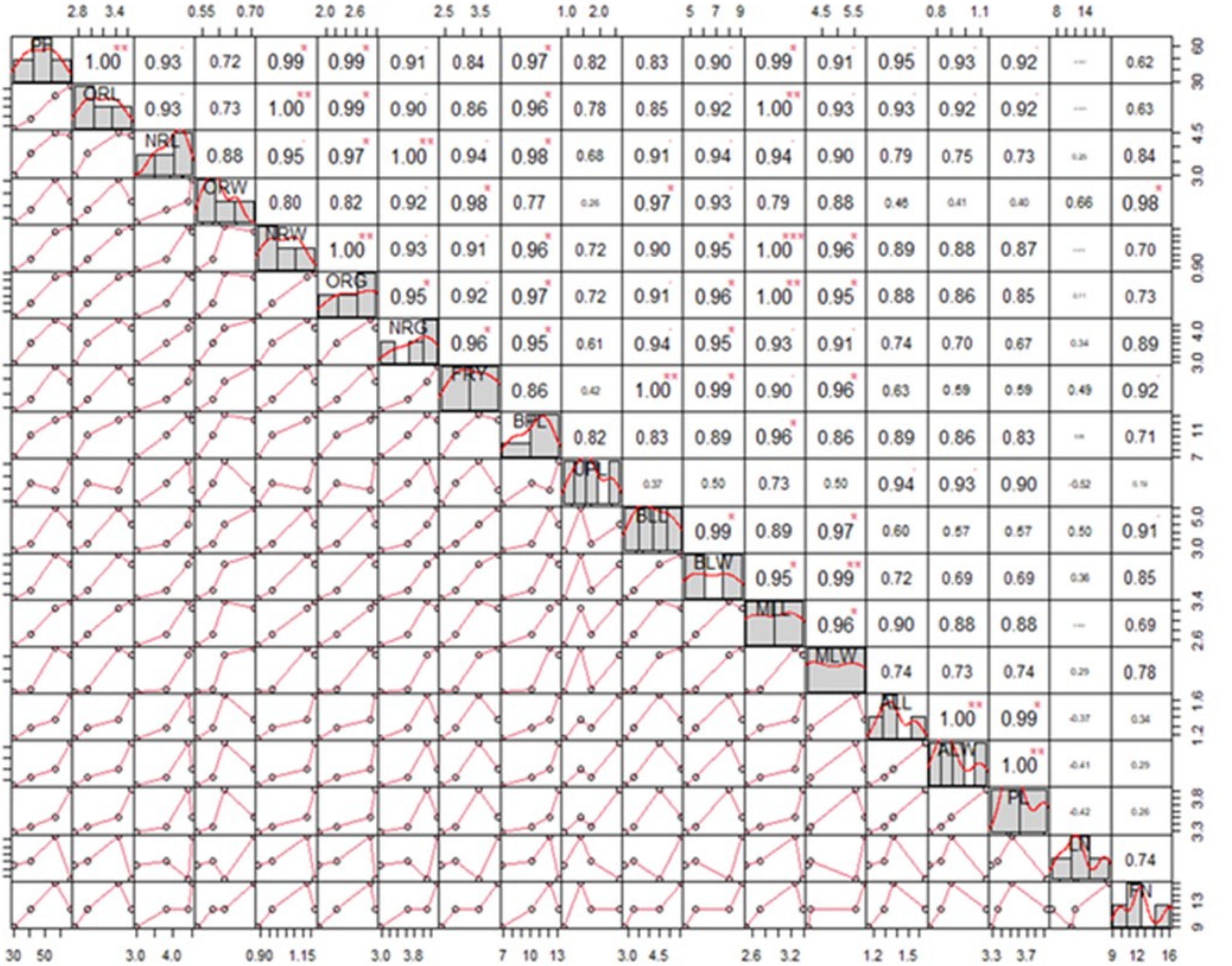


Fig. 4. Scatter plot demonstrating Pearson's correlation matrix for quantitative phenotypic traits among different populations of *A. violaceum*. (***, P < 0.000, **, P < 0.001, *, P < 0.01).

rating them from the rest of the other populations of the study site, whereas the population established at Kashmir University Botanical Garden (KUBG) grew well at the onset of spring; but lost vigorousness as summer temperature rose. This implies that the environmental conditions are not favorable for the luxuriant growth of *A. violaceum* at the control site. The environment and altitude of population-I (Khawous) are highly favorable for the plant species. Our results were in accordance with the former observations (25). It has been stated that reducing plant height and leaf dimension along with altitude is beneficial for the plant species as it prevents them from strong winds (26).

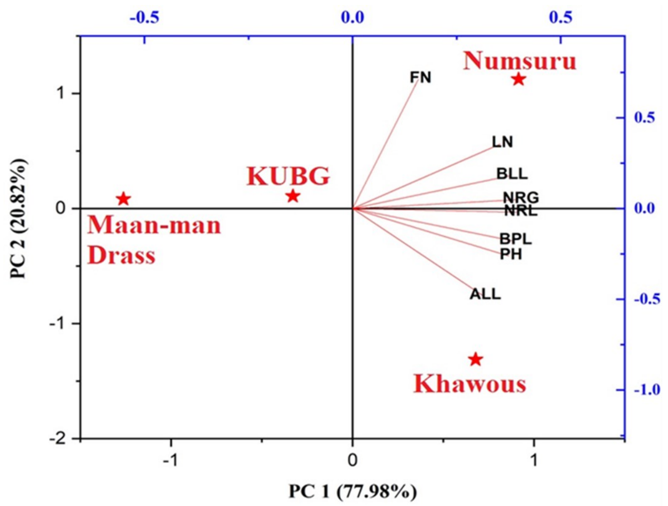


Fig. 5. Principal Component Analysis (PCA) of morphological characteristics of *A. violaceum* across the different study sites. ALL. Apical leaf length; PH. plant height; BPL. Basal petiole length; NRL. new rhizome length; NRG. New rhizome girth; BLL. Basal leaf length; LN. Leaf number.

It has been noticed that there is a tangled relationship between the environment and plants. With the change in altitudinal gradient, a range of environmental factors also vary which imposes a challenge to the plants, to adapt and survive under varying environmental and altitudinal gradients. Plants bring variations in their biochemical and morphological features to acclimate and overcome these stress circumstances (27). The change in morphological traits with varying elevation indicates plasticity and the fixed evolutionary changes (genetic) in these traits may lead to speciation (28).

Conclusion

The present study reveals that variations in the environmental conditions proved to have an enormous impact on the growth pattern and development of *A. violaceum*. The plants growing at altitudinal ranges between 3000-3220 m asl (Population-I) were comparatively much more vigorous and diverse in all morphological characteristics. In addition, flower colours in population-I (khawous) were diverse (violet, blue, light white with blue veins, variegated); whereas the plant specimens grown at (KUBG) grew well in the beginning, however, as the summer temperatures rose, it gradually lost its vigorousness and dried out before reaching the flowering stage. The individuals of population -III (Maan-man, Drass) showed a decreased growth rate in all aspects of morphological traits such as plant height, leaf length, leaf width, number of flowers, rhizome length etc as compared to other population. This study aimed to find a suitable habitat for the establishment, development, cultivation, sustainable economic utilization and conservation of *A. violaceum*, a threatened endemic medicinal plant of the western Himalaya. It can be concluded that the ecology and elevation of population-I (khawous) are suitable for the growth and development of this threatened medicinal plant. Other areas which are also suitable for mass cultivation of this important medicinal plant species in Ladakh are, Nubra valley (Turtuk and Chalunka), Barsoo valley (Tiket, Khandi and Itchoo), Suru valley (Pursa, Thangbo) Drass valley (Matayeen, Prandrass, Mushkoo, Goshan etc) on large scale for commercial purposes as these regions have the same ecological conditions with population-I.

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Authors contributions

This is a collaborative research work of all authors. AH collected the primary data wrote the first draft of the manuscript and prepared graphs. SS edited and designed the manuscript and supervised AH. IAN edited, designed and supervised AH. SR performed the statistical analysis. SA helped in collecting the primary data. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors declared that there is no competing of interest.

Ethical issues: None

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