

## Variation in Growth Dynamics and Reproductive Allocation Across Different Altitudes of Two Species of *Ajuga* – A Medicinally Important Genus

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### Abstract

*Ajuga* is a medicinally important genus of the Himalayan region. This study was carried out on the phenotypic variability and reproductive allocation in two species of genus *Ajuga*, *A. bracteosa* and *A. parviflora* growing in variable habitats of Kashmir valley. Our findings clearly displayed significant divergence among sites which reveals a definite impact of altitude on morphological and reproductive features of the species under study. In *A. bracteosa* the plants growing at low altitudes are comparatively taller and produce more number of leaves, greater leaf dimensions and inflorescence number. But in case of *A. parviflora* a more or less reverse trend was seen. Principal component analysis (PCA) revealed that the habitat of Kangan and Dachigam proved relatively better for the growth of *A. bracteosa* and Brinal 2 and Gogaldara for *A. parviflora*. Maximum resources were allocated to the growth and development of the leaves followed by stem, root tubers and inflorescence in both the species. The reproductive effort was higher ( $40.48 \pm 1.59$ ) for the high altitude Betab valley population in case of *A. bracteosa* and was lowest for the high altitude Brinal 2 population ( $45.88 \pm 1.37$ ) in case of *A. parviflora*. The regression analysis revealed a positive correlation between branch length and root length, branch length and apical leaf length in both the species and a negative correlation between branch length and number of branches per plant in both the species. Our results are very useful to introduce the species into cultivation at a mass scale and developing strategies for conservation.

**Keywords:** *Ajuga bracteosa*; *Ajuga parviflora*; Altitude; Kashmir Himalaya; Morphological variability; Lamiaceae

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### Introduction

Plants are unable to escape the hazards of environment in which they grow because of their sessile habit. To cope with this, many plants undergo variation in one or more morphological characters in response to both abiotic (e.g., climate and weather) and biotic (grazing and competition) factors of the environment as an adjustment to resource availability. This adjustment to environment is generally referred as phenotypic plasticity [1]. Recently more attention has been given to the acclimation of morphological, biochemical and physiological traits of plants along an altitudinal gradient [2-4]. Abiotic factors have a significant effect on the morphological variability, despite the

fact that it may be due to ontogenic programming of the plant as well. Abiotic variables such as water stress, mineral nutrient deficiency and geographical position may have an impact on the overall plant morphology. For example, latitude is negatively correlated with plant height [5], while altitude may also affect the leaf morphology and plant height [6,7]. The plant morphological parameters such as height, leaf area and petiole length vary with different altitudes [8], with a progressive decrease in plant height with an increase in altitude. Many plants have smaller leaves at high altitudes [9-11]. These morphological adaptations are presumably associated with decrease in temperature as well as nutrient and water limitations [10,12]. It may also be an adaptive strategy against the hazardous impact of strong wind that

normally grows at high altitude, thereby improving photosynthetic activities of plant [8,13]. The genus *Ajuga* L. consists of about 40-50 species [14] mostly distributed in the north temperate zone of world [15] also from Europe to Asia and Australia [16]. *Ajuga bracteosa* Wall ex Benth. belongs to family Lamiaceae and is commonly called as Jan e adam. It is perennial, erect or ascending hairy herb, often prostrate with oblanceolate or sub-spathulate leaves [17]. Various crude extracts of this plant have shown activities including antidiabetic, antioxidant, antibacterial, diuretic, stimulant, astringent, rheumatism, febrifuge, headache, earache and is also effective in jaundice, malarial fever and hypertension [18,19]. *Ajuga parviflora* is an annual or short lived perennial herb [20]. It has been used as an astringent and for the treatment of swollen wounds, diarrhea, rheumatism, fever, eye trouble and for the diseases of bladder [21]. They also have the cancer chemo-preventive, hypoglycemic and hypotensive effects [22]. The present study was devised for the first time to understand the variation in growth characteristics and changes in allocation patterns in relation to the environmental conditions in two related species *Ajuga bracteosa* and *A. parviflora* growing at different altitudes of Kashmir Himalaya.

## Materials and Methods

### Study sites

*Ajuga bracteosa* was found growing in Kangan, Dachigam, Langanbal, Betab valley-Pahalgam, Gulmarg, Aharbal, Jawahar tunnel, Ferozpora, Drang and Duksum. *A. parviflora* was found growing in Gogaldara-Gulmarg, Aru-Pahalgam, Upper Munda, Jawahar Tunnel, Naranag and Aharbal. Four natural populations (Kangan, Dachigam, Drang and Betab valley-Pahalgam) of *A. bracteosa* and Brinal 1, Gogaldara, and Aru - Pahalgam and Brinal 2 of *A. parviflora* were selected for the present study on the basis of ease of access and availability (Figure 1).

### Morphological characterization

The studies were carried out by selecting ten mature flowering individuals randomly from each population in order to observe the various morphological parameters of the species. The populations were analyzed for morphological traits like root length, branch length, number of branches per plant, number of leaves per plant, basal leaf length and breadth, apical leaf length and breadth, inflorescence number per plant and number of flowers per inflorescence. The plants were measured *in situ* and most of these parameters were recorded at flowering stage in all the populations. We used linear regression analysis to determine the correlation between various morphological parameters across the altitudinal gradient. Principal component analysis (PCA) was carried out to analyze the morphological traits in relation to habitat dynamics and to infer the coherence between different vegetative and reproductive parameters.

### Resource allocation

Ten mature and healthy flowering plants from all the natural populations were harvested for the study of resource partitioning in different parts of a plant. The plants were fragmented into individual parts such as root, branches, leaves and inflorescence.

Using an electronic balance, fresh weight (weighing as fresh) and dry weight (after oven-drying for 48 h at 80°C) of the plants were determined following Kawano and Masuda [23]. Reproductive effort (RE) was calculated from the estimates of dry weight or biomass allocated to reproductive and vegetative structures following Yaqoob and Nawchoo [24].

RE=dry weight of inflorescence (including stems)/total dry weight of the above and below ground parts × 100

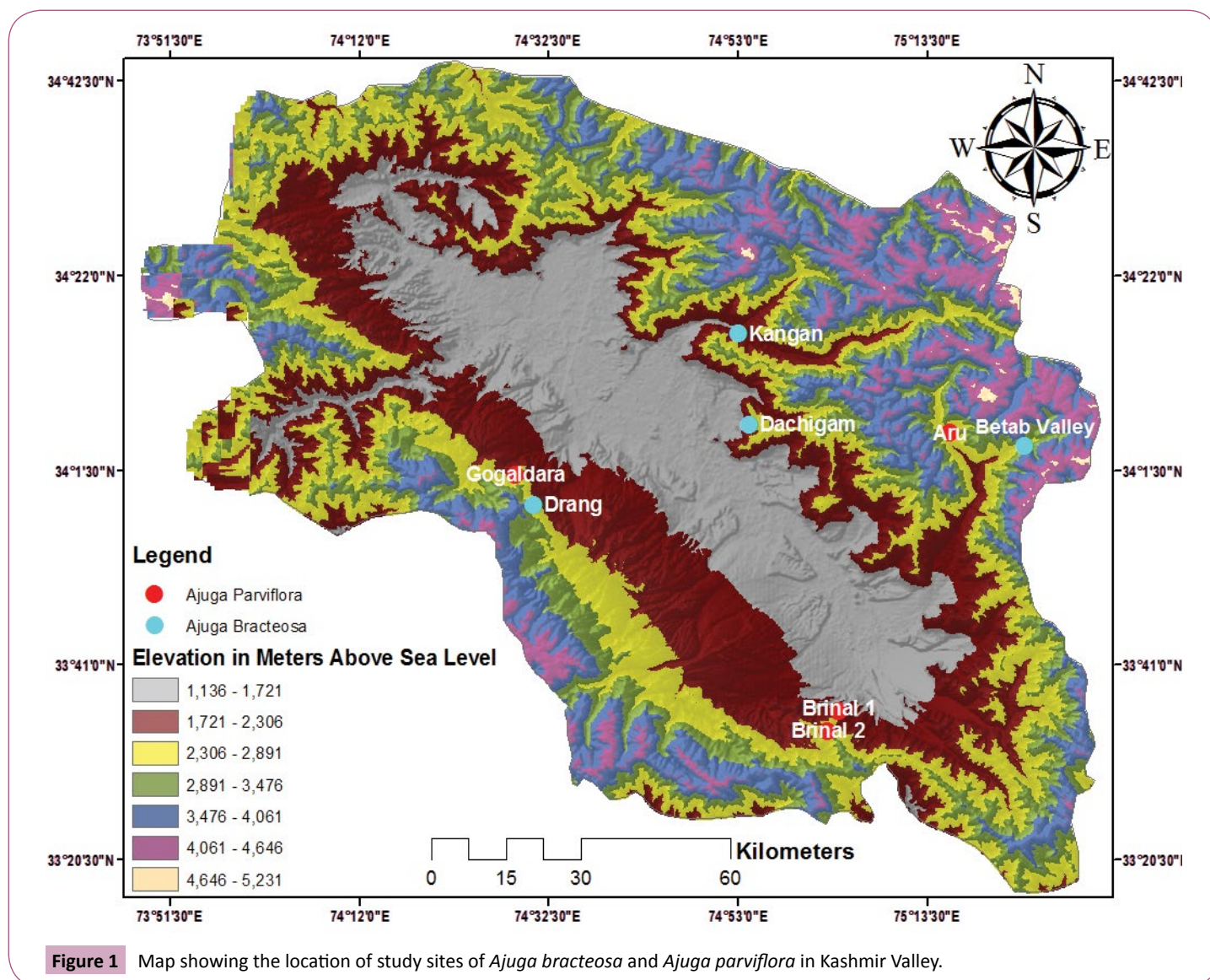
### Data analysis

ANOVA was used to test for differences between populations for all the morphological characters measured using the SPSS 16.0 software. Tukey's multiple comparison of means was used to compare all populations and the differences between individual means were deemed to be significant at  $p \leq 0.05$ .

## Results and Discussion

### Growth characteristics

Present investigation revealed a wide range of suitable habitats for the growth of *A. bracteosa* and *A. parviflora* (Figure 2). *A. bracteosa* thrives best in the open rocky slopes and landslide areas; while as *A. parviflora* prefers moist or rocky shady slopes in association with coniferous forests. *Ajuga bracteosa* is perennial, erect or ascending hairy herb, often prostrate, oblanceolate or sub-spathulate leaves. Flowers are white or purplish-violet tinged from lower surface in distant, axillary whorls in spike. *A. parviflora* is an annual or short-lived perennial. Stems spreading or ascending, usually unbranched, with a sparse to dense eglandular indumentum of long villous multicellular hairs. Leaves may be rosette-forming, obovate-spathulate, petiolate, with multicellular eglandular hairs, thin-texture; inflorescence unbranched verticillaster. It usually flowers between March-June. *Ajuga parviflora* is unusually densely villous in comparison with other species. Both the species exhibits a considerable variability in the morphological traits under different environmental conditions (Tables 1 and 2). Highly significant differences among populations were observed in branch length, branch number per plant, leaf dimensions, leaf number per plant, inflorescence number per plant and flower number per inflorescence. A peculiar variability was found in the branch length of *A. bracteosa* which was highest in plants growing at low altitude sites Dachigam and Kangan ( $12.26 \pm 0.80$  and  $13.47 \pm 0.71$  cm) and lowest in plants growing at Betab valley ( $9.53 \pm 0.89$  cm). Besides, the number of leaves per plant, basal leaf length and inflorescence number per plant were also maximum ( $51.5 \pm 4.60$ ,  $3.91 \pm 0.24$  cm and  $19.6 \pm 1.95$ , respectively) in the plants at low altitude site, Kangan and were minimum ( $31.5 \pm 5.98$ ,  $2.61 \pm 0.37$  cm and  $13.6 \pm 0.96$ ) in the plants at high altitude site, Betab valley. It was found that certain morphological traits like the number of branches per plant and number of flowers per inflorescence showed a different trend; being highest ( $7.6 \pm 1.17$  and  $5.1 \pm 0.73$ ) in plants at high altitude site and lowest ( $4.7 \pm 0.94$  and  $4.8 \pm 0.63$ ) in plants at lowest altitude site. A similar trend was observed in plants of *A. parviflora*, wherein the growth parameters like branch length, root length and number of leaves per plant were maximum ( $13.8 \pm 0.91$  cm;  $11.5 \pm 0.84$  cm and  $24.3 \pm 1.49$  respectively) in plants



**Table 1** Morphological variations (mean  $\pm$  SD) in several plant characters recorded among four different populations of *Ajuga bracteosa* across the study sites.

Plant characteristics	Kangan	Dachigam	Drang	Betab Valley	F value	LSD
Branch length (cm)	13.47 $\pm$ 0.71a*	12.26 $\pm$ 0.80a	10.89 $\pm$ 1.74b	9.53 $\pm$ 0.89c	23.24	0.841
Root length (cm)	9.01 $\pm$ 0.60a	9.16 $\pm$ 0.64a	9.44 $\pm$ 0.71a	9.16 $\pm$ 0.64a	0.76	0.489
No. of branches per plant	4.7 $\pm$ 0.94a	5.2 $\pm$ 0.63a	6.9 $\pm$ 1.19b	7.6 $\pm$ 1.17b	18.35	0.763
No. of leaves per plant	51.5 $\pm$ 4.60a	55.1 $\pm$ 9.59a	35.1 $\pm$ 8.56b	31.5 $\pm$ 5.98b	24.76	5.614
Basal leaf length (cm)	3.91 $\pm$ 0.24a	3.99 $\pm$ 0.21a	3.74 $\pm$ 0.41a	2.61 $\pm$ 0.37b	40.17	0.241
Basal leaf breadth (cm)	1.61 $\pm$ 0.07a	1.6 $\pm$ 0.11a	1.21 $\pm$ 0.19b	1.33 $\pm$ 0.08b	25.64	0.093
Apical leaf length (cm)	1.96 $\pm$ 0.20ab	2.01 $\pm$ 0.16a	1.8 $\pm$ 0.21ab	1.75 $\pm$ 0.13b	4.74	0.136
Apical leaf breadth (cm)	0.345 $\pm$ 0.05a	0.36 $\pm$ 0.07a	0.24 $\pm$ 0.07b	0.25 $\pm$ 0.05b	10.39	0.046
Inflorescence no. per plant	19.6 $\pm$ 1.95a	18.1 $\pm$ 0.99a	12.4 $\pm$ 1.57b	13.6 $\pm$ 0.96b	58.40	1.08
No. of flowers per Inflorescence	4.8 $\pm$ 0.63a	4.4 $\pm$ 0.52a	4.7 $\pm$ 0.67a	5.1 $\pm$ 0.73a	2.0	0.486

growing at low altitude site Brinal 1 and were minimum (10.11  $\pm$  1.96 cm; 9.27  $\pm$  1.21 cm and 20.7  $\pm$  2.45) in the plants growing at high altitude site Brinal 2. The features like the inflorescence number per plant and the number of flowers per inflorescence were however higher (24.5  $\pm$  4.74 and 5.8  $\pm$  0.42) in the plants growing at high altitude as compared to those (21  $\pm$  3.29 and 4.9

$\pm$  0.74) growing at low altitude site. A positive correlation between root length and branch length, branch length and apical leaf length and a negative correlation between number of branches per plant and branch length was observed in both the species (**Figure 3**). Principal component analysis (PCA, **Figure 4**) of all morphological characters across the study sites reveals that the major differences



**Table 2** Morphological variations (mean  $\pm$  SD) in several plant characters recorded among four different populations of *Ajuga parviflora* across the study sites.

Plant characteristics	Brinal 1	Gogaldara	Aru	Brinal 2	F value	LSD
Branch length (cm)	13.8 $\pm$ 0.91a	12.74 $\pm$ 2.47a	11.34 $\pm$ 2.90a	10.11 $\pm$ 1.96b	5.40	1.651
Root length (cm)	11.5 $\pm$ 0.84a	10.45 $\pm$ 1.67abc	9.45 $\pm$ 2.37bc	9.27 $\pm$ 1.21c	3.98	1.227
No. of branches per plant	4.8 $\pm$ 0.91a	5.1 $\pm$ 0.73a	5.2 $\pm$ 0.91a	5.8 $\pm$ 0.91a	2.28	0.66
No. of leaves per plant	24.3 $\pm$ 1.49a	22.6 $\pm$ 3.37ab	22.2 $\pm$ 2.57ab	20.7 $\pm$ 2.45b	3.33	1.929
Basal leaf length (cm)	1.54 $\pm$ 0.27a	5.06 $\pm$ 0.45b	4.12 $\pm$ 1.64b	4.8 $\pm$ 0.38b	33.11	0.666
Basal leaf breadth (cm)	1.14 $\pm$ 0.11a	3.02 $\pm$ 0.29b	2.42 $\pm$ 1.02bc	1.98 $\pm$ 0.48c	18.16	0.442
Apical leaf length (cm)	0.96 $\pm$ 0.11a	1.97 $\pm$ 0.24b	1.3 $\pm$ 0.48a	1.67 $\pm$ 0.19b	22.36	0.221
Apical leaf breadth (cm)	0.7 $\pm$ 0.15a	1.58 $\pm$ 0.11b	1.06 $\pm$ 0.37c	1.44 $\pm$ 0.12b	32.81	0.164
Inflorescence no. per plant	21 $\pm$ 3.29a	23.4 $\pm$ 4.78a	22.7 $\pm$ 2.62a	24.5 $\pm$ 4.74a	1.36	2.994
No. of flowers per Inflorescence	4.9 $\pm$ 0.74a	5.5 $\pm$ 0.71ab	5.0 $\pm$ 0.81ab	5.8 $\pm$ 0.42b	3.81	0.517



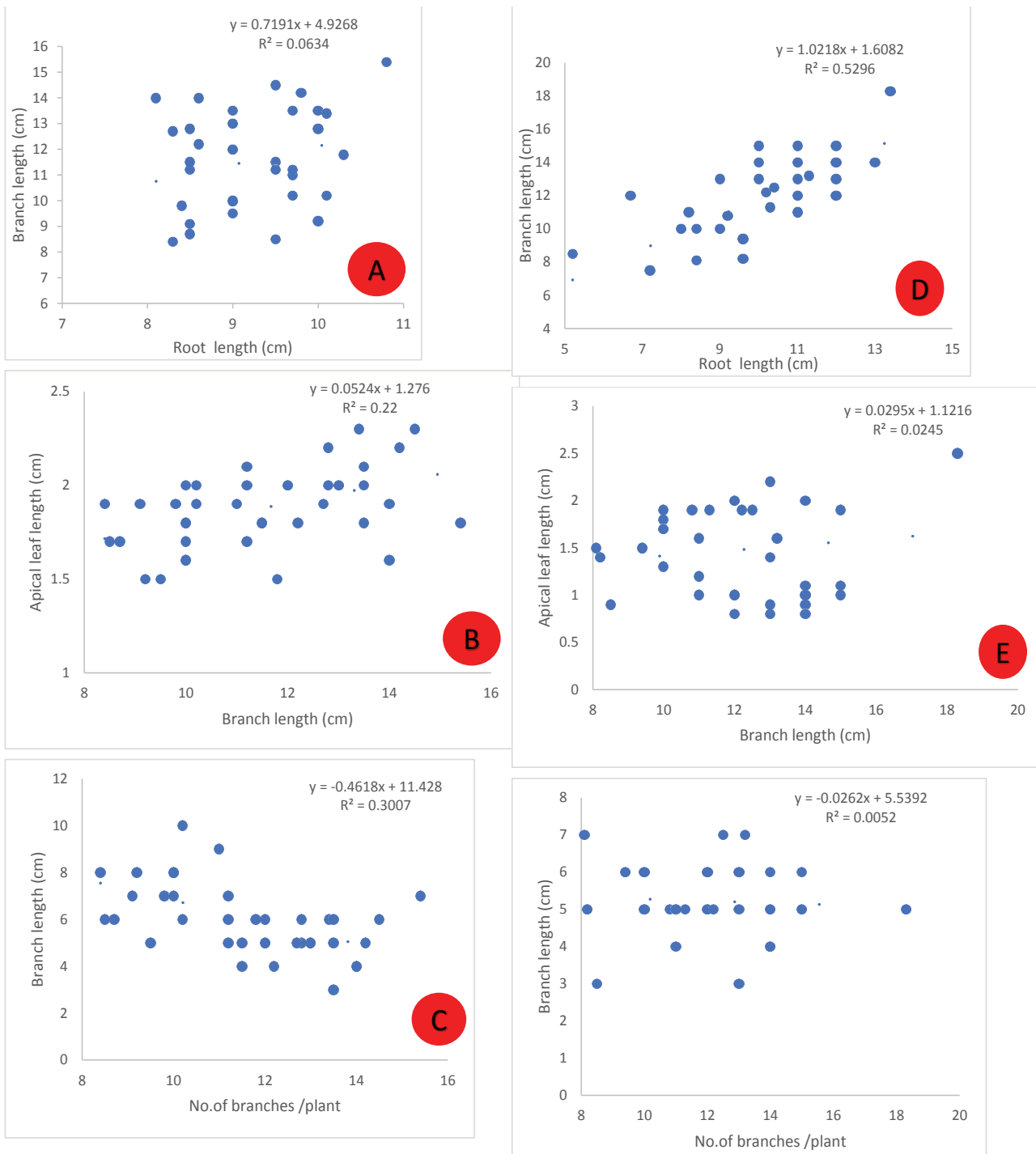
**Figure 2** Different types of habitats of *Ajuga* spp. in Kashmir Valley A. *Ajuga bracteosa* in rocky lands; B. *Ajuga bracteosa* on rocky slope; C. *Ajuga parviflora* near coniferous forests; D. *Ajuga parviflora* in coniferous forest slopes.

between populations were due to size characteristics in both the species. In case of *A. bracteosa*, PCA reveal that the high altitude populations are non-favorable to most of the vegetative and reproductive traits indicating better growth conditions at lower altitudes. In case of *A. parviflora*, a reverse trend was observed. In case of *A. bracteosa*, apical leaf breadth, Inflorescence number per plant and basal leaf breadth were found to be favoring the Kangan population. Basal leaf length, apical leaf length, branch length, number of leaves per plant were found to be favoring the Dachigam population. In case of *A. parviflora*, apical leaf length, apical leaf breadth, number of flowers per Inflorescence, basal leaf length and basal leaf breadth were found to be favoring the Gogaldara population. Inflorescence number per plant and number of branches per plant were found to be favoring the Brinal 2 population. Thus, the habitat of Kangan and Dachigam proved relatively better for the growth of *A. bracteosa* while as the habitat of Gogaldara and Brinal 2 proved relatively better for the growth of *A. parviflora*.

### Reproductive allocation at flowering

The partitioning of resources is non-uniform among different parts

of the plants of *A. bracteosa* (Table 3). There was a remarkable difference in total above ground dry weight biomass and dry weight of different vegetative structures among the plants of studied populations, growing at different altitudes. Maximum resources were allocated towards the growth and development of leaves (0.572  $\pm$  0.12 g to 0.659  $\pm$  0.260 g) followed by root (0.400  $\pm$  0.072 g to 0.514  $\pm$  0.047 g) and branches (0.340  $\pm$  0.041 g to 0.624  $\pm$  0.057 g) while the least resources were allocated towards inflorescence (0.091  $\pm$  0.222 g to 0.208  $\pm$  0.030 g). A somehow similar trend was followed by the plant parts of *A. parviflora* (Table 4). Maximum resources were allocated towards the growth and development of leaves (0.369  $\pm$  0.057 g to 0.521  $\pm$  0.224 g) followed by branches (0.173  $\pm$  0.034 g to 0.437  $\pm$  0.085 g) and root (0.131  $\pm$  0.009 g to 0.413  $\pm$  0.048 g) while the least resources were allocated towards inflorescence (0.087  $\pm$  0.014 g to 0.499  $\pm$  0.130 g). The total resource budget per plant of low altitude and high altitude populations varied to a greater extent in both the species. In case of *A. bracteosa*, the values were maximum in high altitude Betab Valley (1.971  $\pm$  0.389 g) population and were least in the low altitude populations. In case of *A. parviflora* a reverse trend was seen and the values were maximum in the low altitude Brinal 1 (1.829  $\pm$  0.168 g) population and were least in the high altitude populations. Also the percentage allocation to reproductive parts, i.e., reproductive effort showed a similar trend being more (40.48  $\pm$  1.59) for the high altitude Betab Valley population in case of *A. bracteosa* and (51.17  $\pm$  4.01) for the low altitude Brinal 1 population in case of *A. parviflora*. In the present study, considerable phenotypic variability was observed within and across the individuals of different populations of *A. bracteosa* and *A. parviflora* growing along the altitudinal gradient in the Kashmir valley. The morphological characteristics and resource allocation patterns showed both inverse as well as direct correlation with the altitude. Plant height decreased with an increase in altitude, an adaptive phenomenon to protect the plant against the severe conditions at higher altitudes [25,26]. The harsh climatic conditions as well as the shorter growing season results in the overall slow growth rate, which in turn enables the plants to efficiently utilize the available resources [27]. The reduced plant height at higher altitude prevents the damaging effects caused by the strong winds prevalent at high elevations and keeps the leaves closer to warmer soil to enhance its photosynthetic efficiency [13]. However, the decrease in plant height is compensated by a concomitant increase in the



**Figure 3** Regression analysis between several morphological characters A-C. *Ajuga bracteosa* D-F. *Ajuga parviflora*.

number of branches in the plants growing at higher altitudes. The present study revealed that the plants growing at low altitudes are comparatively taller and produce more number of leaves. Our results are supported and confirmed by the observations of Johnson and Cook [28], Hickman [29] and Bresson et al. [30] who have also observed a reduction in length, breadth and area of leaves with an increase in altitude. However, in this study, leaf dimensions show a contrasting trend being greater in low

altitude populations in case of *A. bracteosa* and in high altitude populations in case of *A. parviflora*. The reproductive effort also shows a contrasting trend and in case of *A. bracteosa*, plants growing at higher altitudes had more reproductive effort than the plants growing at lower elevations. These findings are in conformity with Fabbro and Korner [31], Molina-Montenegro et al. [32] and Yaqoob and Nawchoo [33] but in case of *A. parviflora*, a reverse trend was observed.

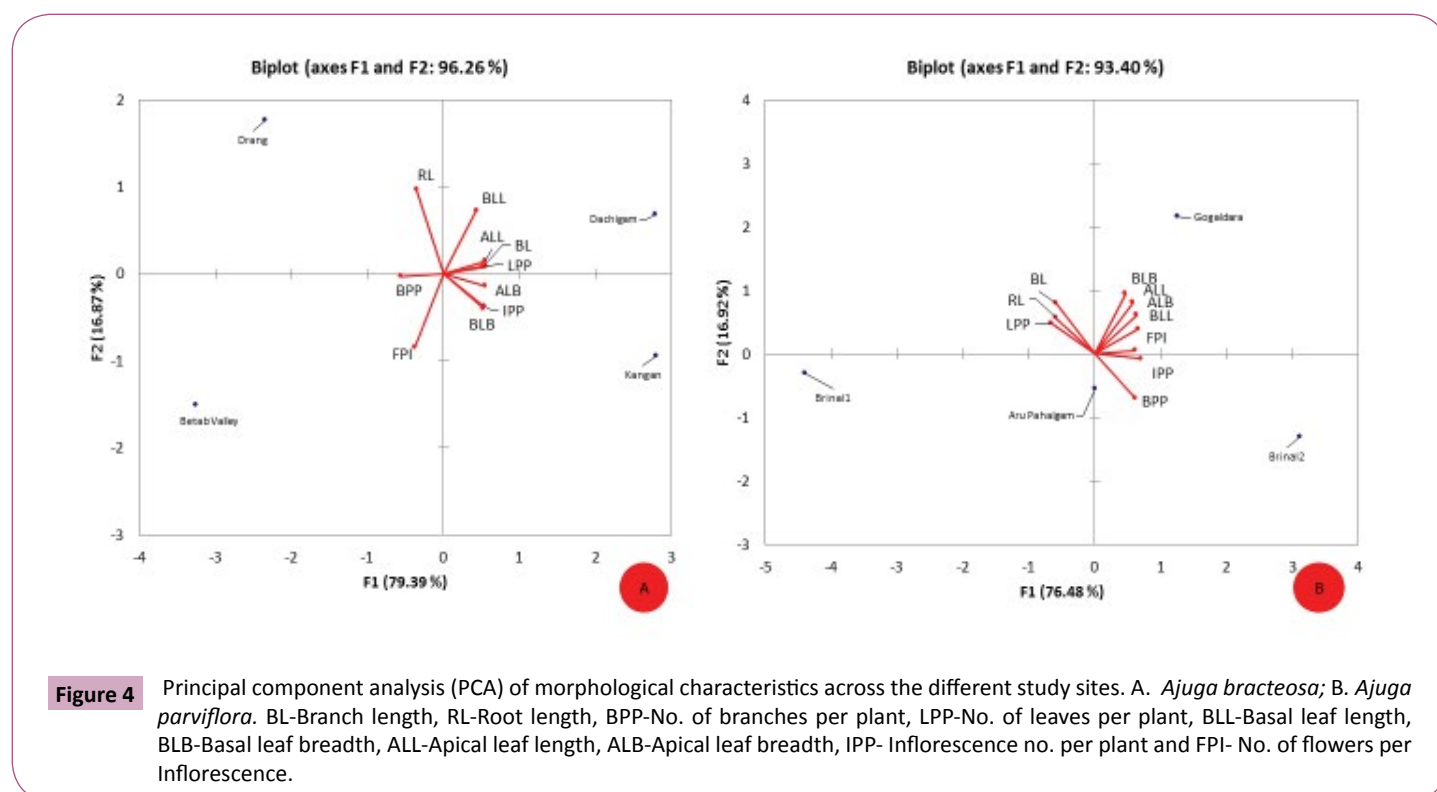
**Table 3** Allocation of resources (mean ± SD) in the form of dry weight (g) toward different plant parts in *Ajuga bracteosa* across the study sites.

Traits	Kangan	Dachigam	Drang	Betab Valley	F value	LSD
Root	0.404 ± 0.059a	0.41 ± 0.045a	0.400 ± 0.072 a	0.514 ± 0.047b	9.36	0.043
Branches	0.345 ± 0.04a	0.340 ± 0.041a	0.476 ± 0.09b	0.624 ± 0.057c	47.70	0.046
Leaves	0.613 ± 0.11a	0.601 ± 0.123a	0.572 ± 0.12a	0.659 ± 0.260a	0.48	0.124
Inflorescence	0.195 ± 0.012a	0.208 ± 0.030a	0.091 ± 0.222b	0.174 ± 0.058a	21.86	0.026
Total resource budget per plant (g)	1.557 ± 0.386a	1.559 ± 0.419a	1.539 ± 0.408a	1.971 ± 0.389a	2.78	0.30
RE	34.68 ± 2.77a	35.15 ± 2.68a	36.84 ± 2.06a	40.48 ± 1.59b	12.73	1.761

**Table 4** Allocation of resources (mean ± SD) in the form of dry weight (g) toward different plant parts in *Ajuga parviflora* across the study sites.

Traits	Brinal 1	Gogaldara	Aru	Brinal 2	F value	LSD
Root	0.372 ± 0.096a	0.131 ± 0.009b	0.332 ± 0.082a	0.413 ± 0.048a	33.66	0.051
Branches	0.437 ± 0.085a	0.173 ± 0.034b	0.336 ± 0.073c	0.362 ± 0.064ac	27.04	0.050
Leaves	0.521 ± 0.224a	0.401 ± 0.024a	0.406 ± 0.232a	0.369 ± 0.057a	1.642	0.124
Inflorescences	0.499 ± 0.130a	0.087 ± 0.014b	0.336 ± 0.09c	0.299 ± 0.037c	42.88	0.061
Total resource budget per plant (g)	1.829 ± 0.168a	0.792 ± 0.164b	1.411 ± 0.303c	1.443 ± 0.281c	32.44	0.180
RE	51.17 ± 4.01a	32.82 ± 2.5b	47.62 ± 2.26c	45.88 ± 1.37c	74.71	2.050

\*Means labeled with the different small letters indicate that they significantly differ from each other among different populations



## Conclusion

We propose that the heterogeneity of the environmental conditions is the main source of phenotypic variation of two *Ajuga* species. Our observation revealed a wide range of suitable habitats for the growth of *A. bracteosa* and *A. parviflora*. With increased altitude, a significant decrease is found in the phenotypic characteristics of *A. bracteosa*, thus it seems that these sites endure a larger environmental stress. But in case of *A. parviflora* a reverse trend was seen. Increasing altitude resulted

in a decrease in the allocation of biomass to reproductive structures in the form of decreasing dry weight. However, future studies should explore the phenotypic plasticity in more detail in plants growing at contrasting environments in order to evaluate precisely the contribution of the phenotypic plasticity to the phenotypic variation in different *Ajuga* species.

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