Indian Journal of Biochemistry & Biophysics
Vol. 60, March 2023, pp. 237-245
DOI: 10.56042/ijbb.v60i3.47166

Variation in morphological, biochemical and antioxidant properties of *Lilium polyphyllum*

Anurag Dhyani[1,4]*, Bhagwati Prasad Nautiyal[2], VK Yadav[3] & Mohan Chandra Nautiyal[4]

[1]Division of Conservation Biology, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Karimancode P.O. Palode, Thiruvananthapuram- 695 562, Kerala, India

[2]VCSG College of Horticulture, Uttarakhand University of Horticulture & Forestry, Bharsar, Pauri Garhwal-246 123, Uttarakhand, India

[3]Department of Botany, Banaras Hindu University, Varanasi-221 005, Uttar Pradesh, India

[4]High Altitude Plant Physiology Research Centre, Srinagar, Pauri Garhwal-246 174, Uttarakhand, India

Received 05 March 2021; *revised 16 February 2023*

Lilium polyphyllum D.Don ex Royle belonging to family Liliaceae is a medicinal plant distributed in temperate to high altitude regions of Himalaya. Its bulbs have been used for anti-aging and vitality properties in >30 formulations of Ayurveda.Therefore, the knowledge on various traits of the plant is necessary so that quality of the finished products could be maintained. Present study investigates, morphological, biochemical and antioxidant properties in different populations of *L. polyphyllum*. A significant variation was recorded in different morphological parameters (Plant height, leaf number, leaf length, leaf width, bulb diameter, bulb fresh weight). Variation was also recorded for total soluble protein (7.81-11.35 mg/g), soluble sugar (96.14-116.14 mg/g), starch (116.37-122.43 mg/g) and total free amino acid (18.22-26.00 mg/g). Antioxidant properties in the bulb were determined and ABTS activity was 4.22 mM /100 g, DPPH activity recorded 0.85 mM/100 g and FRAP activity was 1.50 mM/100 g dw ascorbic acid equivalent, respectively. The diversity in wild populations of the species may serve as a baseline data for future breeding, domestication and conservation program.

Keywords: Altitude, Antioxidant, Conservation, Habitat, Medicinal plant

Morphological and biochemical markers can effectively assess the genetic diversity[1-4]. Morphological traits are primarily indicators of variation and provide clues to genetic diversity. To achieve reliable assessment of variation levels, morphological studies should be supported by biochemical parameters. Studies on medicinal plants have described such an approach[5-7] and all have indicated that variability in morphological and biochemical traits is an interaction between genotype and environment.

Antioxidants naturally found in plants are known to scavenge injurious free radicals from human body and are reported to avert disease expansion by either increasing the human's natural antioxidant defence or by adding with verified dietary antioxidants[8,9]. However, artificial antioxidants have been restricted by judicial rules, due to uncertainties over their toxicity and carcinogenicity[10]. Thus, exploring natural antioxidants is gaining popularity and medicinal plants are viewed as a potent source. Lately, there is an expansion of curiosity in the therapeutic possibilities of medicinal herbs as an antioxidant[11]. Besides famous and conventionally used antioxidants from plants *viz*., wine, tea, vegetables, fruits, and spices, few natural antioxidant (*e.g*., sage and rosemary) have been explored commercially[12,13].

'Astavarga' in Ayurveda is a group of eight plants valued for their medicinal status *viz*., 1) *H. edgeworthii* Hook. f. ex Collet, 2) *Habenaria intermedia* D.Don, 3) *Malaxis acuminata* D.Don, 4) *M. muscifera* (Lindley) Kuntze, 5) *Polygonatum cirrhifolium* Royle, 6) *P. Verticillatum* Allioni, 7) *Fritillaria roylei* H.f., 8) *Lilium polyphyllum* D.Don ex Royle. These plants are recognized for health-boosting properties and to reinforce immune system with cell regeneration capacity[14]. The group is used in more than thirty different Ayurvedic formulations in the form of medicated clarified butter (*Ghritam*), oil (*Taila*) and powder (*Churana*) to treat aphrodisiac, aging, regain youthfulness and arthritis. However recent TMK (traditional medical knowledge) reports more than thirty therapeutic uses (65% sexual problems, 21% physical weakness, 8% strengthen immune system and 6% body ache) in northwest Himalaya, India[14]. Astavarga forms a

*Correspondence:
E-mail: anuragdhyani@gmail.com

chief component of *Chyavanprasha* (a herbal combination) product selling in the global market.

Among others, *Lilium polyphyllum* (family Liliaceae), trade name *Ksirakakoli*, is a bulbous herb, native to the Afghanistan (temperate) and India, Nepal, Pakistan (tropical Asia) of Himalaya. The perennial plant bulb has been reported in therapeutic uses for seminal weakness, haematemesis, rheumatism and intermittent fever[15]. Traditionally, bulbs are shade dried and cooked with potatoes in mustard oil to enhance sexual potency; oil (bulb powder with *Asparagus racemosus*) is used for body pain; bulb powder with Astavarga plants to treat impotency[14]. Due to medicinal properties of *L. polyphyllum*, domestication efforts were conducted in Garhwal Himalaya[16]. Other species of the genus *Lilium* (110 species) has been reported to be used as food and traditional medicines[17,18]. However, *Lilium polyphyllum,* inspite of its importance in traditional healthcare system, detail investigation on its morphological traits, biochemical and antioxidant properties in the samples collected from different populations are lacking, which is an important indicator for determining the diversity. Keeping this in view, here, we attempt to investigate: (i) the variation in morphological, biochemical and antioxidant activity in different populations, and (ii) establish relationship among the different measured parameters. The data generated will form the baseline for future research programme on this species.

Materials and Methods

Plant material and morphological attributes

Field visits were conducted in different parts of Garhwal Himalaya, Uttarakhand, India known for natural populations of *L. polyphyllum* and subsequently three populations were identified for detail investigations. Description of characteristics of sites *i.e*., Dhanaulti, Bhangeli and Gangotri is presented (Table 1). Considering the critically endangered status of species on IUCN Red List[19,20] experiments were designed and conducted by using minimum number of samples to provide optimum information.

Morphological parameters were recorded for ten mature individuals randomly selected from each population. We recorded several parameters of plant morphology, including plant height (PH), leaf number (LN), leaf length (LL), leaf width (LW), seed pod length (SPL), pod diameter (PD), number of seed per pod (NSPP), seed diameter (SD) and seed weight (SW) (n=10 seeds). For bulb morphology, we dug the plants out of the soil, washed the bulbs under running water, and measured various parameters, including bulb length (BL), bulb diameter (BD), number of roots per bulb (NRPB), root length (RL), bulb fresh weight (BFW) and bulb depth in soil (BDIS). We measured bulb length with a scale and bulb diameter with a digital vernier calliper (Mitutoya, Japan). To estimate bulb biomass, we dried the bulbs at 40°C in an oven until they reached a constant weight.

Biochemical analysis

Freshly collected bulbs were brought to the laboratory in an ice box and crushed in liquid nitrogen to make fine powder and stored in ultra-cold freezer (−180°C) for biochemical estimations. The samples were used to estimate variation in different populations for soluble sugars, protein content, and isoenzymes. Soluble sugar and starch were determined using Anthrone method[21-23], total free amino acid and soluble protein[23]. The polypeptide patterns were studied by the SDS-PAGE method as described[24]. Similarly, the soluble protein was extracted by above mentioned method and quantified using the method[23]. Isoenzyme variation was analyzed on 10% polyacrylamide slab gels at a constant current of 20 mA in an electrophoresis apparatus. Esterase, peroxidase and acid phosphatase was detected following the methods[25,26]. Considering the rare status of species, total phenol, flavonoid and antioxidant properties was measured only in the samples of Gangotri and Dhanaulti population.

Extract preparation for total phenol, flavonoid and antioxidant properties

Dried bulb (1 g) was extracted in 100 mL of 80% (*v/v*) methanol in orbital shaker at 22°±1°C for 12 h with non-stop shaking and sonicated at 50 Hz for 10 min. The extract was centrifuged at 8000 rpm for 10 min, supernatant collected and kept at 4°C until use to measure total phenolics, flavonoid and antioxidant activities.

Table 1 —Site characteristics of selected populations of *Lilium polyphyllum*

Population	Altitude (m asl)	Latitude	Longitude	Aspect	Slope (°)	Habitat
Dhanaulti	2200	30°42'N	78°25'E	North east	30	Under canopy of *Cedrus deodara*
Bhangeli	2400	30°91'N	78°68'N	North east	30	Moist habitat
Gangotri	3200	30°99'N	78°94'E	North east	34	Under canopy of *Cedrus deodara*

Quantification of total phenolic contents

Total phenolic content in the methanolic extracts were identified[27] with slight modification[28]. Briefly, 0.25 mL methanolic extract was diluted with 2.25 mL distilled water, followed by the adding 0.25 mL of Folin-Ciocalteu's reagent. The mixture was permitted to stand for 5 min at 22°±1°C. Further, the mixture was neutralised by adding 2.50 mL of 7% (*w/v*) sodium carbonate. This mixture was placed in dark at 22°±1°C for 90 min. Absorbance was then measured at 765 nm with the Hewlett Packard UV-Vis spectrophotometer (Hitachi, Tokyo, Japan). Quantification was completed on the basis of standard curve of gallic acid prepared in 80% methanol (*v/v*) and results were observed in milligrams gallic acid equivalent (GAE) per gram dry weight.

Quantification of total flavonoid contents

Total flavonoid content in the methanolic extract of bulb sample was identified by aluminium chloride calorimetric method with modification[29]. Briefly, 0.50 mL methanolic extract was diluted with 1.50 mL distilled water, followed by the addition of 0.50 mL of 10% (*w/v*) aluminium chloride, 0.10 mL of 1.0 M potassium acetate, and 2.80 mL of distilled water. This mixture was incubated at 22°±1°C for 30 min. Further, the absorbance of mixture (resulting reaction) was recorded at 415 nm UV-Vis spectrophotometer (Hitachi U-2001). Flavonoids quantification was conducted on the basis of standard curve of quercetin prepared in 80% methanol and results were measured in mg quercetin equivalent (QE)/g dw plant material.

Determination of antioxidant activity

The antioxidant activities of the extracts of *Lilium polyphyllum* bulbs was measured by using three different in vitro assays *viz*., ABTS (2,2- Azinobis-3-ethylbenzoth-iazoline-6-sulphonic acid), DPPH (1,1-Diphenyl-2-picryl-hydrazyl radical), and FRAP (Ferric reducing anti-oxidant potential) assays.

The ABTS assay

For the production of ABTS cation ($ABTS^{\cdot+}$), ABTS (7.0 µM) salt and 2.45 µM potassium persulphate was added and placed in dark for 16 h at 23°C. The $ABTS^{\cdot+}$ solution was diluted with 80% (*v/v*) deionized water to obtain an absorbance of 0.70 ± 0.05 at 734 nm. To analyse the sample, 3.90 mL of diluted $ABTS^{\cdot+}$ solution was added to 0.10 mL of methanolic extract. The reaction mixture was carefully mixed and permitted to stand for 6 min (dark, 22°±1°C). Further, absorbance was measured at 734 nm by spectrophotometer. To attain 20-80% reduction in absorbance at 734 nm with respect to blank that was developed with 0.10 mL 80% (*v/v*) methanol, samples were diluted with 80% (*v/v*) methanol. A standard curve of several concentrations of ascorbic acid was developed in 80% (*v/v)* methanol for the equal concentration of antioxidant potential with respect to ascorbic acid. Results were observed in millimole (mM) ascorbic acid equivalent (AAE) per 100 gram dry weight.

The DPPH assay

25 mL of 0.4 mM DPPH reagent was prepared in 80% ethanol (*v/v*) and mixed with 25 mL of 0.2 M 2-(N-morpholino) ethanesulphonic acid (MES) buffer adjusted to pH 6.0 with 1.0 M NaOH, then 25 mL of 20% (*v/v*) ethanol was added. Subsequently, 2.7 mL of this DPPH radical cation preparation was mixed with 0.9 mL of the 80% (*v/v*) methanolic extract of bulb sample and allowed to stand for 20 min at 22°±1°C in the dark. The decline in absorbance at 520 nm was recorded in a U-2001 UV-VIS spectrophotometer (Hitachi). Results were recorded as millimole (mM) ascorbic acid equivalent per 100 gm dry weight.

The FRAP assay

FRAP assay was performed followed by method[30] with minor modification. The stock solutions comprised 300 mM acetate buffer (3.1 g CH_3COONa and 16 mL CH_3OOH), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-*s*-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh solution was developed by adding 25 mL acetate buffer, 2.5 mL TPTZ, and 2.5 mL $FeCl_3 \cdot 6H_2O$ and kept at 37°C. Plant extracts (0.100 mL) allowed to react with 2.9 mL of the FRAP solution for 8 min (dark condition) and measurements were taken at 593 nm. Results expressed as millimole (mM) ascorbic acid equivalent per 100 g dry weight.

Statistical analysis

Morphological parameters were statistically analysed using one-way Analysis of Variance (ANOVA) with Least Significant difference (LSD) at level of significance (5%). The statistical analysis was conducted using SPSS 19.0 program.

Results

Morphological attributes

A significant variation in different morphological traits was recorded among different populations of

L. polyphyllum. Bhangeli population (2400 m asl) had the tallest plants, with a maximum height of 90.40 cm, while the Gangotri population (3200 m asl) had the shortest plants, with a minimum height of 70.70 cm. In terms of seed morphology, plants collected from Gangotri had the longest seed pod length (4.98 cm) compared to all other populations (Table 2). The longest bulb (6.28 cm) was recorded in Bhangeli population (2400 m asl), while the smallest (4.75 cm) was found in Gangotri (3200 m asl) (Table 3). The bulb was located deeper in soil (30.82 cm) in Gangotri compared to Dhanaulti (20.86 cm). A significant variation was found in leaf number, leaf length, leaf width, but no significant variation in seed pod length. Significant variation was also observed in bulb diameter, fresh weight and root number.

Biochemical estimation

Variability in biochemical parameters were also observed in the study, maximum total soluble sugar (116.14 mg/g) and starch content (122.43 mg/g) was found in Gangotri population (3200 m asl). Minimum total soluble protein (7.81 mg/g) reported in Dhanaulti whereas maximum (11.35 mg/g) in Bhangeli. Total free amino acid (26.00 mg/g) was maximum in Bhangeli population (Fig. 1A-1D).

Polypeptide pattern

In *Lilium polyphyllum,* few polypeptide bands were resolved although, band pattern was different in all the populations. The banding pattern revealed that 7 bands appeared in Dhanaulti and Bhangeli population while only 5 bands in Gangotri population. The band intensity was dark in Bhangeli and followed by Dhanaulti population, while poor bands were resolved in Gangotri population (Fig. 2).

Isoenzyme analysis

Peroxidases

Three band were present in Bhangeli population while two bands were observed in Dhanaulti and Gangotri populations. The band intensity was dark in Gangotri followed by Dhanaulti, Bhangeli population (Fig. 3).

Acid phosphatase

Likewise, peroxidase pattern, acid phosphatase band pattern was also similar in all the populations of *L. polyphyllum*. Overall, three bands of different intensity were observed. However, the band intensity was high in Gangotri population, whereas, it appeared poorly in Bhangeli and Dhanaulti populations (Fig. 4).

Esterase

Variation in esterase pattern between the populations was also observed in *L. polyphyllum*. Five bands appear in all the populations. However, all the bands had different band intensity. Comparatively, band intensity was highest in Dhanaulti population followed by Gangotri and Bhangeli populations (Fig. 5).

Total phenol, flavonoid content and antioxidant activity

In bulbs of *L. polyphyllum* total phenol content was estimated 5.45 mg GAE/g dw, total flavonoid content

Table 2 — Plant and seed morphological attributes of *Lilium polyphyllum* individuals in different populations, Uttarakhand

Population	Plant height	Leaf no.	Leaf length	Leaf width	Seed pod length (cm)	Seed pod diameter	Seed/pod	Seed weight (mg) n=10	Seed diameter (mm)
Dhanaulti	78.70±2.03	45.30±4.42	8.35±3.25	1.60±0.32	4.83± 0.10	17.02±0.19	96.00±1.00	186.00±11.53	9.31±0.05
Bhangeli	90.40±5.89	70.90±5.78	10.80±2.70	1.73±0.34	4.84±0.02	16.09±0.20	101.20±2.65	166.80±10.82	8.74±0.16
Gangotri	70.70±1.28	40.00±3.82	7.80±2.23	0.95±0.11	4.98±0.24	17.99±0.20	140.00±9.17	134.60±7.00	7.69±0.21
P value	*P* <0.001	*P* <0.001	*P* <0.05	*P* <0.001	ns	*P* <0.001	*P* <0.01	*P* <0.05	*P* <0.01
LSD at 5%	2.29	2.75	1.53	0.17	0.23	0.24	8.77	15.78	0.21

Source[20,31]

Table 3 —Bulb morphological attributes of *Lilium polyphyllum* individuals in different populations, Uttarakhand

Population	Bulb length	Bulb diameter	Bulb fresh wt.	Bulb biomass	Bulb root length	Bulb root no.
Dhanaulti	5.98±1.92	34.87±3.65	35.61±2.24	12.76±3.88	7.67±0.82	27.33±3.56
Bhangeli	6.28±0.39	39.69±4.52	38.15±3.45	14.30±3.36	7.57±0.76	29.00±2.83
Gangotri	4.75±1.51	23.16±5.34	30.55±2.73	10.61±1.73	7.20±3.75	24.00±2.00
P value	ns	*P* <0.001	*P* <0.001	ns	ns	*P* <0.001
LSD at 5%	0.83	2.72	1.56	1.93	1.28	1.52

Source[20,31]

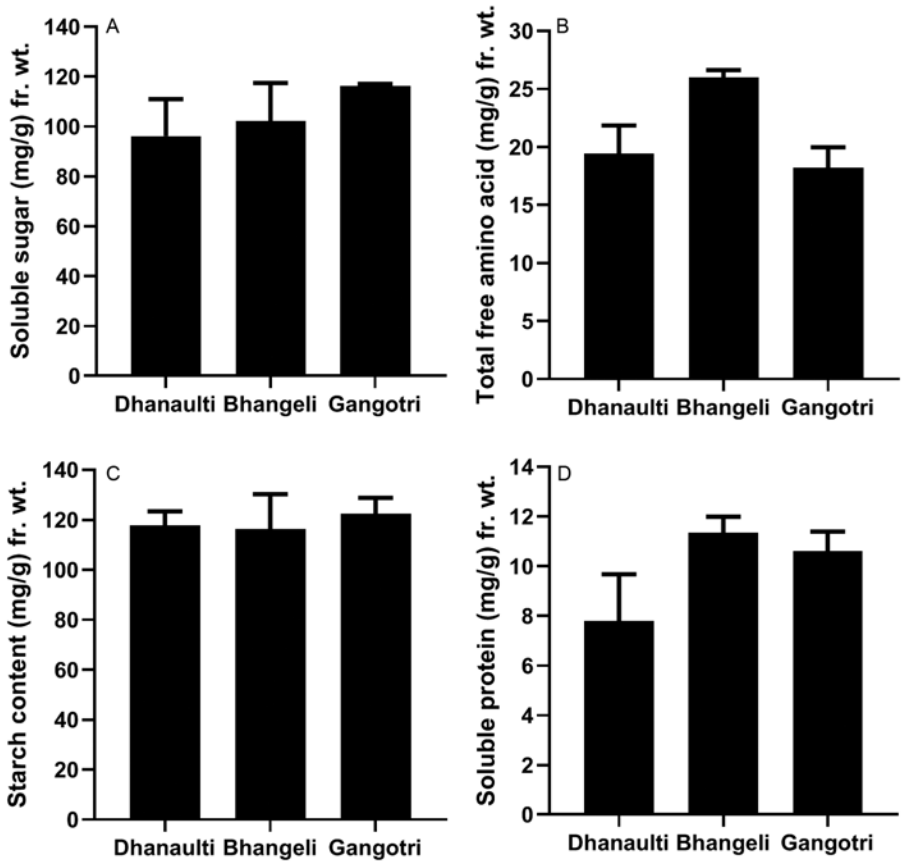

Fig. 1A-D—Biochemical variation in *Lilium polyphyllum* populations, Uttarakhand

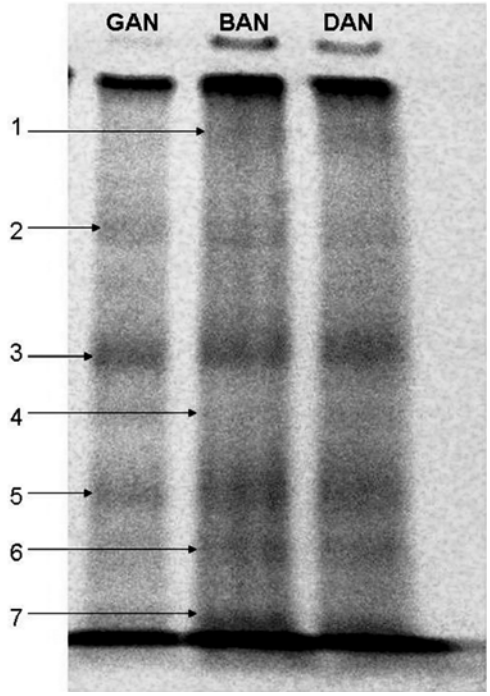

Fig. 2 — Polypeptide pattern in different populations of *L. polyphyllum*

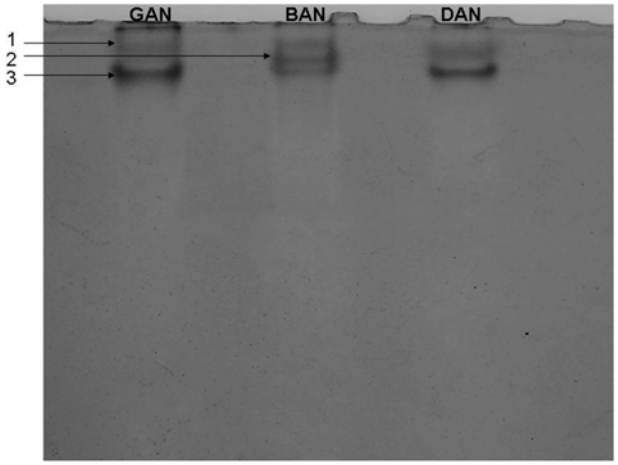

Fig. 3 — Peroxidase activity in different populations of *L. polyphyllum*

was 4.90 mg QE/g dw. ABTS activity was recorded 4.22 mM /100 g, DPPH activity was recorded, 0.85 mM/100 g and FRAP activity was recorded 1.50 mM/100 g dw 1.50 mM/100 g dw ascorbic acid equivalent, respectively, (Table 4).

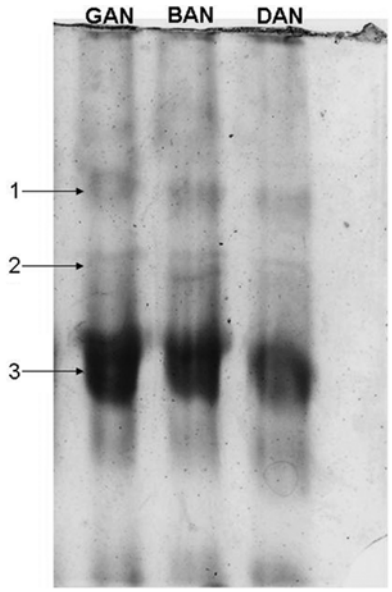

Fig. 4 — Acid phosphatase activity in different populations of *L. polyphyllum*

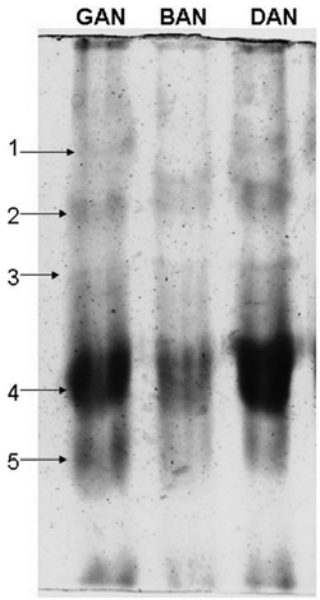

Fig. 5 — Esterase activity in different populations of *L. polyphyllum*

Table 4 — Total phenolics, flavonoids and antioxidant capacity in the bulb of *Lilium* species

Species	Total Phenolic content	Total Flavonoid content	Antioxidant activity			Source
			ABTS	DPPH	CUPRAC/ FRAP*	
L. polyphyllum	5.45 ± 0.06 (mg GAE/g)	4.90 ± 0.12 (mg QE/g)	4.22 ± 0.10 (mM/100 g)	0.85 ± 0.07 (mM/100 g)	1.50 ± 0.09* (mM/100 g)	Present study
L. concolor	3897.60 ± 42.54 c (GAE mg/100g)	413.45 ± 2.03 c (RE mg/100g)	1143.67 ± 11.28 a (TE µmol/100g)	455.31 ± 7.21 d (TE µmol/100g)	1025.14 ± 45.68 b (TE µmol/100g)	
L. davidii var. unicolor	2017.17 ± 140.20 f	150.53 ± 3.66 f	848.49 ± 9.17 b	404.48 ± 14.59 e	595.61 ± 7.24 e	
L. lancifolium	2827.25 ± 55.50 d	227.24 ± 3.66 e	10.75.51 ± 2.94 a	541.27 ± 3.43 b	842.04 ± 8.32 c	[32]
L.leucanthum	2336.00 ± 29.28 e	521.19 ± 17.77 b	889.38 ± 13.42 b	507.64 ± 6.85 c	799.34 ± 5.81 d	
L. pumilum	4177.39 ± 57.19 b	339.13 ± 9.17 d	1091.96 ± 5.70 a	546.51 ± 9.77 b	1044.10 ± 11.30 b	
L. regale	10381.49 ± 49.12 a	1428.21 ± 38.52 a	1173.28 ± 11.41 a	600.33 ± 2.24 a	1438.01 ± 16.56 a	

Discussion

In this study morphological traits of *L. polyphyllum* were studied to understand the variation in wild populations. Morphological traits are an indicator of genetic variability, which may be used to identify superior germplasm. Among the sites, Bhangeli (2400 m asl) population performed best with plant morphology (plant height, leaf length, leaf number, leaf width) and underground bulb parameters (bulb length, diameter, biomass). Considering the medicinal usage of bulbs in Ayurvedic and modern medicines, this population can be considered as an elite source of germplasm for harnessing the higher biomass. As Bhangeli population grows in open site where less competition for resources, while other populations grow under canopy of *Cedrus deodara* (low period of sunlight, more competition for light) indicates that sunlight could be one of the factors influence the morphological traits of the species[20]. Similar habitats and association of the species with *Cedrus deodara* forest have been described earlier from other regions[33,34]. Moreover, studies on *Polygonatum* species[35], *Lilium canadense*[36] and *Picrorhiza kurrooa*[37] also supports that open sites are favourable for obtaining superior morphological traits. Previous studies also reported similar observations on morphology of *Lilium* species *e.g.*, *L. longiflorum, L. formosanum*[38]; *L. lijiangense*[39]; *Lilium canadense*[36]; *L. martagon*[40]. However, fruit and seed morphology, *i.e.* pod length, pod diameter showed moderate variation among three populations of *L. polyphyllum*. Maximum seed weight was due to highest seed moisture content of the seeds in Dhanaulti population. Maximum seeds produced in Gangotri population; it may be a strategy to maintain seed bank in harsh climatic conditions which help to reduce extinction risk

of rare species. However, bulb located deepest in Gangotri compared to other populations might be due to harsh climatic conditions of the region and contractile roots of bulb which anchor and pull the bulb deeper in soil[14].

The carbohydrate and soluble protein content increased with the increase in altitude from Dhanaulti to Gangotri indicating the adaptive significance of these molecules to sustain the harsh climatic conditions at higher altitudes. Similar observation reported in *Polygonum* species[41]. The amino acid content did not follow such trends and highest free amino acid content was observed from Bhangeli, reflecting the variation due to other environmental conditions such as topography, rainfall, temperature, etc. and neglecting the influential role of altitude. This also gives evidence of importance of age and season on the plant.

In the present study, SDS-PAGE of protein in *L. polyphyllum* acclimatized at three different altitudes revealed that high degree of variability was observed in Bhangeli and Dhanaulti populations while low degree of polypeptide variability was observed in Gangotri. In *L. polyphyllum*, heavier and maximum bands were observed in Bhangeli population might be due to open canopy sustaining heavy frost and therefore, adverse conditions existing there, as the certain heat shock protein level enhanced with cold acclimation[42].

Variation in isoenzymes pattern among the three populations of *L. polyphyllum* was noticed in the present study. Although, some shared bands of isoenzymes (peroxidase, esterase, acid phosphatase) were available in three populations, few of the peroxidase, esterase, acid phosphatase bands were population specific. This type of variation in isoenzyme was also recorded among various *Aconitum heterophyllum* populations[43] and two *Angelica* species[44].

Only two common bands among the three populations studied were observed in *L. polyphyllum.* Overall, only three bands were traced. Populations of *L. polyphyllum* showing moderate peroxidase activity suggesting that the species may adapt to a wider range as supported by its distribution from 2,200 m to 3200 m asl[20]. Only one common band of acid phosphatase was observed among populations of *L. polyphyllum*. Variation in the band pattern of acid phosphatase might be due to variation in altitudes as acid phosphatase reportedly rises during heat hardening and an adaptive implication of this enlarged thermo stability has also been recorded in *Angelica* species[44]. A number of esterase bands were observed in *L. polyphyllum* reflecting the genetic diversions due to different populations at different altitudes. Also, the variation showed by isoenzymes through band intensity in diverse populations may be an adaptive character of the populations in different habitats[43,44]. Isoenzyme analysis and polypeptide patterns has been very valuable in conservation of genetic diversity and crop improvement. The degree to which genetic diversity permit a species to endure or to acclimatise climatic variations depends on a positive association among natural and adaptive genetic variation.

The present study revealed that the extract of *L. polyphyllum* contains phenols and flavonoid compounds and show the antioxidant activity. This validated the use of the species as an ingredient of Astavarga component which are known for its vitality strengthening properties. *L. polyphyllum* scavenging property might be due to the presence of hydroxyl group in the phenolic compounds. It is a chemical structure which delivers the essential component as a radical scavenger. The compounds that hold antioxidant activity may impede mutation and cancer because they induce antioxidant enzymes or scavenge free radicals. Antioxidant activities have reported in *Lilium* species[32]and several medicinal plants[2,45-49].

Conclusion

The present study revealed significant difference in morphology, biochemical parameters and confirmed antioxidant property of *L. polyphyllum* bulbs. The range of variation in quantitative traits can be leveraged for initiating breeding, domestication and conservation program in the Himalayan region. The study can also help to identify promising populations, suitable habitat conditions, and altitudes that may be utilized for selecting high-quality plant material of *Lilium polyphyllum*. The antioxidant properties of the species suggest that the consumption of the plant in various formulations can promote vitality and have anti-aging effects. Bhangeli population can be an ideal germplasm for mass multiplication and market demand of the target species. These findings can be further helpful for sustainable utilization and developing agro-technological practices in diverse environmental conditions.

Acknowledgement

This work was supported by Govind Ballabh Pant Institute of Himalayan Environment and Development (GBP/IERP/UA/04-05/12/305), Almora, Uttarakhand,

India. Authors are thankful to Director HAPPRC for facilities. First author thanks Dr. I.D. Bhatt for help in antioxidant analysis and to Dr. Yateesh Mohan Bahuguna for field assistance.

Conflict of interest

All authors declare no conflict of interest.

References

1 Giri L, Jugran AK, Bahukhandi A, Dhyani P, Bhatt ID, Rawal RS, Nandi SK & Dhar U, Population genetic structure and marker trait associations using morphological, phytochemical and molecular parameters in *Habenaria edgeworthii*- a threatened medicinal orchid of west Himalaya, India. *App Biochem Biotech*, (2016) DOI: 10.1007/s12010-016-2211-8.

2 Suyal R, Rawat S, Rawal RS & Bhatt ID, Variability in morphology, phytochemicals, and antioxidants in *Polygonatum verticillatum* (L.) All. populations under different altitudes and habitat conditions in Western Himalayas, India. *Environ Monit Assess*, 191 (2019) 783.

3 Baisakh B, Tripathy SK, Souframanien J, Swain D & Preeti T, Revealing genetic variation in mini core germplasm of urdbean (*Vigna mungo* (L.) Hepper). *Indian J Biochem Biophys*, 58 (2021) 91.

4 Yadav VK, Jha RK & Kaushik P, Biochemical composition of potato biowaste of popular north Indian cultivars. *Indian J Biochem Biophys*, 58 (2021) 100.

5 Jugran AK, Bhatt ID, Rawal RS, Nandi SK & Pande V, Patterns of morphological and genetic diversity of *Valeriana jatamansi* Jones in different habitats and altitudinal range of west Himalaya, India. *Flora,* 208 (2013)13.

6 Yousefzadeh S, Daryai F, Bidgoli AM, Hazrati S, Yousefzadeh T & Mohammadi K, Morphological, essential oil and biochemical variation of *Dracocephalum moldavica* L. populations. *J App Res Med Arom Plants,* 10 (2018) 59.

7 Jenifer DR, Malathy BR &Ariya SS, *In vitro* and *in silico* studies on the biochemistry and anti-cancer activity of phytochemicals from *Plumbago zeylanica*. *Indian J Biochem Biophys*, 53 (2021) 272.

8 Stanner SA, Hughes J, Kelly CN & Buttris JA, Review of the epidemiological evidence for the antioxidant hypothesis. *Public Health Nutr,* 7 (2000) 407.

9 Yadav VK & Kaushik P, Phytochemicals against COVID-19 and gap in clinical investigations: an outlook. *Indian J Biochem Biophys*, 58 (2021) 403.

10 Hao PP, Ni JR, Sun WL & Huang W, Determination of tertiary butylhydroquinone in edible vegetable oil by liquid chromatography/ion trap mass spectrometry. *Food Chem,* 105 (2007) 1732.

11 Huo J, Zhao Z, Hua Z, Fan J, Du J & Guo B, Evaluation of *Juglans regia* L., root for wound healing *via* antioxidant, antimicrobial and anti-inflammatory activity. *Indian J Biochem Biophys*, 57 (2020) 304.

12 Schuler P, Natural antioxidants exploited commercially, In food antioxidants. Hudson BJE (ed.). Elsevier, London, (1990) 99.

13 GaneshpukarA & Saluja A, The pharmacological potential of catechin. *Indian J Biochem Biophys*, 57 (2020) 501.

14 Dhyani A, Nautiyal BP & Nautiyal MC, Importance of Astavarga plants in traditional systems of medicine in Garhwal, Indian Himalaya. *Int J Biodivers Sci Ecosyst Services Manag,* 6 (2010) 13.

15 Warrier PK, Nambiar VPK & Ramankutty C, Indian medicinal plants. a compendium of 500 medicinal plants. Arya Vaidya Sala. Orient Longman (1997).

16 Dhyani A, Nautiyal BP & Nautiyal MC, Seedling performance, phenotypic traits and growing media for domestication of *Lilium polyphyllum*. *Plants People Planet,* 4 (2022) 685.

17 Chau CF & Wu SH, The development of regulation of Chinese herbal medicine for both medicinal and food uses. *Trends Food Sci Tech,* 17 (2006) 313.

18 You X, Xie C, Liu K & Gu Z, Isolation of non-starch polysaccharides from bulb of tiger lily (*Lilium lancifolium* Thumb) with fermentation of *Saccharomyces cerevisiae*. *Carbohyd Polym,* 81 (2010) 35.

19 Saha D, Ved D, Ravikumar K, Haridasan K &Dhyani A, *Lilium polyphyllum*. The IUCN Red List of Threatened Species (2015): e T50126623A79918170.

20 Dhyani A, Nautiyal BP & Nautiyal MC, Distribution, status and conservation of *Lilium polyphyllum* (Liliaceae), a critically endangered medicinal plant from India. *Plant Biosyst,* 152 (2018) 608.

21 McCready RM, Guggolz J, Silviera V & Owen HS, Determination of starch and amylase in vegetables. *Anal Chem,* 22 (1950) 1156.

22 Moore S & Stein WH, A modified ninhydrin method for use in the chromatography of amino acids. *J Biol Chem,* 176 (1954) 367.

23 Bradford MM, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Ann Bioche,* 72 (1976) 248.

24 Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* 227 (1970) 680.

25 Bhadula SK &Sawhney VK, Esterase activity and isozymes during the ontogeny of stamens of male fertile *Lycopersicon esculentum* Mill., a male sterile stamenless -2 mutant and low temperature – reverted mutant. *Plant Sci,* 52 (1987) 187.

26 Welter LR, Isoenzyme analysis of cultured plant cells, In: *Plant Tissue Culture Methods.* (L. R. Welter and F. Constabeleds). IVth R. C. Canada. (1982) 105.

27 Singleton VL, Orthofer R & Lamuela-Raventos RM, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol,* 299 (1999) 152.

28 Rawat S, Bhatt ID & Rawal RS, Total phenolic compounds and antioxidant potential of *Hedychium spicatum* Buch. Ham. Ex D.Don in West Himalaya, India. *J Food Compos Anal,* 24 (2011) 574.

29 Bhatt ID, Dauthal P, Rawat S, Gaira KS, Jugran A, Rawal RS & Dhar U, Characterization of essential oil composition, phenolics content and antioxidant properties in wild and planted individuals of *Valeriana jatamansi* Jones. *Sci Hortic,* 136 (2012) 61.

30 Benzie IFF & Strain JJ, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem,* 239 (1996) 70.

31 Dhyani A, Nautiyal BP & Nautiyal MC, Age determination of the perennial herb *Lilium polyphyllum* (Liliaceae). *Nord J Bot*, 30 (2012) 503.

32 Jin L, Zhang Y, Yan L, Guo Y & Niu L, Phenolic compounds and antioxidant activity of bulb extracts of six *Lilium* species native to China. *Molecules,* 17 (2012) 9361.

33 Samant SS, Pant S, Singh M, Lal M, Singh A, Sharma A &

Bhandari S, Medicinal plants in Himachal Pradesh, north western Himalaya, India. *Int J Biodiver Sci Manag,* 3 (2007) 234.

34 Singh A, Lal M & Samant SS, Diversity, indigenous uses and conservation prioritization of medicinal plants in Lahaul valley, proposed cold desert biosphere reserve, India. *Int J Biod Sci Man,* 5 (2009) 132.

35 Hasegawa T & Kudo G, Comparisons of growth schedule, reproductive property and allocation pattern among three rhizomatous *Polygonatum* species with reference to their habitat types. *Plant Spec Biol,* 20(2005) 23.

36 Nemmer J, *Lilium canadense*. *The Lily Yearbook of North American Lily Society,* 60 (2007) 29.

37 Purohit H., Nautiyal BP & Nautiyal MC, Interpopulation variation in *Picrorhiza kurrooa* Royle ex Benth- Step towards identifying genetic variability and elite strains for crop improvement study. *Am J Plant Physiol,* 3 (2008) 154.

38 Hiramatsu M, Okubo H, Yoshimura K, Huang KL & Huang CW, Biogeography and origin of *Lilium longiflorum* and *L. formosanum* intra and interspecific variation in stem, leaf morphology, flowering rate and individual net production during the first year seedling growth. *Acta Hort,* 570 (2002) 331.

39 Lykkegaard J, *Lilium lijiangense*. *The Lily Yearbook of North American Lily Society* 58 (2005) 96.

40 Balode A, Diversity assessment of *L. martagon* in Latvia and applications in breeding. *The lily yearbook of North American Lily Society,* 60 (2007) 39.

41 Prakash V, Prasad P & Nautiyal MC, Changes in biochemical attributes during acclimatization of *Polygonum* species at different altitudes in Garhwal Himalaya. *Indian J Plant Physiol,* 6 (2001) 244.

42 Neven LG, Haskell DW, Guy CL, Denslow N, Klein PA, Green LG & Silverman A, Association of 70-kilodalton heat-shock cognate proteins with acclimation to cold. *Plant Physiol,* 99 (1992) 1362.

43 Bahuguna R, Purohit MC, Nautiyal BP, Prakash V, Nautiyal MC & Purohit AN, Polypeptide patterns and isoenzymes in different populations of *Aconitum heterophyllum* Wall ex Royle, from Garhwal Himalaya. *Physiol Mol Biol Plants,* 9 (2003) 95.

44 Vashistha RK, Prasad P, Nautiyal BP & Nautiyal MC, Biochemical and electrophoretic variability at population level in two *Angelica* species from Garhwal Himalaya. *Indian J Plant Physiol,* 12 (2007) 246.

45 Joshi K, Adhikari P, Bhatt ID & Pande V, Source dependent variation in phenolics, antioxidant and antimicrobial activity of *Paeonia emodi* in west Himalaya, India. *Physiol Mol Biol Plants*, 28 (2022) 1785.

46 Sharma M, Kumar DA & Prashant K, Phytochemistry and pharmacology of *Calotropis gigantea*- an update. *Indian J Biochem Biophys*, 59 (2022) 611.

47 Mankar GD, Gulave AB, Datkhile KD, Zimare SB, Altitudinal gradients influence the accumulation of pharmaceutically important phenolic compounds in the leaves of *Lobelia nicotianifolia* Roth. and regulates its antioxidant and anticancer property. *Indian J Biochem Biophys*, 58 (2021) 253.

48 Semerci AD, Incecayir D, Konca T, Tunca H & Tunc K, Phenolic constituents, antioxidant and antimicrobial activities of methanolic extracts of some female cones of gymnosperm plant. *Indian J Biochem Biophys*, 57 (2020) 298.

49 Sharaf S, Chinchu JU, Rahitha Devi SJ & Kumar BP, Identification of bioactive compounds with anti-inflammatory potential in the methanolic fruit extract of *Areca catechu* L. (Palmaceae). *Indian J Biochem Biophys*, 58 (2021) 464.