

Morphological studies and development of *ex-situ* protocol for rehabilitation of threatened *Rheum* species under nursery conditions

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ABSTRACT

Rheum is a strong vigorous plant and can live long even in tough conditions. The *Rheum* species have been domesticated as medicinal plants for human consumption. *Rheum* species are perennial stout herbs. Rhubarb is a common English name of the genus *Rheum*. Rhubarb is a plant name for the many different species of *Rheum*, distributed in the temperate and sub-tropical regions of Himalaya from Kashmir to Sikkim and growing wild in the mountains of the Western and North-western provinces of China and adjoining Tibetan territory. It is cultivated much in Europe and the United States. In Kashmir Himalaya, it ranges from an altitude of 1700-5500 m. It is famous for its medicinal uses. Roots are long and stout. The stem is erect, hollow, sulcate, glabrous, or strigose. It is native to Asia-Temperate to Asia-Tropical, from China to India, Nepal, and Pakistan. In India, it is found in Himachal Pradesh, Jammu and Kashmir, and Uttar Pradesh. In Jammu and Kashmir, it grows on open slopes and shrubberies in Kashmir, Leh and Zaskar valley between 3000 and 5000 m, where *Rheum webbianum* is found. The *Rheum spiciforme* is also one of the most known medicinal plant and is found on high altitudes of Gurez valley (3000 m, 5000 m). *Rheum* also prefers a full sunny location and thrives in a moist, fertile soil. Most *Rheum* species can handle heavier clay soils better than many other herbs and makes a striking addition to the spring landscape. Rhubarb is propagated from seeds, seedlings, or pieces of rhizome.

KEY WORDS: Gurez, medicinal plant, Kashmir Himalaya, Leh, *Rheum spiciforme*, *Rheum webbianum*, Rhubarb, Zaskar

INTRODUCTION

The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. These traditional knowledge systems have started to disappear with the passage of time due to the scarcity of written documents and relatively low income in these traditions. Over the past few years, however, the medicinal plants have regained a wide recognition due to an escalating faith in herbal medicine in view of its lesser side effects compared to allopathic medicine, in addition, the necessity of meeting the requirements of medicine for an increasing human population. Many compounds used in today's medicine have a complex structure, and synthesizing these bioactive compounds chemically at a low price is not easy (Shimomura *et al.*, 1997). With deforestation, medicinal wealth is rapidly lost, such that many valuable plants are

threatened with extinction. Medicinal plants have been the subjects of man's interest since time immemorial and play a key role in human health (Constabel, 1990). Medicinal plants have been identified and used throughout human history. Almost every civilization has a history of medicinal plant use (Ensminger *et al.*, 1983). The association of humans with plants obviously originated with the beginning of life on earth when plants provided the oxygen, food, forage, shelter, and medicine needed for higher life forms.

The Valley of Kashmir known for its beauty all over the world is also rich in herbal and floral wealth. The valley of Kashmir is very rich in high value and high altitude aromatic and medicinal plants. Kashmir valley has a great potential for establishing pharmaceutical and essential oil industry based on these high-value plant species. Prominent among the aroma bearing plants

are Lavender, Rose, Clarysage, Peppermint, Rosemary, *Artemisia annua*, *Tagetes minuta*, and Geranium oils. The essential oils derived from these plants form the backbone of perfumery, cosmetic industry. These oils rank among the top 10 essential oils used in aromatherapy throughout the world. The medicinal properties of various herbs and flowers growing in Kashmir need to be publicized so that the local inhabitants particularly villages would not let these plants fall in waste due to lack of awareness.

Rheum species is a perennial stout herb. Rhubarb is the English name of the genus *Rheum* (family Polygonaceae). There are about 60 species of the genus *Rheum* recorded in the world (Agarwal *et al.*, 2001). Rhubarb is a plant name for the many different species of *Rheum*, distributed in the temperate and sub-tropical regions of Himalaya from Kashmir to Sikkim (Nautiyal *et al.*, 2003) and growing in the wild in the mountains of the Western and North-western provinces of China and in the adjoining Tibetan territory and in cultivation in much of Europe and the United States. In Kashmir Himalaya ranges from an altitude of 1700-5500 m. It is famous for its Medicinal uses.

Rheum emodi is a perennial plant that grows from thick short and erected rhizomes. The large, somewhat triangular leaf blades are elevated on long, fleshy petioles. The flowers are small, greenish-white, and borne in large compound leafy inflorescences. It is a leafy perennial herb, streaked green, radical leaves, orbicular or broadly ovate, cordate at base, often long, 5-7 nerved papillose beneath. Petiole long and stout. Inflorescence panicles, flower dark purple, sepals 5, stamens 6-9, ovary 2-4, fruit long, ovoid-oblong, purple. This family of plants contains at least 40 genera and more than 800 species, all with jointed stems. Other characters include leaf stipules: United into a tubular sheath called an ocrea; sepals: Petaloid; petals: Absent; fruit: An achene. *R. emodi*, commonly known as Himalayan rhubarb, is a medicinal herb used in the Indian Ayurvedic system of medicine. Rhubarbs are large perennial herbs. The flower stalk is solid or hollow and reaches a height of 1.5-2 m. The radical leaves, which have long, succulent petioles, form a rosette and are very large and usually entire. Rhubarb is propagated from seeds, seedlings, or pieces of rhizome.

Rheum webbianum Royle is an important medicinal plant belonging to the family Polygonaceae. It is commonly known as "Himalayan Rhubarb" in English, "Ravanchini" in Hindi, "Xu Mi Da Huang" in Chinese, "Chotal" in Pakistan and "Lachhu" or "Chu-rtsa" in Ladakh. It is native to Asia-Temperate to Asia-Tropical, from China to India, Nepal and Pakistan. In India, it is found in Himachal Pradesh,

Jammu and Kashmir, Uttar Pradesh. In Jammu and Kashmir, it is grown on open slopes and shrubberies in Kashmir, Leh and Zaskar valley (Chaurasia *et al.*, 2007). It can be identified having basal and cauline leaves. Basal leaves are orbicular or broadly ovate, large, 30-60 cm long, abaxially pubescent, adaxially papilliferous or muricate, basal veins 5, base subcordate, deeply palmately 5-lobed, middle 3 lobes pinnatisect, apex narrowly acute. Stem leaves are few and smaller than the basal ones. Ocrea is large, membrane, entire and brown. Panicle is large. The pedicels are articulate. The tepals are six, white, purple-red, rarely light red, and orbicular. The stamens are 9. The ovary is broadly ovoid, 1-locular, and 1-ovule. The styles are three, horizontal in axis. It is perennial and 1.5-2 (occasionally up to 2.5) m tall. The root is stout, straight, up to 30 cm in length, up to 10 cm in diameter and dark brown outside and yellow or red-yellow inside in color. The stem is stout, hollow, finely sulcate, and glabrous or pubescent. The species have large somewhat triangular shaped leaves with long, fleshy petioles. The flowers are small, greenish-white to rose-red, and grouped in large compound leafy inflorescences.

Rheum spiciforme is also the stout herbs. Petiole of basal leaf purplish red, subterete, 3-10 cm, stout, glabrous or papilliferous; leaf blade purple-red abaxially, dark green or yellow-green adaxially, ovate or broadly ovate-elliptic, nearly leathery, both surfaces papilliferous or abaxially glabrous, basal veins 5, base rounded or subcordate, margin entire and slightly sinuate, apex obtuse. Leaves all radical, petiole 7-15 cm long, very hard, usually glabrous, blade very leathery, orbicular, broadly ovate or cordate, nerves prominently radiating, red-brown; 15-30 cm long and broad, glabrous or slightly stellate hairy below. Inflorescence pedunculate, glabrous, 10-30 cm long spike-like raceme. Flowers small, 2-3 mm across, pedicel 2-7 mm long; bracteate, bract minute, ovate, scarious. Perianth segments small, entire, obtuse, 2-3 mm long, yellowish or somewhat reddish. Fruit broadly ellipsoid or oblong, 0.5-1.5 cm long, rounded at the apex; wings membranous, broader than the disk.

Other species of *Rheum* found in the different parts of world are *Rheum rhabarbarum*, *Rheum hotaoense*, *Rheum wittrockii*, *Rheum altaicum*, *Rheum forrestii*, *Rheum likiangense*, *Rheum lhasaense*, *Rheum compactum*, *Rheum glabricaula*, *Rheum officinale*, *Rheum palmatum*, *Rheum tanguticum*, *Rheum laciniatum*, *Rheum acuminatum*, *Rheum maculatum*, *Rheum yunnanense*, *Rheum kialense*, *Rheum racemiferum*, *Rheum subanceolatum*, *Rheum inopinatum*, *Rheum pumilum*, *Rheum delavayi*, *Rheum subacaule*, *Rheum uninerve*, *Rheum nanum*,

Rheum tibeticum, *Rheum tataricum*, *Rheum moorcroftianum*, *Rheum przewalskyi*, *Rheum rhizostachyum*, *Rheum reticulatum*, *Rheum globulosum*, *Rheum nobile*, *Rheum alexandrae*, *R. acuminatum*, *Rheum alpinum*, *R. altaicum*, *Rheum coreanum*, *Rheum x hybridum*, and *Rheum ribes*.

MATERIALS AND METHODS

Surveys and Ex-situ Conservation

Surveys were done for the collection of three species of *Rheum* from high altitudes of Kashmir Himalaya. *R. emodi* was collected from various altitudes such as Yousmarg, Dodhpathri, Thajwas Glacier (Sonmarg), Affarwat (Gulmarg), Dagvan (Tarsar Marsar), Aaroo (Pahalgam), Laar, and Dhara. *R. webbianum* was collected from Ladakh region, i.e., Tangsti, Khardungla, Panzila Top (Zanaskar), Tangole (Zanaskar), and Parakhachik (Zanaskar). *R. spiciforme* was collected from Gurez area, i.e., Dawar hills, Dahi Nala, Chakwali, Habbakhaton Mountain, and Tragbal. All the study sites are depicted in Figure 1 and Table 1. The specimens were identified by consulting the Flora of China (Shu *et al.* 2003) and Alpine Flora of Kashmir Himalaya (Dhar and Kachroo 1983). Further, the confirmation of the specimens was done by comparing the specimens in the Herbarium, Department of Botany, University of Kashmir, Kashmir and Punjabi University, Patiala and the authenticated voucher numbers have been obtained from both the universities.

Growth in Nursery

The seeds, roots, rhizomes, and whole plants collected from different locations were planted under natural, shady and suitable environmental conditions in the beds of net house at Naseem Bagh Campus of University of Kashmir, Srinagar. Each bed had its own name plate mentioning the place and date of collection. The plants were monitored on daily basis for their growth responses.

Rheum is also called cliff hanger as it grows on rocks and at high altitude from 2000 to 6000 m, so it needs suitable and favorable conditions when grown *ex-situ*. So far, for the establishment of *Rheum* species in the nursery, the soil was also taken from high altitudes along with the plant which was mixed with the soil of net house. Different soil texture groups were also used with or without fertilizer urea.

Studies of Morphological Features

Morphological traits cover the root length, root thickness, rhizome length, rhizome thickness, leaf length, leaf breadth, leaf number, number of branches, number of



Figure 1: Map showing survey areas

Table 1: Information regarding the different localities of Kashmir visited during present studies

Species (accession number)	Population	Population code	Altitude (m)
<i>R. emodi</i> PUP (59513)/KU (2010-KASH)	Yousmarg	DH	4267.2
	Sonamarg	SM	3462.2
	Pahalgam	DP	3962.4
	Dodhpathri	GM	3862.4
	Gulmarg	LR	4476.8
	Tarsar Marsar	YM	4657.2
	Dhara	TM	3751.6
	Laar	PG	3657.6
	Tissue cultured plants	TC	-
<i>R. webbianum</i> PUP (59515)/KU (2011-KASH)	Khardungla (Leh)	PK	4921.5
	Tangsti (Leh)	TG	4725.8
	Panzila Top (Zanaskar)	KH	4593.8
	Tangole (Zanaskar)	TS	4266.1
	Parkachik (Zanaskar)	PT	3938.4
<i>R. spiciforme</i> PUP (59516)/KU (2012-KASH)	Tissue cultured plants	TC	-
	Dawar hills	SM	4419.6
	Chakwali	CW	4684.8
	Dahi Nala	TB	3810.0
	Satni Mountain	DH	3962.4
	Habba Khatoon Mountain	DN	4756.8
	Tragbal	HM	3352.8
	Tissue cultured plants	-	-

From the list of surveyed localities, it is clearly reflected that vast area presenting variety of vegetation types has been covered with altitude ranging from 3352.8 to 4921.5 m. +++: Fast response, ++: Moderate response, +: Slow response, *R. emodi*: *Rheum emodi*, *R. webbianum*: *Rheum webbianum*, *R. spiciforme*: *Rheum spiciforme*

nodes, and plant height. These parameters were studied for scoring data regarding morphological features of the plant species. The nodes are absent in the case of *R. webbianum* and *R. spiciforme*. Hence, this parameter was not studied in these two species.

Survey and Collection

Rheum species are perennial, stout herbs, distributed in the temperate and sub-tropical regions of Himalaya from

Kashmir to Sikkim lying at an altitude of 2800-3800 m. For this study, three species, i.e., *R. emodi*, *R. webbianum*, and *R. spiciforme* were collected from different habitats of Kashmir Himalaya (Plates 1-3).

Maintenance of Germplasm (*Ex-situ*) in Nursery

For germplasm establishment, plants, seeds, roots and rhizomes collected from different altitudes were grown in the different beds in Nursery at University of Kashmir (Plate 4 - Figures 22-25).

Response of *Rheum* Species in Nursery

In nursery, good seed germination ability was observed in natural soil, different soil texture groups and under the influence of urea. Sand was also mixed in the soil. However, maximum germination was recorded in sandy soil treated with urea in the ratio of 1:2.

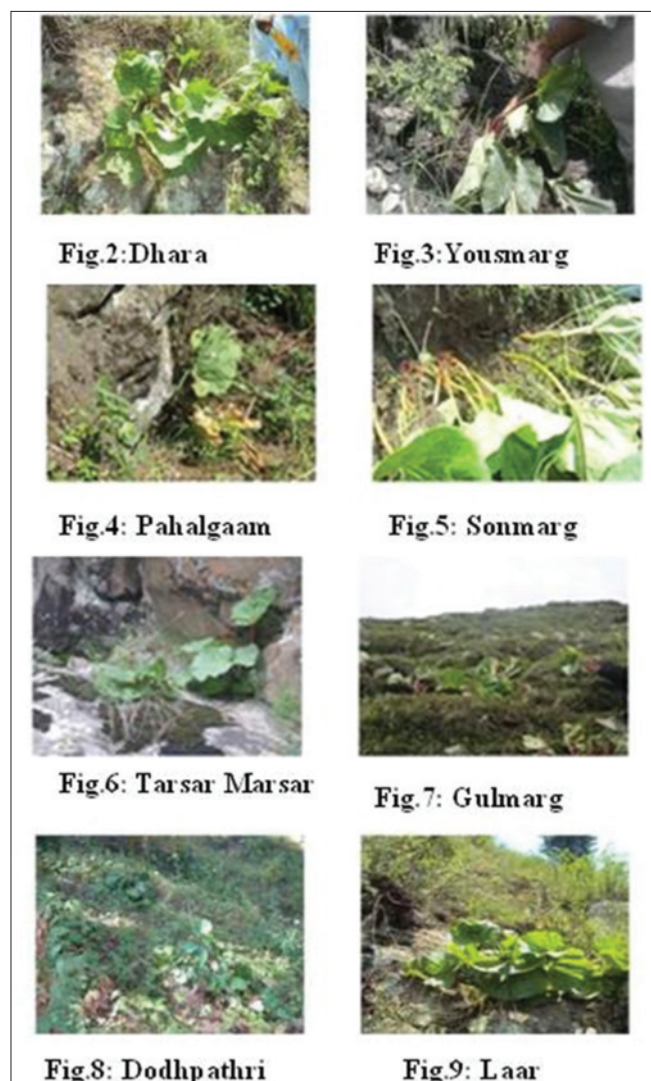


Plate 1: *Rheum emodi* collected from different altitudes of Kashmir Himalaya

R. emodi

The germplasm of *R. emodi* collected in 2010 showed rapid growth response in the nursery. The survival of the whole plants showed increment from 70% to 90% from 2011 to 2014 (Table 2, Plate 5 - Figures 26-28).

R. webbianum

The germplasm of *R. webbianum* showed slow growth response as compared to *R. emodi* in nursery. The survival rate during the year 2011-14 was 50-75% (Table 2, Plate 5 - Figures 29-31).

R. spiciforme

The germplasm of *R. spiciforme* collected in 2011 also showed fast and good growth response and the survival rate observed in 3 years was 60-85% (Table 2, Plate 5 - Figures 32-34).

Morphology of *Rheum* Species

In this study, *R. emodi*, *R. webbianum* and *R. spiciforme* collected from different altitudes (natural habitats) were studied using different morphometric parameters (using standard techniques).

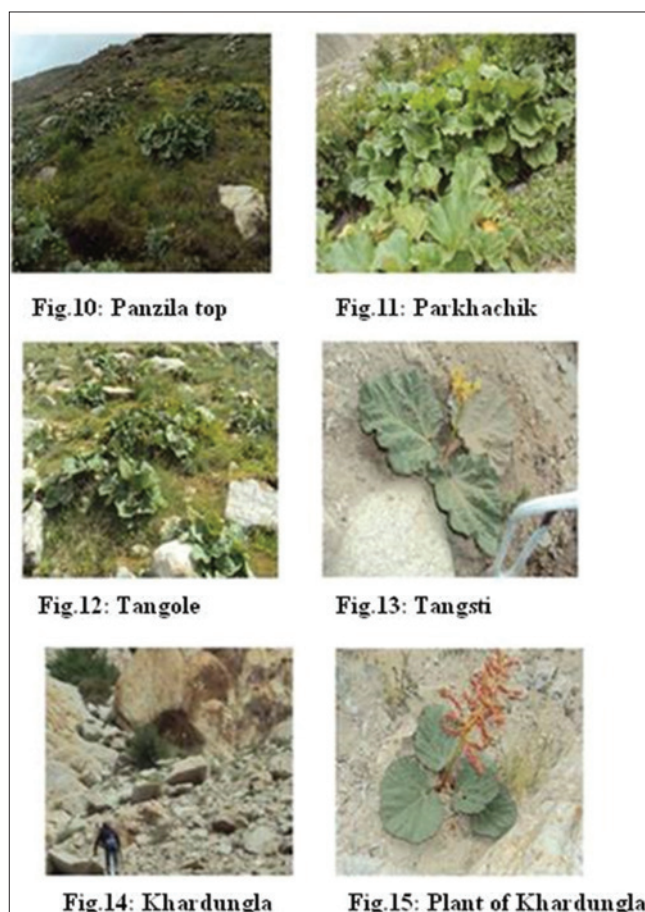


Plate 2: *Rheum webbianum* collected from different altitudes of Ladakh and Zaskar valley

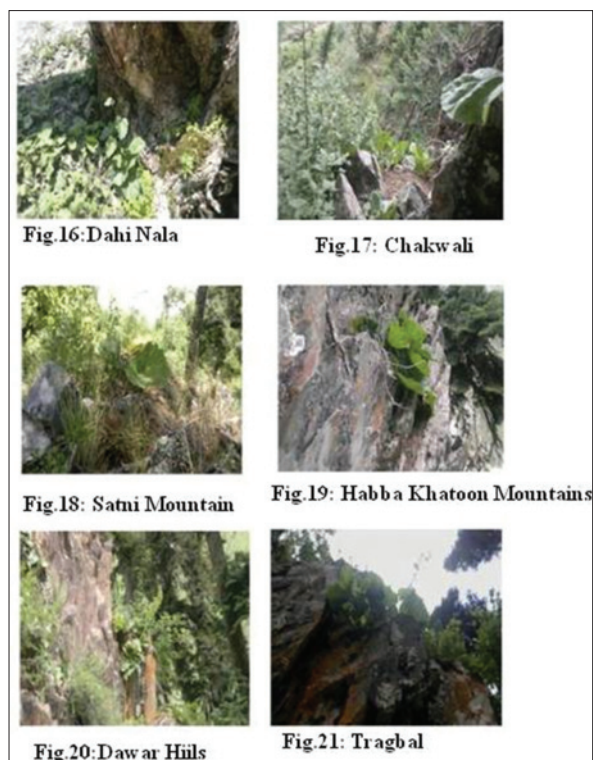


Plate 3: *Rheum spiciforme* collected from different altitudes of Gurez valley and nursery development



Plate 4: Ex-situ conservation of *Rheum* species

Table 2: Ex-situ conservation of genetic diversity of *Rheum* species in net house

<i>Rheum</i> species (germplasm)	Germination/ growth response	Survival rate (%)			
		2011	2012	2013	2014
<i>R. emodi</i>					
Seeds	+	85	85	80	80
Whole plants	+++	70	85	90	90
Roots and rhizomes	++	60	70	80	80
<i>R. webbianum</i>					
Seeds	+	60	60	55	50
Whole plants	+++	60	60	70	75
Roots and rhizomes	++	50	60	70	75
<i>R. spiciforme</i>					
Seeds	+	-	80	75	75
Whole plants	+++	-	85	85	85
Roots and rhizomes	++	-	60	70	80

+++ : Fast response, ++ : Moderate response, + : Slow response,

R. emodi: *Rheum emodi*, *R. webbianum*: *Rheum webbianum*,

R. spiciforme: *Rheum spiciforme*

R. emodi

In the present studies, it was noticed that *Rheum* grows from both thick rhizomes and long roots. The maximum root, length, i.e., 67 cm and maximum shoot length, i.e., 52 cm were observed in Sonmarg plant and the maximum root thickness, i.e., 7.3 cm was found in Yousmarg plant as shown in plate 6. The maximum root area, i.e., 5.3 cm was also found in Yousmarg plant; the maximum leaf area, i.e., 22.7 cm was also found in Sonmarg plant. The maximum leaf length and leaf breadth, i.e., 46 cm and 54 cm was found in Gulmarg plant. The *R. emodi* of Laar in its natural habitat was found in large number in quadrants 7/m². Fresh weight of root, i.e., 6 g was found in Sonamarg plant while as dry weight of root, i.e., 168 g was found in Dodhpathri plant. The maximum plant height in meters, i.e., 5.6 m was observed in Dodhpathri plant. Maximum number of branches, i.e., 19 and leaves, i.e., 28 were observed in Gulmarg plant. The maximum number of nodes, i.e. 10 was found in Laar plant (Tables 3 and 4).

R. webbianum

In the present studies, the maximum root length, i.e. 98 cm and thickness of root, i.e. 59 cm of *R. webbianum* were found in Tangsti plant. The maximum rhizome thickness, i.e. 68 cm of *R. webbianum* was found in Panzila Top and maximum rhizome length was found in Tangsti plant. The maximum leaf length, i.e. 34 cm and leaf breadth, i.e. 46 cm of *R. webbianum* were observed in Parkachik plant as shown in plate 6. The maximum height, i.e. 1.4 m of *R. webbianum* was observed in Panzila Top plant. The maximum number of branches, i.e. 14 and leaves, i.e. 18 of *R. webbianum* were found in Parkachik plant. The *R. webbianum* found in Parkachik area was more in number, i.e. 17/m²

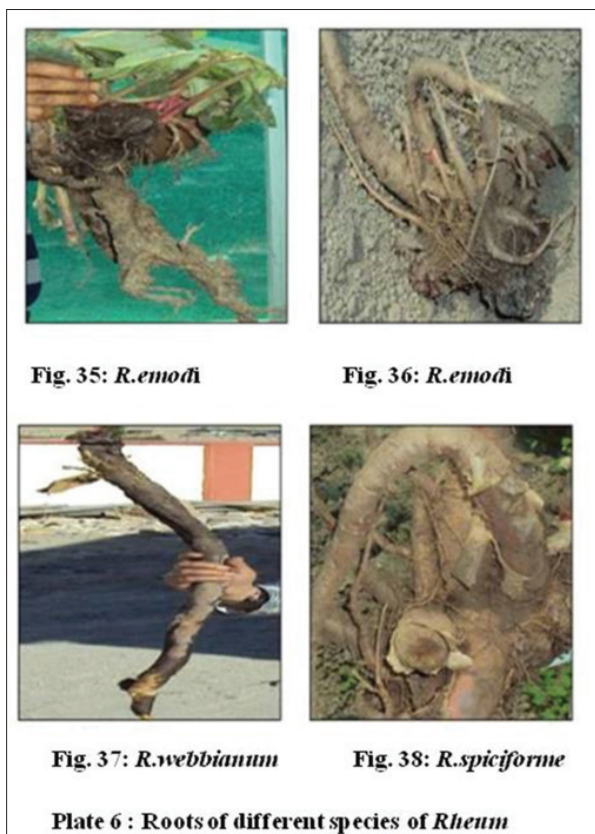
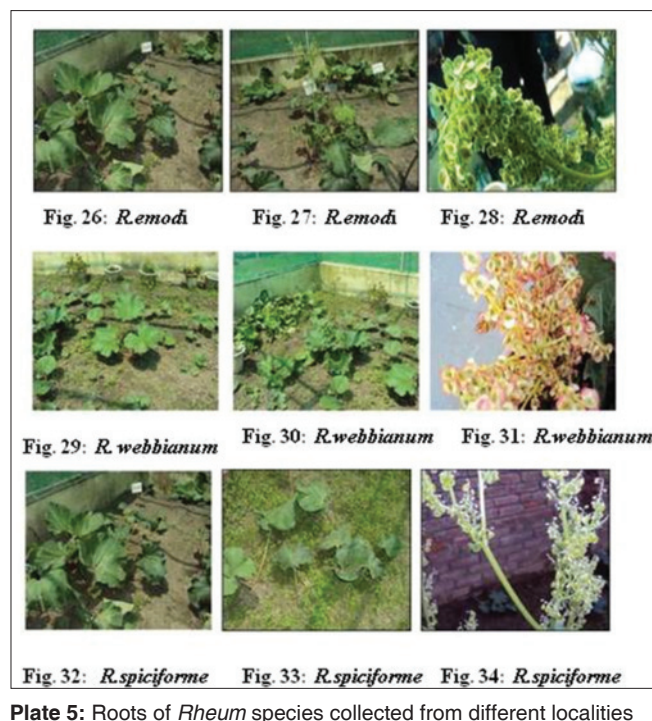


Plate 6: Roots of different species of *Rheum*

(Tables 5 and 6).

R. spiciforme

In present studies, the maximum root length, i.e. 64 cm

Table 3: Morphological features (underground parts) of *R. emodi* growing under nursery conditions

Altitudes (m)	Root length (cm)		Root thickness (cm)		Root area (l×b) (cm)		Rhizome length (cm)		Rhizome breadth (cm)		Rhizome area (l×b) (cm)		Leaf length (cm)		Leaf breadth (cm)		Leaf area (l×b) (cm)	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Gulnarg/4476.8	^a 47.6±2.51	^a 39±1	^b 4±1	^b 2±1	^a 2.4±0.1	^a 1.1±0.1	^b 14±1	^b 9±1	^a 74±1	^a 39±1	^a 25.7±0.1	^a 12±1	^a 46±1	^a 29±1	^a 54±1	^a 41±1	^b 11.2±0.01	^a 3±1
Dhara/3751.6	^b 37.6±2.0	^b 28.3±1.5	^a 1.3±0.5	^a 1.5±0.1	^b 59±1	^b 74±1	^a 24±1	^a 15±1	^a 65±1	^a 38±2	^b 16.4±0.1	^a 5±1	^a 43.3±1.5	^a 26.6±4.1	^a 32.6±2.5	^a 17.6±2.7	^b 15.7±0.1	^a 4±1
Pahalgam/3962.4	^b 38.3±1.5	^b 22±3	^a 6.6±1.5	^b 2.3±0.5	^a 3±1	^a 4.7±0.01	^a 12.6±2.0	^b 6±2	^b 33.3±1.5	^a 19±1	^a 5.2±0.1	^a 1.5±0.1	^b 26±3.6	^b 16.6±1.5	^a 41.3±3.2	^b 24.6±4.7	^b 13.4±0.1	^a 5.3±0.1
Sonamarg/3462.2	^b 67±1	^a 42.6±2.5	^a 5.6±1.5	^a 4±1	^a 4±1	^a 2.2±0.01	^a 38.3±2.0	^a 24.6±4.7	^b 70±1	^a 45±1	^a 28.3±0.1	^a 13.7±0.1	^a 32±2.6	^a 24±1	^a 63±2	^a 50±1	^b 22.7±0.01	^b 12.7±0.01
Yousmarg/4267.2	^a 59±1	^a 39±2.6	^a 7.3±1.5	^a 3±1	^a 5.3±0.1	^a 1.5±0.1	^a 23±2.6	^a 13.6±2.0	^a 42±4.3	^a 30±1	^b 11.2±0.1	^a 4.9±0.1	^a 26.3±1.5	^b 16±1	^a 27±7.2	^a 20.6±1.5	^a 9.7±0.1	^a 3.7±0.1
Dodhpatri/3862.4	^a 49±2	^a 32.3±2.5	^a 3.3±1.5	^a 1.6±1.1	^a 2.5±0.1	^a 1.04±0.01	^a 17±2.6	^a 14.6±1.5	^a 38±7	^a 22.3±1.5	^a 8±1	^a 3.8±0.1	^a 19.3±1.5	^a 14±2	^a 37±5	^a 34.3±2.0	^a 3.8±0.1	^a 8.8±0.1
Tasar Marsar/4657.2	^a 58±2	^a 42±2.6	^a 4±2	^a 2±1	^a 3.5±0.1	^a 1.3±0.1	^a 28.3±2.0	^a 22±3.6	^a 52.6±2.0	^a 36.6±1.5	^b 16.4±0.1	^a 9.8±0.1	^a 24.6±3.0	^a 14±3	^a 31±3	^a 27±2	^b 10.63±0.1	^a 4.9±0.1
Laav/3657.6	^a 47.3±3.0	^a 37.6±2.0	^a 6.3±1.5	^a 4±1	^a 3±1	^a 2±1	^a 36.6±1.5	^a 22.6±2.0	^a 45.3±3.0	^a 33.3±1.5	^b 18.2±0.01	^a 8.7±0.01	^a 50±1	^a 39.6±3.7	^a 45±3.6	^a 31.6±4.1	^a 24.9±0.1	^a 24.1±0.1

Values are represented as mean±SD (n=3). Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at P<0.005. SD: Standard deviation, *R. emodi*: *Rheum emodi*

Table 4: Morphological features (above ground) *R. emodi* under nursery conditions

Collection area	Number of branches		Number of leaves		Number of nodes		Fresh root weight (g)		Dry root weight (g)		Quadrants (Sq.) (1 m)		Plant height (m)	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Number 1	Number 2	Maximum	Minimum
Gulmarg	^a 19±1	^b 8.3±1.5	^c 28.3±1.5	^b 16±1	^a 8.3±1.5	^a 4±1	^a 5±1	^a 2±1	^a 89±1	^a 69±1	^a 4±1	^a 6±1	^a 2.1±0.1	^a 1.6±0.1
Dhara	^a 12±1	^a 5±1	^b 14±1	^a 7±1	^a 8±1	^a 5±1	^a 4±1	^a 2±1	^b 69±1	^b 28±1	^a 6±1	^a 3±1	^a 1.4±0.1	^a 1.1±1
Pahalgaam	^a 10±1	^a 3±1	^a 7±1	^a 3±1	^a 7±1	^a 4.6±1.5	^a 3±1	^a 2±1	^c 79±1	^a 49±1	^a 4±1	^a 2±1	^a 1.6±0.1	^a 1.0±1
Sonamarg	^a 11±1	^a 7±1	^a 24±1	^b 15±1	^a 9±1	^a 6±1	^a 6±1	^a 4±1	^c 79±1	^a 89±1	^a 6±1	^a 4±1	^a 2.2±0.1	^a 1±0
Yousmarg	^a 7±1	^a 4±1	^a 9±1	^a 5±1	^a 4±1	^a 2±1	^a 4±1	^a 2±1	^a 38±1	^b 24±1	^a 4±1	^a 2±1	^a 1.5±0.1	^a 1.2±1
Dodhpatri	^a 11±1	^a 6±1	^b 9±1	^a 6±1	^a 8±1	^a 5±1	^a 4±1	^a 2±1	^a 168±1	^a 109±1	^a 4±1	^a 2±1	^b 5.6±0.1	^a 1.5±0.1
Tarsar	^a 9±1	^a 5±1	^b 14±1	^a 5±1	^b 12±1	^b 8±1	^a 4±1	^a 2±1	^d 89±1	^c 39±1	^a 5±1	^a 3±1	^a 1.6±0.1	^a 1.2±1
Marsar														
Laar	^a 11±1	^a 5±1	^b 15±1	^b 10±1	^b 11±1	^b 7±1	^a 5±1	^a 4±1	^c 79±1	^a 49±1	^a 7±1	^a 3±1	^a 2±1	^a 1±0

Values are represented as mean±SD ($n=3$). Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at $P<0.005$. Data scored after 12 weeks. SD: Standard deviation, *R. emodi*: *Rheum emodi*

Table 5: Morphological features (underground parts) of *R. webbianum* under nursery conditions

Altitudes (m)	Root length (cm)		Root thickness (cm)		Root area (l×b) (cm)		Rhizome length (cm)		Rhizome breadth (cm)		Rhizome area (l×b) (cm)		Leaf length (cm)		Leaf breadth (cm)		Leaf area (l×b) (cm)	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Panzila Top/4593.8	^b 38±1	^a 27±1	^a 4±1	^a 2±1	^a 2±1	^a 83±1	^c 68±1	^b 39±1	^b 24±1	^b 18±1	^c 16.2±0.1	^b 7.5±0.1	^a 19±1	^a 10±1	^b 39±1	^b 29±1	^a 7±1	^a 3.2±0.1
Tangole/4266.1	^b 34±1	^a 24±1	^a 3±1	^a 2±1	^a 1.3±0.1	^a 49±1	^a 44±1	^b 30±1	^b 26±1	^b 16±1	^b 12.1±0.1	^b 5.2±0.1	^b 24±1	^a 14±1	^a 44±1	^b 31±1	^a 11.2±0.1	^a 4.7±0.1
Parkachik/3938.2	^a 18±1	^a 12±1	^a 3±1	^a 2±1	^c 7.9±1	^b 23±1	^a 49±1	^b 34±1	^b 25±1	^b 15±1	^b 12±1	^b 5.5±0.1	^c 34±1	^a 14±1	^a 46±1	^b 28±1	^a 12.5±0.1	^a 4.4±0.1
Tangsti/4725.8	^a 98±1	^a 59±1	^b 14±1	^a 8±1	^b 13.4±0.1	^a 5.3±0.1	^a 64±1	^a 44±1	^a 29±1	^b 15±1	^c 19.4±0.1	^b 7.1±0.1	^b 24±1	^b 17±1	^a 24±1	^a 18±1	^a 7.4±0.1	^a 3.4±0.1
Khardungla/4921.5	^a 49±1	^a 34±1	^a 5±1	^a 2±1	^a 2±1	^a 1.04±0.01	^a 39±1	^a 19±1	^a 14±1	^a 7±1	^a 5±1	^a 1.5±0.1	^b 27±1	^a 24±1	^a 27±1	^a 17±1	^a 9.7±0.1	^a 4.4±0.1

Values are represented as mean±SD ($n=3$). Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at $P<0.005$. SD: Standard deviation, *R. webbianum*: *Rheum webbianum*

Table 6: Morphological features (above ground parts) of *R. webbianum* under nursery conditions

Collection area	Number of branches		Number of leaves		Fresh root weight (g)		Dry root weight (g)		Quadrants (Sq.) (1 m)		Plant height (m)	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Number 1	Number 2	Maximum	Minimum
Panzila top	^a 9±1	^a 6±1	^b 11±1	^b 7±1	^a 4±1	^a 2±1	^a 38±1	^a 24±1	^a 14±1	^a 7±1	^a 1.4±0.1	^b 89±1
Tangole	^b 11±1	^b 8±1	^b 14±1	^b 8±1	^a 6±1	^a 4±1	^c 89±1	^c 70±1	^a 11±1	^b 8±1	^a 1.2±0.1	^b 85±1
Parkachik	^b 14±1	^b 8±1	^b 18±1	^b 10±1	^a 7±1	^a 3±1	^b 69±1	^b 39±1	^a 17±1	^a 6±1	^a 1.1±0.1	^b 89±1
Tangsti	^a 9±1	^a 6±1	^a 8±1	^a 4±1	^b 8±1	^a 5±1	^a 209±1	^a 89±1	^a 2±0	^a 4±0	^a 1.2±0.1	^c 95±1
Khardungla	^a 6±1	^a 3±1	^a 5±1	^a 3±1	^a 4±1	^a 2±1	^a 39±1	^a 19±1	^a 2.6±0.5	^a 3.6±0.5	^a 1.1±1	^a 49±1
									^a 3±0	^a 3.1±0.4		
									^a 2±1	^a 3±0		

Values are represented as mean±SD ($n=3$). Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at $P<0.005$. SD: Standard deviation, *R. webbianum*: *Rheum webbianum*

and thickness, i.e. 8 cm of *R. spiciforme* were found in Chakwali plant. The maximum rhizome length, i.e. 79 cm and thickness, i.e. 59 cm of *R. spiciforme* were also found in Chakwali plant. The maximum leaf length, i.e. 54 cm and breadth, i.e. 59 cm of *R. spiciforme* were also found in Chakwali plant as shown in plate 6. The maximum plant height, i.e. 2 m was also found in Chakwali plant. The maximum fresh, i.e. 14 g and dry root weight, i.e. 149 g were found in Tragbal area. The *R. spiciforme* in its natural habitat in Chakwali was found higher in number, i.e. 14/m². The maximum number of branches, i.e. 14 and leaves, i.e. 18 was found in Habba Khatoon Mountain area (Tables 7 and 8).

DISCUSSION

Morphological features are important in classifying the genotypes into different groups and records of these traits in different populations give the estimate of the range of these traits found in a species. Further, many of the morphological features may have a positive correlation with the synthesis and production of any phytoconstituent/ phytochemical of the plant. Thus, study of morphology becomes more important as it can indicate the best genotypes in relation to some active principles found in plants. Therefore, in this study, we recorded at least nine morphological features in all the three species of *Rheum*. These three species of *Rheum* (*R. emodi*, *R. webbianum*, and *R. spiciforme*) were collected, i.e. from different altitudes of Kashmir Himalaya and studied using different morphometric parameters (using standard techniques). *R. emodi* is a perennial plant that grows from thick and erected rhizomes and as well as from long roots. The leaves are large, triangular and broadly ovate with fleshy petioles. The flowers are small, greenish-white on large compound leafy inflorescences. Nodes are also present. *R. webbianum* is also having basal and cauline leaves. Basal leaves are orbicular or broadly ovate and are triangular in shape with long, fleshy petioles. Flowers are reddish pink in color. Nodes are absent in this species. *R. spiciforme* is also stout herb. Leaves are also broadly ovate and triangular in shape. It has also thick rhizomes and long roots. Flowers are small and yellowish green. Nodes are absent in this species. These species were grown in net house (Kashmir University) under suitable conditions as they have been listed under endangered category and are under threat. *Rheum* species are one of the known medicinal plants. *Rheum* has been vanished from lower localities, and now are found only on higher altitudes.

In net house, all the three *Rheum* species showed good response and survival rate was also good but *R. emodi*

Table 7: Morphological features of (underground parts) *R. spiciforme*

Altitudes (m)	Root length (cm)		Root thickness (cm)		Root area (l×b) (cm)		Rhizome length (cm)		Rhizome breadth (cm)		Rhizome area (l×b) (cm)		Leaf length (cm)		Leaf breadth (cm)		Leaf area (l×b) (cm)	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Satni Top/3962.4	^b 49±1	^a 34±1	^a 6±1	^a 4±1	^a 5±1	^a 1.74±0.1	^a 29±1	^a 29±1	^c 29±1	^c 19±1	^a 13.4±0.1	^a 5±1	^a 29±1	^a 19±1	^a 31±1	^a 17±1	^a 8±1	^a 3.5±0.1
Dawar hill/4419.6	^c 59±1	^a 34±1	^a 5±1	^a 3±1	^a 6.5±0.1	^a 1.3±0.1	^a 44±1	^a 49±1	^c 39±1	^c 21±1	^a 19.0±0.1	^a 7.7±0.01	^a 44±1	^a 29±1	^a 54±1	^b 24±1	^c 24.7±0.1	^a 7.4±0.1
Chakwali/4684.8	^a 64±1	^b 50±1	^a 8±1	^a 4±1	^b 7.7±0.1	^a 2.9±0.1	^a 79±1	^a 79±1	^c 59±1	^a 42±1	^a 47±1	^c 21.9±0.1	^a 54±1	^c 39±1	^c 59±1	^a 47±1	^a 32±1	^b 18.2±0.1
Habba Khatoon mountain/4756.8	^c 59±1	^b 48±1	^a 8±1	^a 5±1	^a 5.4±0.1	^a 2.9±0.1	^a 64±1	^a 43±1	^c 54±1	^a 38±1	^c 35.7±0.1	^b 17.1±0.1	^a 44±1	^c 38±1	^c 49±1	^a 40±1	^c 22.5±0.1	^b 15.9±0.1
Dahi Nala/3810.0	^a 39±1	^a 31±1	^a 8±1	^a 4±1	^a 3.2±0.1	^a 1.6±0.1	^b 54±1	^b 42±1	^a 44±1	^a 34±1	^b 24.7±0.1	^b 15.1±0.1	^a 44±1	^b 32±1	^a 50±1	^a 43±1	^c 22.9±0.1	^b 14.5±0.1
Tragbal/3352.8	^b 49±1	^a 37±1	^a 6±1	^a 3±1	^a 3±1	^a 1.5±0.1	^a 61±1	^a 52±1	^a 49±1	^a 44±1	^a 31±1	^c 23.5±0.1	^a 34±1	^a 21±1	^b 44±1	^a 37±1	^b 15.7±0.1	^a 8.3±0.1

Values are represented as mean±SD (n=3), Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at P<0.005. SD: Standard deviation, *R. spiciforme*: *Rheum spiciforme*

Table 8: Morphological features (above ground parts) of *R. spiciforme*

Collection area	Number of branches		Number of leaves		Fresh root weight (g)		Dry root weight (g)		Quadrants (Sq.) (1 m)		Plant height (m)	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Number 1	Number 2	Maximum	Minimum
Satni top	^a 10±1	^{ab} 7±1	^a 5±1	^a 3±1	^a 7±1	^a 3±1	^{bc} 90±1	^{ab} 79±1	^a 7±1	^a 3±1	^a 1.6±0.1	^a 1.1±0.1
Dawar hill	^a 9±1	^a 6±1	^{ab} 11±1	^a 7±1	^a 6±1	^a 2±1	^a 79±1	^{ab} 70±1	^a 9±1	^{ab} 7±1	^a 1.7±0.1	^a 1.2±0.1
Chakwali	^a 11±1	^b 8±1	^b 14±1	^{ab} 8±1	^a 9±1	^a 4±1	^c 95±1	^b 78±1	^b 14±1	^b 9±1	^a 2.1±0.1	^a 1.9±0.1
Habba Khatoon mountain	^{ab} 14±1	^a 8±1	^b 18±1	^b 10±1	^a 8±1	^a 5±1	^b 89±1	^a 67±1	^a 8±1	^a 4±1	^a 2.0±0.1	^a 1.8±0.1
Dahi Nala	^a 9±1	^a 4±1	^a 7±1	^a 3±1	^{ab} 10±1	^{ab} 7±1	^c 98±1	^c 89±1	^a 9±1	^{ab} 7±1	^a 1.7±0.1	^a 1.3±0.1
Tragbal	^a 7±1	^a 3±1	^a 6±1	^a 3±1	^b 14±1	^b 8±1	^d 149±1	^d 99±1	^a 7±1	^a 5±1	^a 1.8±0.1	^a 1.6±0.1

Values are represented as mean±SD ($n=3$). Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns are statistically significant at $P<0.005$. SD: Standard deviation, *R. spiciforme*: *Rheum spiciforme*

and *R. spiciforme* showed good survival rate as compared to *R. webbianum*. The species of *Rheum*, i.e. *R. nobile* and *R. alexandrae* possesses large bracts and these bracts have been found to protect reproductive organs from damage by the high levels of UV-B radiations associated with the high altitudes of the plateau (Terashima *et al.*, 1993; Omori and Ohba, 1996; Omori *et al.*, 2000). This type of bract also occurs in other alpine plants, e.g., *Sassurea* of *Asteraceae* (Wang and Liu 2004).

The *ex-situ* germplasm of *R. emodi* showed rapid and fast growth response. The germplasm of *R. webbianum* showed slow growth response in net house. The germplasm of *R. spiciforme* also showed fast and good growth response. During survey, 10 morphological features of *R. emodi* were observed. Of these, maximum root length, i.e., 67 cm was observed in Sonamarg population while minimum root length, i.e. 37.6 cm was observed in Dhara plant. The maximum root thickness, i.e. 7.3 cm was found in Yousmarg plant. The maximum leaf length and leaf breadth, i.e. 46 cm and 54 cm, respectively, were found in Gulmarg plant. The maximum plant height (5.6 m) was observed in Dodhpathri plant.

In the case of *R. webbianum*, the maximum rhizome thickness, i.e. 68 cm was found in Panzila Top and maximum rhizome length was found in Tangsti plant. The maximum leaf length, i.e. 34 cm and leaf breadth, i.e. 46 cm were observed in Parkhachik plant. The maximum height, i.e. 1.4 m was observed in Panzila Top plant. The maximum number of branches, i.e. 14 and leaves, i.e. 18 of *R. webbianum* were found in Parkhachik plant. Likewise, in *R. spiciforme* the maximum root length, i.e. 64 cm and thickness, i.e. 8 cm were found in Chakwali plant. The maximum rhizome length, i.e. 79 cm and thickness, i.e. 59 cm of *R. spiciforme* were also found in Chakwali plant. The maximum leaf length, i.e. 54 cm and breadth, i.e. 59 cm were also found in Chakwali plant. The maximum plant height, i.e. 2 m was also found in Chakwali plant.

When all the three species taken into account, *R. emodi* exhibited maximum diversity in six (rhizome breadth, leaf length, leaf breadth, no. of branches, no. of leaves, and plant height) morphological traits while *R. webbianum* showed maximum diversity in two (root length and root thickness) morphological traits and *R. spiciforme* showed maximum diversity in one (rhizome length) morphological trait. As it is well-known fact that phenotypic traits are influenced by environmental conditions, the diversity detected in specific traits in all these species may be attributed to the climatic conditions prevailing in habitats of each species. Further, when mean altitudinal collections analyzed we found that *R. webbianum* showed its occurrence in higher altitudes than other two species, and we also found that roots of this species were showing greater diversity as compared to other two species which may be the result of its adaptive behavior toward alpine region and higher endurance toward harsh cold temperature. Likewise, the diversity in branches, leaves and plant height in the case of *R. emodii* can be attributed to suitable and nutrient rich environment of low altitude as compared to *R. webbianum*. All these morphological traits in addition to others are required to study with larger sampling and covering diverse populations so that some standard descriptor can be developed to identify elite germplasm from the natural strands of *Rheum* species.

Rheum species contains anthraquinone derivatives, anthrones and tannins, etc., in which anthraquinone derivatives including emodin, aloë-emodin, rhein, physcion, chrysophanol and their glucosides are the accepted important active components. Aloe-emodin is a natural active compound present in the *Rheum* species. It has also been found that aloë-emodin has numerous biological properties including antiviral, antimicrobial, and hepatoprotective activities. Aloe-emodin has been reported to exhibit anticancer activity on neuroectodermal tumors, lung squamous cell carcinoma, and hepatoma cells. Another compounds found in *Rheum*

species are emodin, rhein, chrysaphanol, physcion, etc., and therefore, conservation of *Rheum* species is very important as this plant is under great threat and listed as endangered plant.

CONCLUSION

The *Rheum* species, i.e. *R. emodi*, *R. webbianum* and *R. spiciforme* are identified on the basis of main features, i.e. *R. emodi* is having nodes whereas nodes are absent in *R. webbianum* and *R. spiciforme*. The flowers of *R. webbianum* are reddish pink whereas the flowers of *R. emodi* are greenish white and flowers of *R. spiciforme* are yellowish green. The *R. emodi* and *R. spiciforme* are cliff hangers, they are mostly found in rocky areas, whereas *R. webbianum* is found mostly on plain slopes of hills and they are short herbs. The collected germplasm was grown in net house of Kashmir University and at least nine quantitative traits were recorded in all the three species. Of the nine traits studied, *R. emodi* exhibited maximum diversity in six (rhizome breadth, leaf length, leaf breadth, number of branches, no. of leaves, and plant height) morphological traits while *R. webbianum* showed maximum diversity in two (root length and root thickness) traits and *R. spiciforme* showed maximum diversity in one (rhizome length) morphological trait. The diversity detected in studied traits in all these species may be attributed to adaptive behavior of each species toward the climatic conditions prevailing in habitats of each species.

The conservation of *Rheum* species is very necessary as they are one of the most important medicinal plants. *Rheum* species contains anthraquinone derivatives, anthrones and tannins, etc., in which anthraquinone derivatives including emodin, aloe-emodin, rhein, physcion, chrysophanol, and their glucosides are the accepted important active components. Aloe-emodin is a natural active compound present in the *Rheum* species. It has also been found that aloe-emodin has numerous biological properties including antiviral, antimicrobial and hepatoprotective activities. Aloe-emodin has been reported to exhibit anticancer activity on neuroectodermal tumors, lung squamous cell carcinoma, and hepatoma cells. Another compounds found in *Rheum* species are emodin, rhein, chrysaphanol, physcion, etc. The *Rheum* species are also used by tribal and local people for many diseases such as, wounds and boils, Jaundice, cough and asthma muscular pain/body ache freckle/skin eruptions/skin diseases swelling, inflammation/rheumatic pain, constipation piles bone fracture/backache/joint pain rheumatic pain

stomachache/intestinal infections/and dysentery. Its leaves are taken as Food.

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