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Morphological studies and meiotic chromosome analysis of *Epimedium elatum* (Morr & Decne)- Rare endemic medicinal plant of the Northwestern Himalayas in India

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ABSTRACT

Epimedium elatum (Berberidaceae) is a rare endemic medicinal herb of the Northwestern Himalayas in India. Recent ethnopharmacological reports have demonstrated its traditional medicinal use against various bone-related diseases in the Kashmir Himalayas. It owes its pharmaceutical importance due to high concentration of flavonoid glycosides like Epimedin A, B, C and Icariin which are known mainly for aphrodisiac, antiosteoporosis, anticancer, antioxidant, antiaging, antifatigue, and antiviral activities. It is a neglected medicinal plant in the Northwestern Himalayan region and may fall in the list of endangered species due to continuous anthropogenic pressures in its native habitats. In this study, we investigated distributional and altitudinal range of this prized species from 20 diverse eco-geographical zones of Kashmir Himalayas for the first time. We also report here its diversity in morphological attributes both in wild and captive cultivation. The species has a very small population size in most of the surveyed habitats with no natural protection. Under cultivation it showed increased plant height (63.09 ± 4.9 cm), more number of leaves (95.53 ± 11 cm) and flowers (160.76 ± 20 cm), indicating importance of high altitude medicinal garden for its immediate *ex situ* conservation. Further, the acetocarmine staining and squashing of young anthers confirmed it as a diploid species ($2n = 12$) like other *Epimedium* species. Chromosome number and meiotic abnormalities are also reported for the first time in the species. Finally, constant anthropogenic pressures in the Northwestern Himalayas demand immediate *in situ* and *ex situ* conservation programs for *E. elatum*.

KEY WORDS: Accession, conservation, chromosomes, *Epimedium elatum*, Kashmir Himalayas

INTRODUCTION

Epimedium elatum (Berberidaceae) is a genus with more than 65 accepted species (The Plant List 2013). They are mostly perennial species with heart shaped leaves and majority of them have 4-parted "spider-like" flowers in spring season (Ma *et al.*, 2011). They are distributed in North Temperate Zones in China, Japan, Korea, Europe and some African countries (Zhang *et al.*, 2016). Medicinally, they are known as Herba Epimedii, a famous botanical supplement prepared from 5 *Epimedium* species, viz., *Epimedium brevicornum*, *Epimedium sagittatum*, *Epimedium koreanum*, *Epimedium pubescens,* and *Epimedium wushanense* (Ma *et al.*, 2011). Epimedin-A, Epimedin-B, Epimedin-C, and Icariin are the major chemical constituents in almost all *Epimedium* species (Chen *et al.*, 2015). They are known to possess a number of pharmacological actions such as aphrodisiac (phosphodiesterase-5 inhibition), antiosteoporosis, antioxidation, antitumor, and antiaging activities (Ma *et al.*, 2011).

E. elatum (Morren and Decne) is a sparsely branched, low growing, perennial, and deciduous medicinal herb, which reaches a height of about 100 cm in shady habitats in Kashmir Himalayas (www.srgc.net). According to recent reports, *E. elatum* is the only medicinal species in

Epimedium genus which is exclusively endemic to high altitude shady coniferous forests of the Northwestern Himalayas in India and Pakistan (www.gbif.org; Nasir and Ali 2005; Perveen and Qaiser 2010; Tantry *et al.*, 2012; Naseer *et al.*, 2015, Arief *et al.*, 2015, 2016; Lone *et al.*, 2016). It has several local common names such as Saul Sumbal, Chhal Kambli, and "mosquito herb" and its extract is sprayed as rodent and mosquito repellent in some Himalayan communities (Arief *et al.*, 2015). Traditionally, *E. elatum* has been used to treat cold, cough, running nose, toothache, tooth-decay, diseases of bones and joints (Arief *et al.*, 2015). Phytochemically, *E. elatum* contains high concentration of Epimedin A, Epimedin B, Epimedin C, Icariin, Icariside-Iand same has been confirmed by its recent bio prospection in Kashmir Himalayas (Sofi *et al.*, 2014; Naseer *et al.*, 2015; Arief *et al.*, 2015, 2016).

Phenotypic plasticity in plants is defined as the ability of a single genotype to develop multiple phenotypes under different eco-edaphic environmental conditions (Palacio-Lopez *et al.*, 2015). High altitude medicinal plants are known to show a significant phenotypic plasticity in the Northwestern Himalayas (Badola and Aitken 2003). Epimedium species too are known to show phenotypic plasticity both in wild and cultivated conditions (Quan *et al.*, 2011; Xu *et al.*, 2013b; Xu *et al.*, 2014; Chen *et al.*, 2015b; Pan and Guo 2016; Xuemei *et al.*, 2016). It indicates the existence of different phenotypes and genotypes in Epimedium species under different environmental conditions. In our earlier study, we have reported its diversity at genetic, phytochemical, and biochemical level. It shows wide phenotypic plasticity according to the habitat conditions. Under open sunny conditions, it shows "dwarf habit" whereas in shady conditions, it shows robust growth with maximum herbage, favorable for the plant (Lone *et al.*, 2016). However, several medicinal plants have also become threatened in the Northwestern Himalayas due to various anthropogenic threats (Tali *et al.*, 2015). *E. elatum* too can become threatened in near future if timely conservation measures are not taken. In this regard, conserving germplasm of *E. elatum* was highly important. There is need to study this medicinal plant in detail from various botanical aspects such as phenotypic plasticity, local distributional, and altitudinal pattern in Kashmir Himalayas and basic chromosome number. These classical efforts will help to generate more research interests toward this neglected medicinal plant species in Kashmir Himalayas. This medicinal plant could be explored further for its nutraceutical and pharmacological properties in coming decades due to its high content of prenylated flavonoids.

MATERIALS AND METHODS

Study Area

This study was mainly done in the Kashmir Valley in the Northwestern Himalayan biogeographic zone, situated on the northern edge of India. It extends from 33°.20′ and 34°.54′ N latitudes and from 73°.55′ and 75°.35′ E longitudes covering an area of 15,948 km^2. Topographically, it is a deep elliptical bowl-shaped valley surrounded by mountains of the Pir Panjal in the South and Southwest and by the Greater Himalayan range in the North and East (Tali *et al.*, 2015). The areas surveyed included 20 eco-geographical zones from Pir Panjal ranges to Gurez valley of higher Himalayas in Kashmir, India (Table 1).

Eco-geographical Survey and Diversity in Morphology

An eco-geographic survey was undertaken within Kashmir Himalayan forests for determining the distributional and altitudinal range of *E. elatum*. Various floras (Singh and Kachroo., 1987; Sharma and Jamwal, 1998), herbarium records, research articles and internet research tools like Google scholar, Scopus, etc.) were consulted for working out its distribution in Kashmir Himalayas. The information collected was used to plan the time table and routes for field studies. The study area was thoroughly surveyed for ensuring collection of the required material. Several parameters such as altitude, latitude, longitude, including morphological parameters such as plant height (cm), branch and leaf number, leaf length and breadth, petiole length, inflorescence length, flower length and breadth, etc., were recorded. Same measurements were done on randomly selected mature individuals from each accession. Depending on availability, suitable numbers of specimens were collected, and their field information was recorded under a specific collection number. The plant specimens were dried and preserved via standard taxonomic methods. They were identified and subsequently at Centre of Plant - Taxonomy, University of Kashmir and Janaki Ammal Herbarium (Figure 1), Indian Institute of Integrative Medicine, Jammu and Kashmir, India.

Transplantation at Experimental Farms

E. elatum with immature rhizome buds were sampled at random from mountain forests of Gulmarg (2700 m) and Aharbal (2500 m) during early winter. They were transplanted under shade at two experimental field stations at Yarikhah (Tangmarg) and Sanatnagar (Srinagar), respectively. Forest mulch brought from wild was subsequently mixed with soil to mimic natural habitats during transplantation. Morphological measurements were made between 2013 and 2015. All parameters on

Table 1: Accession numbers, geographic location, and habitat status of the 20 studied populations of *Epimedium elatum* in Kashmir Himalayas

Accessions	Locality/District	Codes*	Vouch[1]	Vouch[2]	Alt.asl[3]	Latitude	Longitude	Habitat status
Gulmarg	Baramulla	GL	2094	22304	2725	34.02°	74.22°	Nature reserve
Babareshi	Tangmarg Baramulla	BR	2099	22306	2694	34.03°	74.23°	Nature reserve
Drang	Tangmarg Baramulla	DR	2105	22305	2301	33.55°	74.29°	No protection
Dangarpora	Sheeri Baramulla	DG	2103	22308	2592	34.05°	74.32°	No protection
Boniyar	Uri Baramulla	BY	2107	22307	2148	34.15°	74.21°	Nature reserve
Yusmarg	Budgam	YS	2101	22310	2383	33.49°	74.40°	No protection
Dodipathri	Budgam	DP	2095	22309	2432	33.53°	74.34°	Nature reserve
Naranag	Kangan Ganderbal	NAR	2102	22303	2272	34.21°	74.58°	Nature reserve
Gagangir	Kangan Ganderbal	GG	2109	22314	2435	34.17°	75.12°	No protection
Dachigam	Harwan Srinagar	DGM	2100	22312	2912	34.08°	75.02°	Nature reserve
Pahalgam	Anantnag	PGM	2093	22319	2206	34.00°	75.18°	No protection
Kokernag	Anantnag	KNG	2092	22311	2343	33.34°	75.17°	No protection
Verinag	Anantnag	VNG	2108	22320	1935	33.32°	75.14°	Nature reserve
Khillanmarg	Gulmarg Baramulla	KMG	2104	22315	3133	34.02°	74.21°	Nature reserve
Chaknala	Gurez Bandipora	CNG	2097	22316	2508	34.37°	74.51°	No protection
Sheikhpora	Tilayl Gurez Bandipora	SPG	2096	22317	2646	34.35°	74.59°	No protection
Kanzalwan	Gurez Bandipora	KZG	2098	22318	2431	34.38°	74.42°	Nature reserve
Badwan	Gurez Bandipora	BDG	2106	22321	2521	33.39°	74.46°	Nature reserve
Hirpora	Shopian	HP	2090	22322	1818	33.39°	74.57°	No protection
Aharbal	Kulgam	AB	2091	22313	2425	33.38°	74.74°	Nature reserve

*Accession codes of *Epimedium elatum*. [1,2]Voucher specimens deposited at Kashmir University Herbarium (Srinagar) and Janaki Ammal herbarium (IIIM Jammu). [3]Altitude is given in meters above sea level

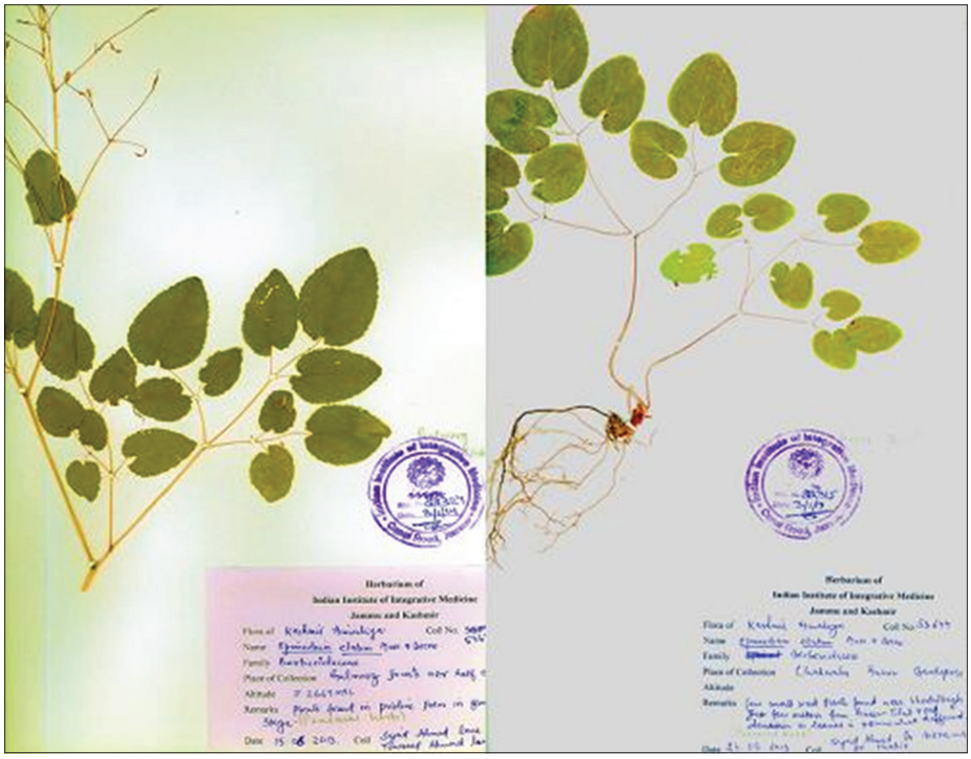

Figure 1: Taxonomic identification of *Epimedium elatum* with characteristic "three branches and nine leaved morphology"

morphology were statistically analyzed for mean and standard error.

Meiotic Chromosome Analysis

For meiotic studies, young and immature flower buds were collected randomly from wild eco-zones (Drang and Gulmarg) and also from plants cultivated at experimental field stations in months of April and May 2014. Plants having ideal buds were selected at different times of the day from early morning to ensure accuracy in getting perfect meiotic stages. They were fixed in freshly prepared Carnoy's fixative (ethanol/chloroform/

acetic acid - 6:3:1, v/v/v) for 24 h, washed thrice with 70% ethanol to remove the fixative and stored at 4°C until use. The meiotic studies were carried out on young flower buds using standard acetocarmine smear technique. For slide preparation the anthers were squashed in 1% acetocarmine and slides were studied for chromosome counts, and meiotic behavior in pollen mother cells (PMCs) at early prophase-I, diakinesis, metaphase-I (MI), anaphases-I/II (AI/II), telophases.

I/II (TI/II) and sporad stages, respectively. All meiotic phases were evaluated and abnormalities if any were recorded. The gametic chromosome number was determined in at least 10 PMCs at diakinesis from all four populations (two wild and two cultivated). In populations with abnormal meiotic course, a total of 30-50 PMCs were examined.

Photomicrographs

The PMCs with perfect stages (for chromosome counts, meiotic abnormalities, and sporads) were initially observed with light microscope and later under Carl Zeis Microscope supported by Labomed digital camera. Photomicrographs reported in this study were taken via ×100 magnification lens using immersion oil for better resolution.

RESULTS

Phenotypic Plasticity in Wild Habitats

During exhaustive eco-geographical surveys, 20 accessions of this species were collected from Kashmir Himalayas, India (Figure 2). Small population size and localized distribution in specific pockets reflect its critically rare status in Kashmir Himalayas. It showed wide phenotypic plasticity in all 20 accessions, collected from diverse eco-geographical zones. The highest plant height (100-110 cm), more number of branches and leaves were found in Gulmarg, Verinag, Naranag, and Aharbal accessions while minimum values (12-18 cm) were recorded for Kokernag, Pahalgam, Yusmarg, and Gagangir accessions (Figure 3). Moreover, cool-moist environment, deep shade of bushes, thickets and shrubs was found to favor tall habit of this species in naturally protected habitats (Table 2), whereas open sunny environment was found to favor dwarf phenomenon, as reported by Quan *et al*. 2011 in Epimedium species. It is considered to be the tallest (4 feet) Epimedium species (www.srgc.net) and unique eco-edaphic environment of the Northwestern Himalayas could be the reason for this phenotype plasticity. This is the first report wherein the morphology and distribution of this species have been studies in diverse habitats in Kashmir valley, India.

Morphological Variability under Cultivation

E. elatum transplanted at two field stations were measured for diversity in their phenotypic plasticity. Plants at Yarikhah farm (Tangmarg) were better adapted as showed increased plant height (63.09 ± 4.9 cm), maximum number of leaves (95.53 ± 11 cm) and flowers (160.76 ± 20 cm) per plant (Table 3). Because of the high relative light intensity at Srinagar farm, dwarf

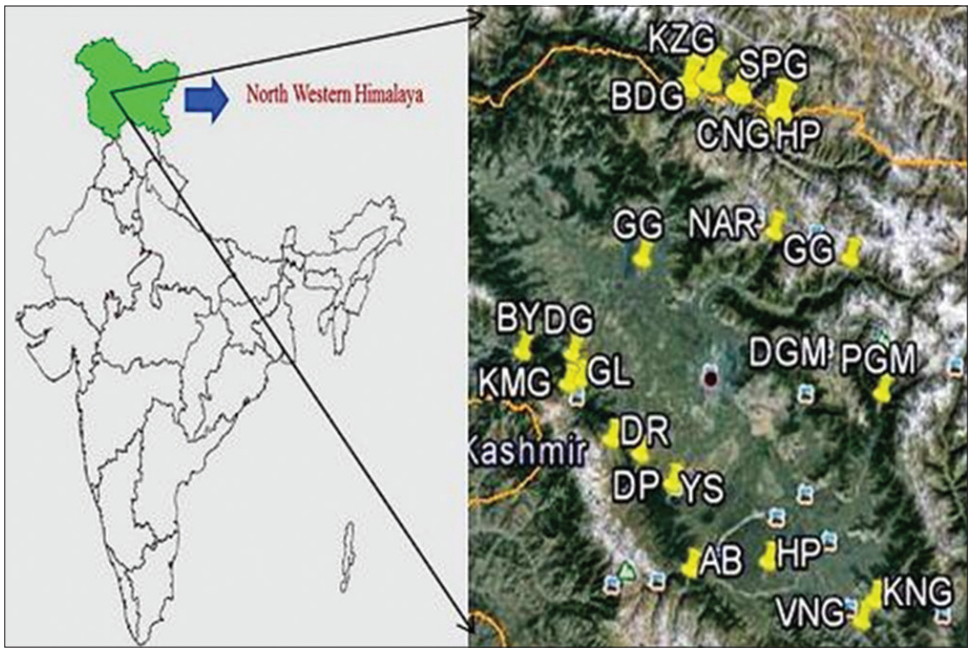

Figure 2: Geographical map showing the geographical distribution of 20 accessions of *Epimedium elatum* in Kashmir Himalaya, J & K, India (Table 1 for accession codes)

Table 2: Morphological comparison of different accessions of *E. elatum* recorded during eco-geographical surveys

Acc codes[a]	Plant height (cm)	Branches/plant	Leaves/branch	Petiole length (cm)	Leaf size (cm) L/B	Inflorescen. length (cm)	Flower size (cm) L/B	Pod length (cm)	Habit (Herb)
GL	62.2-110	16-22	18–54	2.5-3.8	4.5-7.0/4.0-4.8	18-29	0.9-1.0/0.8-1.0	2.2-3.3	Tallest
BR	48-77	12-18	18-45	1.9-2.6	5.1-8.5/4.0-4.7	11-21	0.8-1.0/0.9-1.0	1.8-2.9	Tall
DR	28-58	6-12	18-36	1.5-3.0	4.1-5.5/3.1-4.2	9.0-12	0.9-1.0/0.8-0.9	2.1-3.0	Medium
DG	32.1-48	3-06	12	1.1-1.3	4.8-5.3/4.1-4.3	12.1-16.1	0.8-0.9/0.8-0.9	1.9-2.0	Small
BY	49.2-101.2	15-24	18-36	1.4-1.9	5.1-6.4/4.0-4.7	12-17	0.8-0.9/0.7-1.0	1.2-1.7	Tall
YS	20-39.2	3-5	9-11	0.9-1.3	2.4-4.1/2.0-2.8	7.5-9.1	0.8-0.9/0.6-0.9	1.1-1.9	Small
DP	42-86	13-16	18-36	1.1-1.8	5.0-6.6/4.0-6.3	15-18.1	0.8-0.9/0.8-0.9	2.9-3.3	Tall
NAR	86.0-101.2	16-20	27-55	2.1-2.9	5.8-6.3/4.0-4.7	19-26	NA	2.0-2.3	Tallest
GG	18.1-42.1	6	14-13	0.9-1.2	4.0-4.9/2.3-2.9	12.1-16.1	0.9-1.1/0.8-0.9	1.9-2.0	Small
DGM	85.3-102.3	13-29	27-36	1.3-1.5	7.3-7.4/4.9-5.1	18.3-25	0.8-1.0/0.9-1.0	2.1-2.7	Tallest
PGM	18.1-42.8	6-12	9-12	1.1-1.2	4.2-5.6/2.9-3.8	12.1-16.1	0.8-0.9/0.8-0.9	1.9-2.0	Small
KNG	12-23.4	4-08	12-16	1.1	4.0-4.8/2.8-2.9	NA	NA	NA	Small
VNG	48-76	16-17	36-65	1.0-1.4	5.8-6.4/4.0-4.8	9.8-16.1	0.7-1.0/0.9-1.0	1.9-2.9	Tall
KMG	21.1-29	4	9–16	1.1-1.3	3.2-3.9/2.1-2.9	NA	NA	NA	Small
CNG	28.1-50.2	8-18	16-36	1.1-1.3	3.2-5.6/2.3-2.9	NA	NA	1.9-2.0	Small
SPG	48.1-58.1	14-21	27-36	1.1-1.3	4.7-5.2/3.1-3.8	NA	NA	NA	Medium
KZG	28.3-70.3	13-19	26-56	1.1	4.0-5.2/3.1-3.5	NA	NA	NA	Medium
BDG	78.8-100.1	18-21	18–63	1.1-1.3	5.1-5.7/4.1-4.8	NA	NA	NA	Tall
HP	15-25.3	5-15	9-12	1.1-1.3	4.1-4.2/2.2-2.4	NA	NA	NA	Small
AB	95-110	23-32	45-95	2.4-3.5	5.8-8.2/5.8-6.1	29-39	0.8-1/0.9-1.0	3.1-3.9	Tallest

[a]Accession codes of *Epimedium elatum* collected from wild habitats of Kashmir Himalayas, India (Table 1 for details of 20 accessions).
E. elatum: Epimedium elatum, NA: Not available

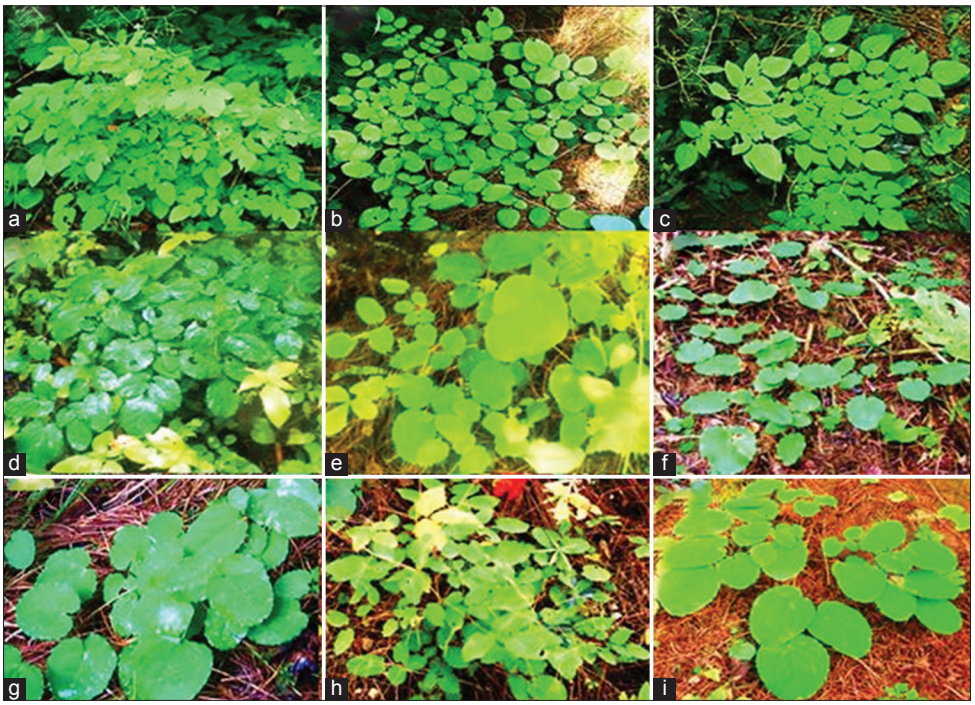

Figure 3: Representative populations of *Epimedium elatum* growing in wild (eco-geographical zones) habitats of Kashmir Himalayas in India. (a) Aharbal, (b) Verinag, (c) Naranag, (d) Gulmarg, (e) Boniyar, (f) Khillanmarg, (g) Pahalgam, (h) Sheikhpora, (i) Chaknala. Note the tall and small habit of the same plant species under different habitat conditions indicating phenotypic plasticity prevalent in this species

phenomenon occurred in *E. elatum*. This was observed in wild habitats where partial shade and sunny conditions were found to induce dwarfness in *E. elatum*. Similar observations were made previously in *E. pubescens* and *E. wushanense,* in both wild and cultivation (Quan *et al.*, 2011). According to specimen examination, field investigation, and observations of material in cultivation, *E. elatum* is a small flowered taxon (Figure 4). The botanical description of this medicinal plant is given in Table 4. It prefers cool shady environment for acclimatization even at low altitude conditions. The phenology of the plant was recorded, and it was found to flower differently in wild and cultivated conditions. At low altitude habitats like Dachigam National Park (1600 m) it was observed to flower in the month of

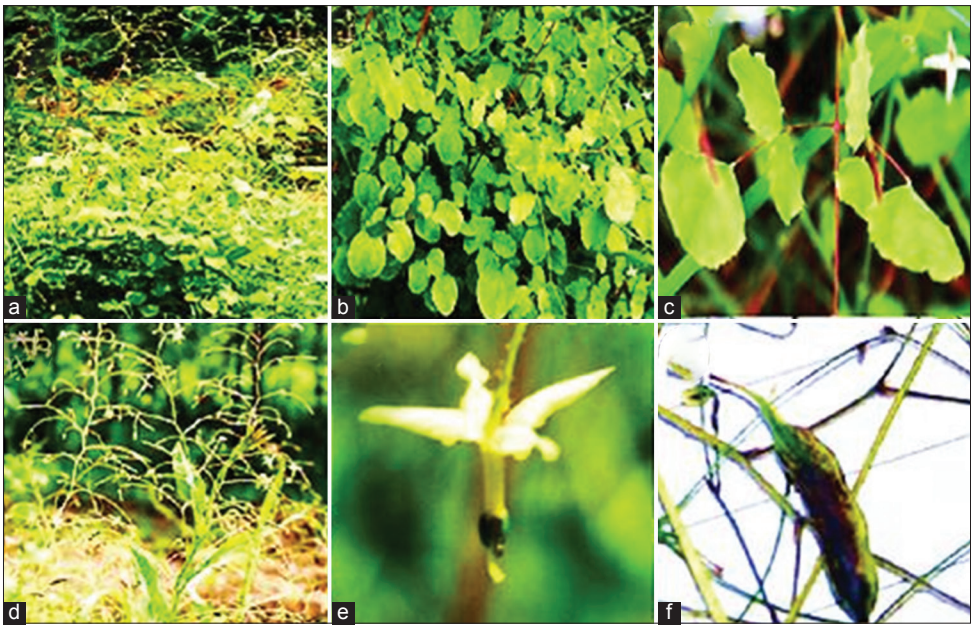

Figure 4: Morphological variability of transplanted *Epimedium elatum* at IIIM Yarikhah farm. (a and b) Whole plant along with inflorescence, (c) typical branch showing "three leaflets" arising from a node, (d) inflorescence with flowers and pods, (e) a typical flower, (f) pod with seeds

Table 3: Quantitative morphological parameters of *E. elatum* under cultivation

Morphological parameters	Mean±SE	
	IIIM Srinagar farm	IIIM Yarikhah farm
Plant height (cm)	42.82±3.0	63.09±4.9
Leaf length	3.73±0.32	4.56±0.16
Leaf breadth	3.13±0.04	3.16±0.4
Petiole length	1.18±0.13	1.98±0.93
Internode length	7.59±0.23	10.39±1.8
No of branches/plant	04.45±2.4	60.53±11
No of leaves/branch	9.87±0.04	27.07±1.8
No of flowers/plant	6.65±0.72	160.76±20
Flower length	1.13±0.04	1.03±0.94
Flower breadth	0.86±0.04	0.89±0.00
Inflorescence length	13.69±1.1	28.53±8.54

E. elatum: Epimedium elatum, SE: Standard error

April to May whereas at high altitudes like Khillanmarg (2800-3100 m) this plant was observed to flower in the months of June-July due to altitudinal and temperature differences.

Meiotic Chromosome Analysis

In this meiotic investigation, chromosome number of *E. elatum* has been confirmed to be 2n = 12 including a brief outline of its meiotic behavior. At prophase I, the chromosomes and homologous pairing was prominent with apparent crossing over and chaismata formation (Figure 5a). Six discrete bivalents, linked by chaismata were visible at diplotene and diakinesis, respectively, confirming diploid chromosome number of *E. elatum* (Figure 5b and c). The bivalents were characterized by interstitial and terminal chaismata. Besides, ring-shaped bivalents were observed at diakinesis (Figure 5d and e). As meiosis advanced into metaphase I, the highly condensed bivalents became aligned on the meiotic spindle and clustered in the equatorial plate of the cell (Figure 5f and g). Some of the prominent meiotic abnormalities observed at meiosis I were interbivalent connections, multivalent formation of unoriented bivalents, chromosomal bridges, laggards (Figure 6h), chromosome stickiness (Figure 5i-k). Telophase I was found be normal in most of the pollen mother cells (Figure 5l). However, it is surprising to see meiotic abnormalities both in wild as well as in cultivated populations of this plant. Some of the prominent abnormalities observed during meiosis II were interbivalent connections (Figure 6a and b) and ring bivalents (Figure 6c and d) at metaphase II. Anaphase II showed many abnormalities like interchromosomal connections and chromosomal connections (Figure 6e) and congested chromosomes (Figure 6f,k,l), interbivalent connections (Figure 6g and k), scattered chromosomes at three poles (Figure 6h), interbivalent connection and abnormal segregation (Figure 6i,j,m). Telophase II is shown from Figure 6n-p with normal configuration.

DISCUSSION

Epimedium species grow on mountain cliffs under moist forests, near streams, and wet lands at altitudes ranging from 200 to 3700 m asl (Ying and Chen, 2001). Similar types of habitat preferences have been observed for *E. elatum*, an endemic monotypic taxon in Kashmir Himalayas. Endemic taxa of an area are most important because of their restricted

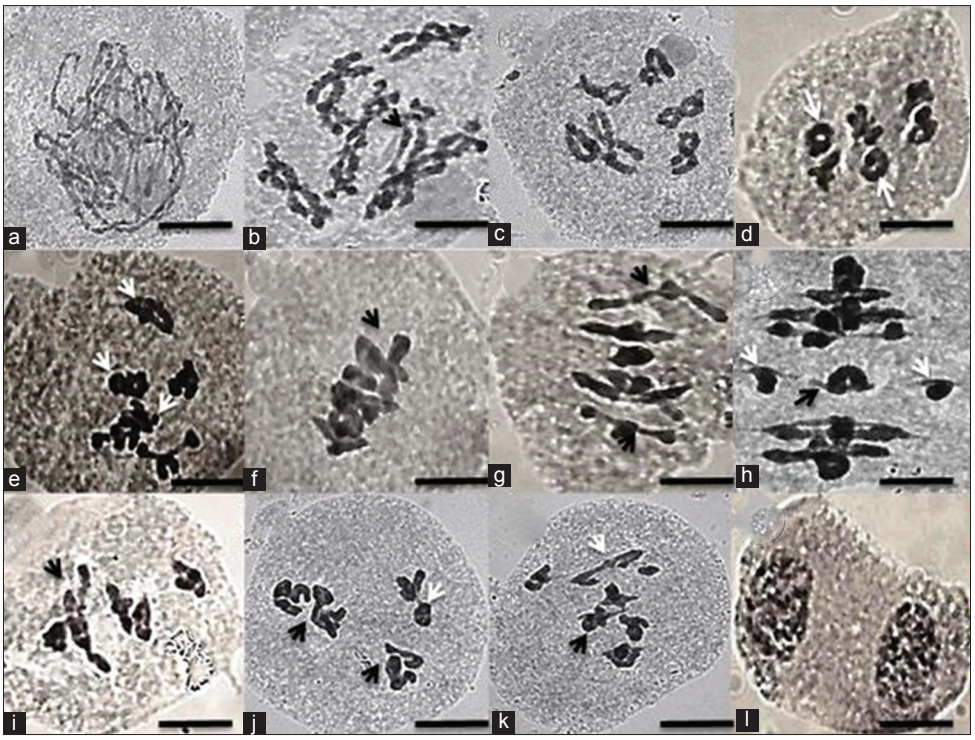

Figure 5: Different stages of meiosis I in *Epimedium elatum* with clear *n* = 6 and *2n* = 12 chromosomes and few meiotic abnormalities. (a) Prophase I, (b and c) Diakinesis with prominent pairing, crossing over and chiasmata formation, (d) Diakinesis with sticky chromosomes and ring shaped bivalents (white arrow), (e-g) metaphase I with few abnormalities like chromatin stickiness (white arrows in E), (h) metaphase I with few univalents (white arrows), (i) metaphase I with chromatin stickiness, (j and k) metaphase I showing chromatin stickiness (white arrows) (l) telophase I (Bar-10 µm)

Table 4: Botanical description of *E. elatum*

Plant part	Botanical description of characters
Synonyms	*Epimedium hydaspidis* Falc.
Habitat	Temperate perennial herb growing at altitudes from 1800-3300 m
Rootstock	Consists of perennial creeping and scaly underground rhizome with thick growth of adventitious roots
Stem	Glabrous, solitary, base squamate and branches are pale straw colored
Leaves	Ternately compound, each pinnae with 3 leaflets (three bran, petioluled, ovate-cordate, symmetrical or oblique, Terminal leaves larger than the lateral ones, subserrately toothed with spinulosus tips, submembranous, apex obtuse to rounded; petiolules shorter than the blade, thin, slightly swollen at base
Inflorescence	Panicle irregularly branched, glabrous to glandular-hairy
Flowers	Bracteates, pedicellate, complete, hermaphrodite, actinomorphic, (2) merous, hypogynous, small pale yellowish or yellowish white in color, linear-lanceolate. Floral nectaries present, Pollination entomophilous Flowering period: Early April at IIIM Srinagar and Mid-April to May at IIIM Yarikhah Fruiting period: Mid-April to May at IIIM Srinagar and Late April to May at IIIM Yarikhah
Sepals	Polysepalous, sepals ovate-lanceolate and acute
Petals	Polypetalous, petals lanceolate-elliptic with innermost honey bearing pair shorter and shoe-shaped
Androecium	Stamens 4 in number, opposite to petals. Anthers linear half as long as filaments, opening by 2 upcurved valves, crowded together
Gynoecium	Ovary simple, solitary, linear, multicarpellary (ovules 5-15), style slender, persistent, stigma dilated
Fruit	Capsules 5-10 mm long, 2-3 mm broad, usually 2-3-seeded, submembranous, with the dorsal suture continued by a style 3-5 mm long
Seed	Seeds biseriate, small (2 mm in diameter), reniform, black

E. elatum: Epimedium elatum

distribution to a particular region and they are not found anywhere else in the world (Ali 2008). The low population size and a single distribution on a small scale might trigger their extinction. Hence, endemic species deserve immediate attention (Alam and Ali, 2010). *E. elatum* thus needs immediate action from conservationists, botanists, and concerned authorities involved in medicinal plant research in the Northwestern Himalayas.

The plant species showed wide morphological variation in most of the surveyed habitats. Variation in morphological characters in a species might be due to differences in ecotypes, which are often indicative of genetic differences (Clausen *et al.*, 1948). Therefore, further various studies are needed to screen different ecotypes/chemotypes/genotypes of this species from the Northwestern Himalayas for its early germplasm characterization and conservation.

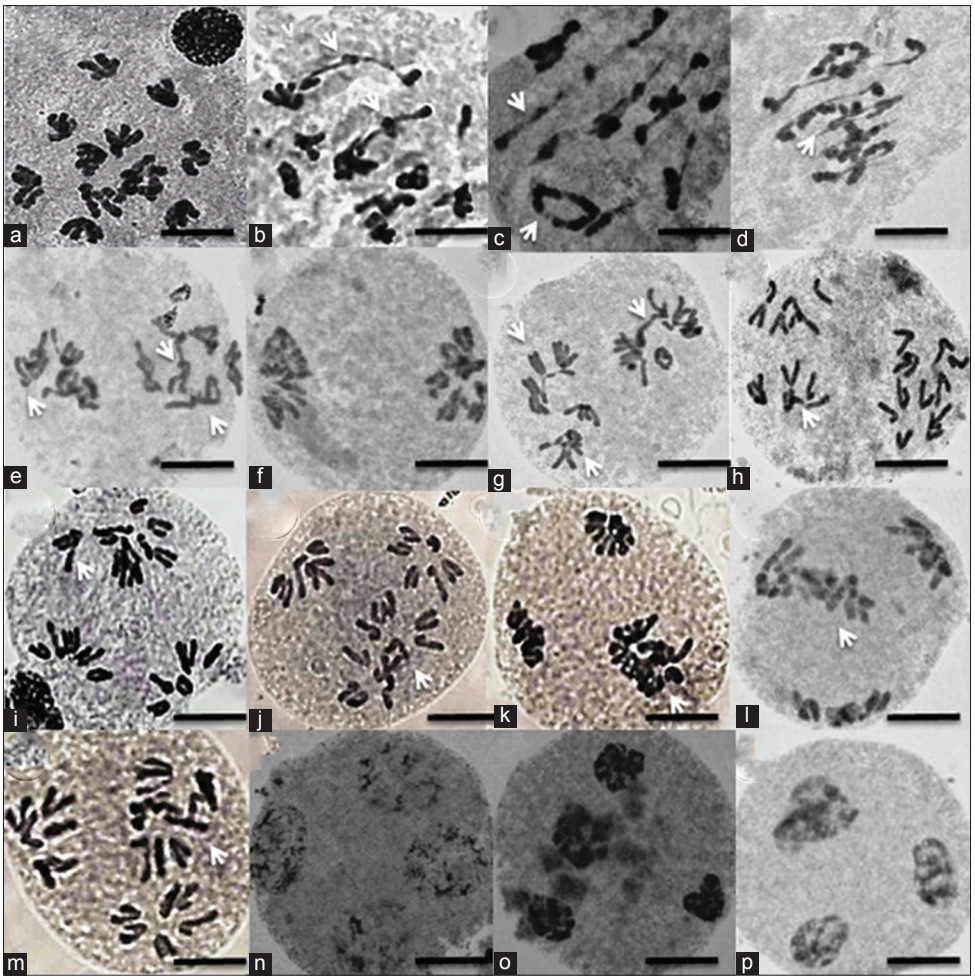

Figure 6: Various meiotic abnormalities observed during cytological characterization of *Epimedium elatum.* (a) Metaphase II, (b) metaphase II with interbivalent connections. (c and d) Metaphase II with ring bivalents and interbivalent connections (white arrows). (e) Anaphase I with abnormalities like interchromosomal connections (white arrows). (f) Normal anaphase I, (g) anaphase I with inter bivalent connections (white arrows). (h) Anaphase II with mixing of chromosomes at one pole. (I and j) Anaphase II. (k) Anaphase II with chromosome stickiness (l and m) anaphase II (n-p) telophase II (Bar-10 µm)

Phenotypic variation can enable a plant species to change its growth pattern as it encounters different stresses (Guo *et al.*, 2007). These resulting selection pressures will vary across environmental conditions and can differently favor genotypic characters and affect phenotypic traits in different populations (Petit and Thompson., 1998). Usually, a minimum population size is required for the long-term viability of rare species and the latter occupy small geographic ranges and specific habitats (Cunningham *et al.*, 2001; Rabinowitz, 1981; Samant *et al.*, 1996; Mills and Schwartz., 2005; Ricketts *et al.*, 2005). The captive cultivation of *E. elatum* proved successful in establishing its germplasm at high altitude medicinal garden. Shade of trees and shrubs including cool moist environment under cultivated conditions seems to be the favorable habitat for its large scale propagation. Domestication of medicinal plants is a sustainable alternative and offers the prospect to solve the problem of genetic resource erosion. Hence, by bringing *E. elatum* into cultivation, conventional and biotechnological techniques can be applied to improve its pharmaceutical properties for commercial purposes. Moreover, many other research activities can be conducted in future for highlighting medicinal potential of this species in Kashmir Himalayas, India.

Most of the Epimedium species have been reported to be diploid with $2n = 12$ except *E.yingjiangense* which is a tetraploid species with $2n = 4\times = 24$ (Sheng *et al.*, 2010). In related cytological studies from China and Japan, chromosome numbers have been studied for 18 and 11 Epimedium species, respectively, (Sheng *et al.*, 2010; Kuroki, 1967, 1970). The cytological studies of *E. elatum* confirm it as a diploid species with prominent meiotic abnormalities. Meiotic abnormalities have been previously reported in five Epimedium species from China (Jiang *et al.*, 2011) and in few hybrid populations of this

genus (Sheng *et al.*, 2011). Various explanations have been given for causes of meiotic abnormalities in plants. Chromosome stickiness is observed from early prophase-I to anaphase-II, but it is more frequent during metaphase-I (Jeelani *et al.*, 2014). It also results in the delayed separation of bivalents/chromatids at anaphase-I and II and decreases pollen viability in some species (Rao *et al.*, 1990; Consolaro and Pagliarini., 1996). Primary cause and biochemical basis of this phenomenon are still unknown, and genetic as well as environmental factors are suggested to be the main causes (Nirmala and Rao., 1996; Baptista-Giacomelli *et al.*, 2000). The chromatin bridges have also been noted in many PMCs and it seems to be the result of chromatin stickiness and late disjunction of bivalents. Various researchers have given their own explanations for laggards and bridge formation. Reasons given for the same phenomenon are interlocking of bivalents (Bhattacharjee, 1953), paracentric inversions (Sinha and Godward., 1972), abnormal spindle formation (Tarar and Dnyansagar, 1980) or delayed terminalization (Kumar and Tripathi, 2007). Data on pollen fertility assessment of this species could be helpful in understanding the overall effect of these abnormalities on its fruit and seed set.

CONCLUSION AND CONSERVATION IMPLICATIONS

Medicinal plants in the Northwestern Himalayas have been harvested in an unsustainable manner to meet the demand of pharmaceutical companies. The eco-geographical surveys showed that most of the habitats of *E. elatum* have been destroyed by human disturbance. Damage to its natural habitats would lead to a decrease in its population size and probably a subsequent increase in inbreeding, decreasing its genetic diversity. Different studies are needed for assessing exact nature of its endangerment, morphoecological behavior, reproductive biology and regional and local distribution patterns. Understanding its adaptive biology is important for development of effective agro-technology for this medicinal species. Knowledge of the adaptive mechanisms of threatened medicinal plants is crucial for both *in situ* and *ex situ* conservation and other research programs. It is also useful for attempting a large-scale cultivation program to meet the bulk demand of pharmaceutical companies because growing plants within their suitable agro-climatic environment not only helps in producing higher yields but also high levels of active components (Badola and Aitken 2003). Furthermore, to avoid human over collection of medicinal plants from Kashmir Himalayas, greater awareness for protection of *E. elatum* must be emphasized and related research centers and forest departments should be encouraged to undertake its conservation through an integrated conservation strategy based on demographic, ecological, and genetic aspects. Extensive collection and subsequent cultivation in a shade garden and reintroduction into their original wild habitats seems feasible for *E. elatum*. The new environment may have important ecological differences compared with the original habitat, and the approach is technically challenging and is expensive. Therefore, *ex situ* conservation is recommended only to supplement *in situ* conservation. *In vitro* techniques may prove to be an effective alternative means of propagation and recovery of the rare *E. elatum*.

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