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Effect of altitudinal variation on floral morphology in *Valeriana jatamansi*, a critically endangered medicinal plant of north east Himalayan region

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Abstract

Countless medicinal plants are endemic to Indian Himalayan region. Over exploitation of *Valeriana jatamansi* for its rhizome these species are now came under endangered species. Rhizomes and roots of this species have secondary metabolite like valerenic acid and valepotriates that can cure obesity, skin diseases, epilepsy, insanity and snake poisoning etc. Present research deals with the flower morphological trait study over control Kalimpong environment with four lower altitude exposed treatments. From the independent sample t test it has been cleared that the flower diameter has been gradually decreased in the exposure of lower altitude. Moreover length of style was found to be of highest value for control K plants. Research findings visibly indicated that alternate exposure of higher and lower altitude gives a significant impact on anther size. From these findings it may be concluded that lower altitude exposure enhance cross pollination and there is a positive correlation of petal breadth over the maximum flower diameter.

Keywords: Altitude, endangered, endemic, flower morphological

Introduction

The Great Himalaya is the main, origin, quarry and biodiversity centre for many valuable medicinal and aromatic plants. Therapeutically medicinal plant species acclimatize themselves with varying soil, topography and climatic conditions for their growth and development. Many of the medicinal plants are endemic to Indian Himalayan region. Due to this cause some sublime medicinal plants species are in the queue of extinction. It would be very difficult for these species to counter the anthropogenic destruction to re-establish again. Interestingly *Valeriana jatamansi* have now been enlisted as an endangered species in the National Medicinal Plant Board, New Delhi (www.nmpb.nic.in). Cultivation of wild species and its sustainable harvesting (Ticktin, 2004) ^[11] are two important measures to conserve species germplasm. Moreover by adopting scientific method of harvesting and some of cultivation practices will neutralize the current crisis as well as future demand.

The name Valeriana might originate from the Latin 'valere' meaning courage, or 'valeo' which mean to be strong. Its Common names are valerian, All-heal, Garden Heliotrope. It belongs to family Valerianaceae or Caprifoliaceae i.e. Honey suckle. (Li, 1994) ^[8]. The family Valerianaceae has traditionally been placed close to Dipsacaceae, and it's often suggested to constitute a link between the families Dipsacaceae and Caprifoliaceae. (Backlund and Moritz, 1998) ^[1]. *V. jatamansi* Jones, is the native of North-eastern Himalayan region with distribution range from Afghanistan to south-west China, India, Bhutan, Nepal and Burma. The plants are cultivated in subtropical and temperate Himalaya between 1000 to 3000 m Above Sea Level (ASL). It generally prefers to grow on hill slopes, moist places, damp woods, ditches and along the streams. Basically hills were natural mine for these medicinal plants, but the major limitation was water resources and availability space. Whereas in the foot hill region of these north east Himalayan region that is Terai region is full of water resources and large fields. It is commonly known as Indian valerian (English), Mushkbala (Kashmiri) and in Sanskrit Sughanthdawal or Tagar (Raina and Srivastava, 1992) ^[9].

Habitat degradation and other biotic interferences in its distribution ranges are the primary reasons for the extinction of this herb from natural habitat. World Health Organization (WHO) recognized and advocated the use of herbal medicines as vide alternative system of therapy in the form of pharmaceuticals and functional foods etc. Due to demand of medicinal plants for both domestic consumption by local inhabitants and for preparing drugs by pharmaceutical industries there is rapid depletion of stock of wild population from natural habitat. Indiscriminate collection of rhizomes from the plants has created large scale depletion over the years and necessitated its replenishment and cultivation (Kaur et al. 1999)^[6]. The threat of extinction of the herbal plants can be minimised by conservation and efficient mass multiplication techniques. As per the report of Wyatt given in 1981 the population assessment of Valeriana species has revealed as decrease of 30-40 plants per 100 m² and the decline in number is increasing with passage of time. The Valeriana species from some previously recorded localities are totally wiped out (Wyatt, 1981) ^[12]. Genetic diversity is influenced by habitat types and the altitudinal range (Jugran et al., 2013, 2015)^[5, 4]. This species has more genetic diversities than any other endemic species because of its wide geographical distribution. Reports were there to depletion of V. jatamansi at a fast pace is sought after for its rhizome. Hence conservation methods should be adopted to control its further depletion from its natural habitats.

Valeriana jatamansi plants were native to north east Himalayan region, due to over exploitation of these plants for its medicinal purpose these species are now came under endangered species. Rhizome being the economic as well as propagating material for the plant, men exploits these rhizomes for medicinal manufacture rather for propagation. It's time to orient research in such a way that first and foremost priority to conserve these plant species and save them from the endangered edge. Some of the propagating materials were brought from the hill zone to plains for its survival purpose. These plants were well established as well as performing very well to the plain area. The major objective of the present study is to find out the floral variation of the species in different altitude.

Botanical description

issinca	uon
-	Plantae
-	Tracheophyta,
-	Magnoliopsida
-	Dipsacales
-	Caprifoliaceae
-	Valeriana
-	jatamansi
	- - - - - - - - -

Botanical Name	-	Valeriana jatamansi Jones
Synonym	-	Valeriana wallichii DC. (Bennet,
1987) [2]		

Material and method

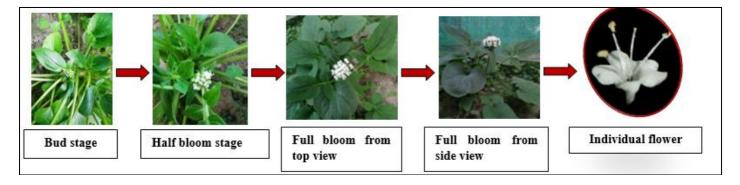
V. jatamansi Jones cutting materials were collected from the native hill zone and were brought to plains (planted) for acclimatization. Flowers were collected with the help of forceps and are immediately kept inside ice box filled half with ice crystals. According to its exposure different levelling was given and the flower sample is taken to Image analyser for detail study. Flowers kept in a posture to observe its maximum parts and feasible for characters study. Care should be taken to keep at least on anther intact so that its measurements can be recorded. The instrument image analyser (Dewinter Technologies, ISO; 9001:2008 model name and number: Zoom star III) was used for taking the flower morphologic data. The instrument was self calibrated to measure the desirable distance in micro meter.

Experimental site and its Geographical location

The research trial have been conducted in the year 2018-2019 and 2019-2020 at Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar field covered with 75 GSM HDPE shed net under partial shade of plant canopy were made for suitable plant growth. The exact replica copy of shed was provided at higher altitude Regional Research Station (Hill Zone) Kalimpong. UBKV farm is located at 28° 19' N Latitude and 89° 23' E longitude and at an altitude of 43 Meter above Mean sea level was considered as Lower altitude region where as Kalimpong farm is situated at 27° 32' N latitude and 88° 28' E longitude and at an altitude of 1097 meter above mean sea level is considered as higher altitude for *Valeriana jatamansi*.

Stem cuttings were brought form the hill zone Kalimpong and were planted on plain area of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar farm. In between this transfer there was a buffering period of on an around one and half moth of planting in a controlled plant growth room (temperature of 22 ± 2 ⁰C, relative humidity of 65 ± 5 and 3000 lux of led light with a light period of 14 hr. followed by 10 hr. of dark period). Once the cutting materials planted on artificial soil were fully established and starts growing these plants were transferred to field covered with 75 gsm of green shade net below tree canopy shed.

The exposure itself is treated as treatments here. Basically five treatments were there such as, firstly the plant grown on Hill zone of Kalimpong (control K), secondly two successive year grown UBKV plants (represented as 2^{nd} UU), thirdly second year Kalimpong plants through one year exposure to UBKV (represented as 2^{nd} UK), fourthly second year.





Flowers collected with the help of forceps Flowers kept inside the Ice box and lid kept closed Flower pictures taken in Image analyzer



Flower picture of higher altitude (Kalimpong) plants



Flower picture of lower altitude (UBKV Farm) plants

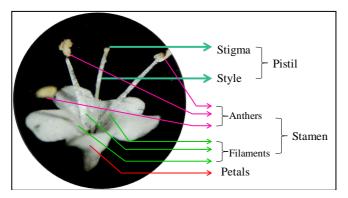


Fig 1: various parts of the Valeriana jatamansi flower

UBKV plant through one year exposure to Kalimpong (that represented as 2nd KU) and lastly two consecutive year exposed Kalimpong plant (that is 2nd KK). Various characters of flower were considered to study its change such as, maximum flower diameter, maximum length of petal, breadth of largest petal, length of filament, diameter of filament, length of style, diameter of style, length of anther and breadth of anther.

Maximum flower diameter

Size of the flower were studied with this parameter, after taking picture of the individual flower cross length wise line were drawn to measure the calibrated outer diameter of the flower. Being a tiny flower measurements were recorded in micron.

Maximum length of petal

Largest petal length will specify the largest petal length in micron, which might indicate the lower altitude and higher altitudinal variation.

Breadth of largest petal

Among the 5 petals, largest petal breadth was recorded in micron to study the pattern of petal shape variation acceding to change in altitude.

Length of filament

Out of the three filaments Total length of the filament from the bottom part anther up to its origin space from flower petal were recorded in micron to study the variation in its length in between the lower and higher altitude flower.

Diameter of filament

Thickness of the filament was measured in micron by drawing line above the filament that is visible on the system display.

Length of style

From stigmatic surface up to the above part ovary the total length of the female reproductive part was taken in micron to study the pattern of behaviour over longitudinal variation.

Diameter of style

The style tube diameter was recorded in micron for both the cases of altitude for each treatment to study the thickness pattern of the style tube.

Length of anther

Visually line drawn above the anther structure in micron was recorded. There might variation for the size of anther according to its deviation in exposure over altitude.

Breadth of anther

Line drawn against the cross section of the anther length determines its breadth in micron.

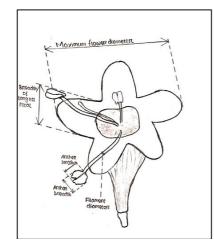


Fig 2: Schematic diagram of some of flower traits under study]

Statistical tools used

T-test for equality of means of the independent samples and correlation study Graph Pad Prism.

Observations

The trait petal length of flower was observed to be differing significantly in case of two consecutive year exposed to UBKV (2nd UU) of V. Jatamansi from the control K plants that was native to hill zone Kalimpong. 2nd UU plant showed larger petals of 1497.17 µm length. But the largest petal breadth was significantly higher in case of control K than 2nd UU. A length of 789.77 µm was recorded for the trait largest petal breadth in case of control K plants and a lower value of 747.54 μ m was observed for 2nd UU plants. Moreover length of filament was observed to be shorter (1787.80µm) in case of flowers of Kalimpong control plot and this length was increased to 1904.65µm in case of 2nd UU plants. In the contrary filament diameter for this particular comparative study did not differ significantly. Length of style and its diameter were differ significantly with a value of 1928.47 µm (higher) and 1606.00 μm (lower) in case of control Kalimpong plot and 2nd UU plants respectively. Where as a reverse trend was observed, such as $68.13 \ \mu m$ and $85.45 \ \mu m$ for the style diameter trait. Anther length differ significantly, with a value of 356.59 μ m and 228.99 μ m in case of control K and 2nd UU plants respectively and a similar trend was also observed for the trait anther breadth (table 1).

Filament length and style diameter were non-significant and rest of all flower traits were found to be significantly differ for the comparative study over control K and the second year exposed Kalimpong plant via one year exposure to UBKV (2^{nd} UK). Maximum flower diameter were found to be higher for 2^{nd} UK and lower for control K i.e. 2620.27 µm and 2437.89 µm respectively. Length and breadth of petal were found to be higher in case of 2^{nd} UK (i.e. 1466.06 µm, 837.69 µm) and lower in case of control K (i.e. 1362.90 µm, 789.77

μm) plant respectively. Diameter of the filament increases from 120.07 μm to 133.55 μm when the plant brought from control Kalimpong environment to 2^{nd} UK. Moreover the length of style reduced from 1928.47 μm to 1796.45 μm under the exposure of 2^{nd} UK. Larger sized (length and breadth) anthers were spotted in case of 2^{nd} UK (i.e. 387.22 μm, 242.43 μm) and smaller in case of control K plants (i.e. 356.59 μ, 228.22 μ) respectively (table 2).

For the comparative study among control Kalimpong plant and second year exposed UBKV plant through previous exposure to Kalimpong (2nd KU) showed significant values for all the traits except filament length. Flower size (i.e. maximum flower diameter) increased from the control plant to 2nd KU. Petal length and diameter also increased accordingly, such as length of 1493.08 µm was recorded in case of 2nd KU and 1362.90 µm in case of control K plants. But petal breadth increases from 789.77 µm to 877.50 µm in case of 2nd KU. 120.07 µm of filament diameter was recorded in case of control K that was significantly increased to 155.76 µm in case of 2nd KU. On the contrary length of style was higher in case of control K (i.e. 1928.47 µm) and lower in case of 2nd KU (i.e. 1772.35 µm). In continuation to that style diameter was higher in case of 2nd KU that is 73.16 µm and lower in case of control K that is 68.13 µm respectively. Enhanced Anther size was spotted in case of 2nd KU (i.e. length 407.65 µm and breadth 239.91 µm) whereas smaller size was sowed in case of control K with a length of 356.59 μm and a breadth of 228.99 μm (Table 3).

Furthermore the comparisons between control Kalimpong plants and the two consecutive year grown Kalimpong plant (i.e. 2nd KK) revealed all of the flower traits that were considered significantly different from each other except the traits anther length and anther breadth. Size of the flower decreases in case of 2nd KK from the control K plants. Maximum flower diameter was observed to be 2437.89 µm and 2291.91 µm for control K and 2nd KK respectively. Petal length and breadth were significantly higher for control K with the value of 1362.90 µm, 789.77 µm and lower in case of 2nd KK with the value of 1340.90 µm and, 760.27 µm. Similarly length of filament was higher in case of control K (i.e. 1787.80 µm) and lower in case of 2nd KK (i.e. 1625.50 µm). In the contrary filament diameter was higher in case of 2nd KK (i.e. 134.27 µm) and lower in case of control K (i.e. 120.07 µm). Moreover style length and style diameter were significantly higher in case of control K (Table 4).

Among all of correlation study from table number 5 and figure X it has been clear that none of the traits were significantly correlated with the flower size, except petal breadth, which has significant positive correlation with flower size (0.78). Although anther length and petal breadth (0.88); anther length and filament diameter (0.85) are moderately correlated. Moreover petal length was found to be moderately correlated (0.69) with style diameter.

Table 1: Mean table of the flower traits among control K and 2nd UU, Valeriana jatamansi

Observations	Maximum flower	diameter (µm)	Petal length (µm)		Largest petal breadth (µm)		Filament length (µm)		Filament diameter (µm)	
	Control K	2 nd UU	Control K	2nd UU	Control K	2 nd UU	Control K	2 nd UU	Control K	2 nd UU
Obs-1	2387.41	2338.71	1349.11	1490.35	791.11	745.32	1670.62	1880.61	114.49	111.35
Obs-2	2266.23	2299.78	1362.42	1489.48	780.44	736.02	1847.34	1981.04	113.29	108.51
Obs-3	2493.61	2308.57	1364.28	1498.70	801.41	741.71	1794.46	1867.74	104.34	106.16
Obs-4	2519.11	2499.19	1359.50	1501.66	788.12	734.96	1811.59	1888.04	131.62	125.26
Obs-5	2597.81	2458.93	1362.33	1495.93	795.36	751.34	1878.16	1907.02	124.17	119.46
Obs-6	2442.06	2482.74	1374.75	1466.30	808.93	762.84	1766.84	1888.45	112.12	109.04
Obs-7	2237.19	2395.27	1378.00	1513.77	750.42	754.91	1706.07	1902.94	124.11	113.07
Obs-8	2539.71	2392.69	1352.82	1521.21	796.39	759.22	1827.30	1921.34	130.44	103.46
Obs-9	2416.59	2361.56	1358.83	1495.05	792.77	749.50	1781.00	1904.36	123.43	117.07

Obs-10	2479.19	2432.41	1366.97	1499.30	792.77	739.57	1794.59	1904.94	122.71	116.26
Mean	2437.89	2396.98	1362.90	1497.17	789.77	747.54	1787.80	1904.65	120.07	112.96
SD	115.53	70.58	8.87	14.70	15.76	9.64	62.20	30.91	8.69	6.63
SEM	36.53	22.32	2.80	4.65	4.98	3.05	19.67	9.78	2.75	2.10
t Score	0.96	j	-24.7	3**	7.23**	*	-5.32	**	2.06	
Prob. Value	0.35		0.0	0	0.00		0.0	0	0.06	
Mean Difference	40.9	1	-134	-134.27 42.23 -116.85		-116.85		7.11		
Std. Error Diff.	42.8	1	5.4	.3	5.84		21.97		3.46	

*Significant at 5% level

**Significance at 1% level

Table 1: Continuation

Observations	Style leng	th (µm)	Style diame	ter (µm)	Anther leng	gth (μm)	Anther brea	dth (µm)
	Control K	2 nd UU	Control K	2 nd UU	Control K	2nd UU	Control K	2nd UU
Obs-1	1881.03	1592.86	63.27	81.26	351.67	320.04	223.13	199.04
Obs-2	1733.26	1669.85	59.93	89.40	370.87	337.66	228.95	216.93
Obs-3	1948.05	1571.10	76.58	88.41	358.61	323.65	226.08	223.33
Obs-4	1981.70	1711.54	68.61	86.99	372.56	340.45	231.19	197.56
Obs-5	2071.93	1509.82	71.68	72.87	348.79	336.24	232.84	212.43
Obs-6	1897.40	1505.29	72.11	81.63	350.19	341.10	231.96	217.13
Obs-7	1947.23	1676.27	64.99	93.23	361.88	356.83	221.37	222.26
Obs-8	1967.19	1611.28	67.89	89.76	346.16	325.44	242.36	214.59
Obs-9	1886.01	1636.34	67.10	86.52	360.92	348.45	227.34	201.71
Obs-10	1970.94	1575.67	69.17	84.37	344.26	354.15	224.63	206.60
Mean	1928.47	1606.00	68.13	85.45	356.59	338.40	228.99	211.16
SD	88.56	68.96	4.75	5.76	9.98	12.60	6.05	9.40
SEM	28.00	21.81	1.50	1.82	3.16	3.98	1.91	2.97
t Score	9.09	**	-7.33*	**	3.58*	*	5.04*	*
Prob. Value	0.0	0	0.00)	0.00)	0.00	
Mean Difference	322.	47	-17.31		18.19		17.83	
Std. Error Diff.	35.4	49	2.36	,	5.08		3.54	

*Significant at 5% level **Significance at 1% level

Table 2: Mean table of the flower traits among control K and 2nd UK, Valeriana jatamansi

Observations	Maximum flow		Petal leng	gth (μm)	Largest peta		Filament		Filament di	
	<u>(μm</u>)			and TITZ	<u>(μm</u>		<u>(μn</u>	/	<u>(μm</u>)	
	Control K	2 nd UK	Control K	2 nd UK	Control K	2 nd UK	Control K	2 nd UK	Control K	2 nd UK
Obs-1	2387.41	2532.83	1349.11	1462.40	791.11	825.32	1670.62	1721.01	114.49	128.52
Obs-2	2266.23	2759.97	1362.42	1444.25	780.44	836.02	1847.34	1743.84	113.29	121.47
Obs-3	2493.61	2571.54	1364.28	1456.54	801.41	831.71	1794.46	1770.67	104.34	138.63
Obs-4	2519.11	2656.36	1359.50	1480.38	788.12	844.96	1811.59	1886.97	131.62	133.68
Obs-5	2597.81	2706.66	1362.33	1473.71	795.36	821.34	1878.16	1815.94	124.17	135.03
Obs-6	2442.06	2532.95	1374.75	1486.07	808.93	842.84	1766.84	1736.61	112.12	137.07
Obs-7	2237.19	2762.43	1378.00	1474.47	750.42	848.94	1706.07	1804.42	124.11	139.69
Obs-8	2539.71	2439.41	1352.82	1450.69	796.39	828.79	1827.30	1646.63	130.44	134.32
Obs-9	2416.59	2630.17	1358.83	1460.89	792.77	849.50	1781.00	1780.62	123.43	128.07
Obs-10	2479.19	2610.36	1366.97	1471.24	792.77	847.48	1794.59	1750.90	122.71	139.03
Mean	2437.89	2620.27	1362.90	1466.06	789.77	837.69	1787.80	1765.76	120.07	133.55
SD	115.53	104.90	8.87	13.35	15.76	10.43	62.20	63.85	8.69	5.87
SEM	36.53	33.17	33.17	4.22	2.80	3.30	4.22	20.19	4.98	1.86
t Score	-3.70*	*	-20.3	6**	-8.02	**	0.7	8	-4.06*	**
Prob. Value	0.00		0.0	0	0.00)	0.4	5	0.00)
Mean Difference	-182.3	38	-103	.16	-47.92		22.04		-13.48	
Std. Error Diff.	49.35	5	5.0	7	5.98	3	28.1	9	3.32	

*Significant at 5% level **Significance at 1% level

Table 2: Continuation

Observations	Style leng	th (µm)	Style diamet	ter (µm)	Anther leng	gth (μm)	Anther brea	Anther breadth (µm)		
	Control K	2 nd UK	Control K	2 nd UK	Control K	2 nd UK	Control K	2 nd UK		
Obs-1	1881.03	1885.49	63.27	68.59	351.67	387.15	223.13	236.72		
Obs-2	1733.26	1786.71	59.93	72.17	370.87	378.12	228.95	231.36		
Obs-3	1948.05	1783.92	76.58	75.21	358.61	374.89	226.08	240.39		
Obs-4	1981.70	1798.29	68.61	63.01	372.56	379.17	231.19	239.65		
Obs-5	2071.93	1756.97	71.68	67.79	348.79	383.45	232.84	253.96		
Obs-6	1897.40	1789.48	72.11	68.18	350.19	400.37	231.96	243.28		
Obs-7	1947.23	1768.57	64.99	71.25	361.88	403.08	221.37	240.69		
Obs-8	1967.19	1802.20	67.89	74.44	346.16	399.29	242.36	255.42		

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Obs-9	1886.01	1813.60	67.10	69.75	360.92	369.83	227.34	237.03
Obs-10	1970.94	1779.31	69.17	70.41	344.26	396.80	224.63	245.84
Mean	1928.47	1796.45	68.13	70.08	356.59	387.22	228.99	242.43
SD	88.56	35.26	4.75	3.53	9.98	11.92	6.05	7.55
SEM	3.30	11.15	19.67	1.12	20.19	3.77	2.75	2.39
t Score	4.38	**	-1.04		-6.23*	**	-4.39*	*
Prob. Value	0.0)	0.31	0.31		1	0.00	
Mean Difference	132.	02	-1.95	-1.95 -30.62 -13.4		-13.45	5	
Std. Error Diff.	30.1	4	1.87	1.87 4.92 3.06		4.92		
×C: 'C' / / / / 1 1								

*Significant at 5% level **Significance at 1% level

Table 3: Mean table of the flower traits among control K and 2nd KU, Valeriana jatamansi

Observations	Maximum flowe (µm)	er diameter	Petal len	gth (µm)	Larges breadt	-	Filament (µn		Filament diameter (μm)	
	Control K	2 nd KU	Control K	2 nd KU	Control K	2 nd KU	Control K	2 nd KU	Control K	2 nd KU
Obs-1	2387.41	2603.17	1349.11	1502.06	791.11	899.26	1670.62	1807.65	114.49	159.08
Obs-2	2266.23	2541.06	1362.42	1483.82	780.44	859.46	1847.34	1794.64	113.29	150.82
Obs-3	2493.61	2598.52	1364.28	1486.51	801.41	878.98	1794.46	1802.09	104.34	160.37
Obs-4	2519.11	2566.21	1359.50	1497.13	788.12	878.28	1811.59	1800.67	131.62	158.24
Obs-5	2597.81	2579.97	1362.33	1488.33	795.36	870.03	1878.16	1793.31	124.17	156.96
Obs-6	2442.06	2609.99	1374.75	1493.52	808.93	888.52	1766.84	1788.05	112.12	149.33
Obs-7	2237.19	2597.88	1378.00	1499.84	750.42	876.98	1706.07	1801.96	124.11	153.34
Obs-8	2539.71	2588.30	1352.82	1499.25	796.39	879.31	1827.30	1798.21	130.44	160.43
Obs-9	2416.59	2575.24	1358.83	1491.88	792.77	864.24	1781.00	1803.01	123.43	151.63
Obs-10	2479.19	2606.54	1366.97	1488.48	792.77	879.96	1794.59	1806.89	122.71	157.38
Mean	2437.89	2586.69	1362.90	1493.08	789.77	877.50	1787.80	1799.65	120.07	155.76
SD	115.53	21.48	8.87	6.28	15.76	11.37	62.20	6.16	8.69	4.12
SEM	36.53	6.79	2.80	1.99	4.98	3.60	19.67	1.95	2.75	1.30
t Score	-4.00*	*	-37.89**		-14.2	27**	-0.6	50	-11.73	**
Prob. Value	0.00		0.00		0.0	00	0.56		0.00)
Mean Difference	-148.8	0	-13	0.18	-87.73		-11.85		-35.69	
Std. Error Diff.	37.16		3.	44	6.1	15	19.77		3.04	

*Significant at 5% level **Significance at 1% level

Table 3: Continuation

Observations	Style leng	th (µm)	Style diamet	ter (µm)	Anther leng	th (µm)	Anther brea	dth (µm)
	Control K	2 nd KU	Control K	2 nd KU	Control K	2 nd KU	Control K	2 nd KU
Obs-1	1881.03	1776.41	63.27	78.84	351.67	409.17	223.13	238.07
Obs-2	1733.26	1764.68	59.93	76.05	370.87	411.40	228.95	241.77
Obs-3	1948.05	1775.91	76.58	69.17	358.61	412.94	226.08	242.72
Obs-4	1981.70	1760.37	68.61	68.49	372.56	399.01	231.19	239.12
Obs-5	2071.93	1772.48	71.68	74.43	348.79	398.29	232.84	244.67
Obs-6	1897.40	1768.76	72.11	76.64	350.19	398.90	231.96	242.05
Obs-7	1947.23	1789.85	64.99	78.42	361.88	414.74	221.37	240.90
Obs-8	1967.19	1763.92	67.89	67.66	346.16	418.44	242.36	237.79
Obs-9	1886.01	1763.84	67.10	70.89	360.92	400.13	227.34	236.42
Obs-10	1970.94	1787.25	69.17	71.00	344.26	413.51	224.63	235.60
Mean	1928.47	1772.35	68.13	73.16	356.59	407.65	228.99	239.91
SD	88.56	10.09	4.75	4.21	9.98	7.75	6.05	2.96
SEM	28.00	3.19	1.50	1.33	3.16	2.45	1.91	0.94
t Score	5.54	**	-2.50	*	-12.78	**	-5.13*	**
Prob. Value	0.0	0	0.02		0.00		0.00	
Mean Difference	156.	13	-5.03	-5.03		-51.06		3
Std. Error Diff.	28.1	8	2.01		4.00		2.13	

*Significant at 5% level **Significance at 1% level

Table 4: Mean table of the flower traits among control K and 2nd KK, Valeriana jatamansi

Observations	Maximum flower diameter (μm)		Petal length (µm)		Largest petal breadth (µm)		Filament length (µm)		Filament diameter (µm)	
	Control K	2 nd KK	Control K	2nd KK	Control K	2 nd KK	Control K	2 nd KK	Control K	2 nd KK
Obs-1	2387.41	2287.41	1349.11	1339.11	791.11	751.11	1670.62	1620.62	114.49	134.49
Obs-2	2266.23	2296.23	1362.42	1332.42	780.44	760.44	1847.34	1627.34	113.29	132.29
Obs-3	2493.61	2293.61	1364.28	1334.28	801.41	761.41	1794.46	1624.46	104.34	134.34
Obs-4	2519.11	2292.11	1359.50	1340.50	788.12	763.12	1811.59	1621.59	131.62	137.62
Obs-5	2597.81	2304.01	1362.33	1342.33	795.36	755.36	1878.16	1628.16	124.17	135.17

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Obs-6	2442.06	2282.06	1374.75	1343.75	808.93	758.93	1766.84	1626.84	112.12	133.12
Obs-7	2237.19	2277.19	1378.00	1338.00	750.42	750.42	1706.07	1629.07	124.11	130.11
Obs-8	2539.71	2309.71	1352.82	1342.82	796.39	756.39	1827.30	1627.30	130.44	131.44
Obs-9	2416.59	2297.59	1358.83	1358.83	792.77	792.77	1781.00	1625.00	123.43	136.43
Obs-10	2479.19	2279.19	1366.97	1336.97	792.77	752.77	1794.59	1624.59	122.71	137.71
Mean	2437.89	2291.91	1362.90	1340.90	789.77	760.27	1787.80	1625.50	120.07	134.27
SD	115.53	10.61	8.87	7.29	15.76	12.22	62.20	2.78	8.69	2.56
SEM	36.53	3.35	2.80	2.31	4.98	3.86	19.67	0.88	2.75	0.81
t Score	3.98*	**	6.06	j**	4.68	**	8.24	**	-4.96*	**
Prob. Value	0.00)	0.0	00	0.0	0	0.0	0	0.00)
Mean Difference	145.9	98	22.	00	29.5	50	162.30		-14.2	0
Std. Error Diff.	36.6	9	3.6	53	6.31		19.69		2.87	
*0	(1 1									

*Significant at 5% level

**Significance at 1% level

Table 4: continuation

Observations	Style length (µm)		Style diameter (µm)		Anther length (µm)		Anther breadth (µm)	
	Control K	2 nd KK	Control K	2 nd KK	Control K	2 nd KK	Control K	2 nd KK
Obs-1	1881.03	1811.03	63.27	65.27	351.67	361.67	223.13	226.13
Obs-2	1733.26	1813.26	59.93	58.93	370.87	360.87	228.95	229.95
Obs-3	1948.05	1818.05	76.58	61.58	358.61	358.61	226.08	226.08
Obs-4	1981.70	1820.70	68.61	63.61	372.56	362.56	231.19	230.19
Obs-5	2071.93	1817.93	71.68	62.68	348.79	358.79	232.84	230.84
Obs-6	1897.40	1815.40	72.11	63.11	350.19	354.19	231.96	229.96
Obs-7	1947.23	1817.23	64.99	64.99	361.88	361.88	221.37	227.37
Obs-8	1967.19	1814.19	67.89	65.89	346.16	356.16	242.36	228.36
Obs-9	1886.01	1816.01	67.10	64.10	360.92	360.09	227.34	226.34
Obs-10	1970.94	1809.94	69.17	59.17	344.26	364.26	224.63	229.63
Mean	1928.47	1815.37	68.13	62.93	356.59	359.91	228.99	228.49
SD	88.56	3.34	4.75	2.41	9.98	3.05	6.05	1.86
SEM	28.00	1.06	1.50	0.76	3.16	0.96	1.91	0.59
t Score	4.04**		3.09*		-1.01		0.25	
Prob. Value	0.00		0.01		0.33		0.81	
Mean Difference	113.10		5.20		-3.32		0.50	
Std. Error Diff.	28.02		1.68		3.30		2.00	

*Significant at 5% level

**Significance at 1% level

Table 5: Correlation table of maximum flower diameter over all of the rest flower traits over the seven treatments

	Petal breadth	Filament length	Filament diameter	Style length	Style diameter	Anther length	Anther breadth	Maximum flower diameter
Petal length	0.42	0.69	0.17	-0.63	0.69	0.31	0.00	0.54
Petal breadth		0.04	0.74	0.21	-0.12	0.88*	0.74	0.78*
Filament length			-0.29	-0.45	0.73	-0.17	-0.32	0.36
Filament diameter				0.20	-0.35	0.85*	0.64	0.43
Style length					-0.56	0.20	0.43	0.09
Style diameter						-0.24	-0.42	0.12
Anther length							0.75	0.60
Anther breadth								0.52

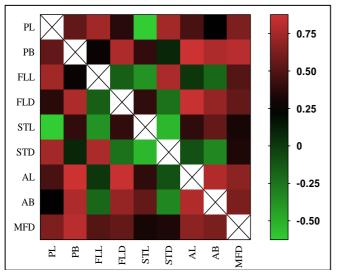


Fig 3: Heat map of the correlation study of maximum flower diameter over rest of all other flower traits.

Discussion

Highest maximum flower diameters were observed in case of 2^{nd} UK and lowest in case of 2^{nd} KK. Furthermore control K plant shows 2437.89 µm that decreases to 2396.98 µm in case of 2^{nd} UU. But in case of largest petal length were found in case of 2^{nd} UU that is 1497.17 µm which was significantly higher than control plant. In continuation to that largest petal breadth was observed in case of 2^{nd} KU.

The 2^{nd} UU exposure showed significantly higher value of filament length than other environments whereas 2^{nd} KU showed highest value of filament diameter. In control K plants the trait style length was observed to be highest among all of the environments mentioned in this study, which indicates that the plant exhibits trend of cross pollination in its ex situ exposure. In contrast to that style diameter was highest in case of 2^{nd} UU which follows the similar trend as observed for other flower traits. Kumari *et al.*, 2020 ^[7] reported that in case of female flower the floral size was reduced to get pollinated maximum number of flowers by the pollinator in a single visit. Similar trend has also been found in our study

where we found flower diameter has been reduced in ex situ condition.

The highest value of anther length was observed in case of 2nd KU and highest value of anther diameter was observed in case of 2nd UK. From these observations we can conclude that alternate exposure of higher and lower altitude give a significant impact on anther size.

Moreover another trait petal breadth also showed a positive significant correlation with anther length (0.88). Similar correlation studies have been found by Chakraborty *et al.*, 2015^[3] breadth of the sepal and length of the sepal was found to be moderately correlated (0.472).

From RAPD profiling by Sing *et al.*, 2015 ^[10] the variation is higher among the population of different location than within population, from which they concluded that the effect of local environment is responsible for this variability.

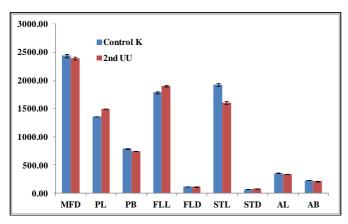
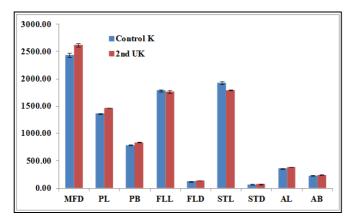


Fig 4: Comparison of flower traits with control K and 2nd UU



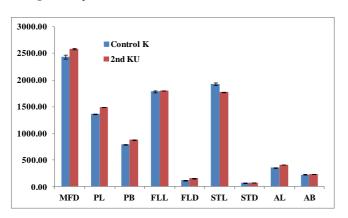


Fig 5: Comparison of flower traits with control K and 2ndUK

Fig 6: Comparison of flower traits with control K and 2nd KU

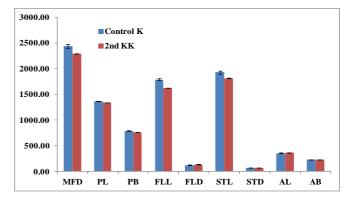


Fig 4: Comparison of flower traits with control K and 2nd KK

Conclusion

From the table 4 it has been indicated that over exposure to the hill environment (2^{nd} KK) reflects negative impact on different flower parameters. From the independent sample t test it has been cleared that the flower diameter has been gradually decreased in the exposure of lower altitude. Moreover Length of style was found to be of highest value for control K plants. Research findings visibly indicated that alternate exposure of higher and lower altitude gives a significant impact on anther size. From these findings it may be concluded that lower altitude exposure enhance cross pollination. From the correlation table it has been further concluded that there is a positive correlation of petal breadth over the maximum flower diameter.

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