Rhododendrons conservation in the Sikkim Himalaya

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Rhododendrons form a major plant group at upper temperate locations having a characteristic slow growth rate and sizable horticultural value. Asia is the homeland for rhododendrons and many species had been hunted out of the region during British rule. About 98% of the Indian species are found in the Himalayan region, among which 72% are found in Sikkim. Due to human interference the natural populations of rhododendrons in the entire Himalaya are gradually diminishing. The major threats to rhododendrons are deforestation and unsustainable extraction for firewood and incense by local people. A set of rhododendrons which are classified as rare/endangered may be wiped out from the biota in the near future if proper conservation measures are not made. The present work incorporates biotechnological and conventional methods to counter the threat on survival of these plants. Studies on in vitro Rhododendron maddeni from the cotyledonary nodal segments, young leaves and stems, have yielded positive results. The goal of the work is to find out means of conservation through in vitro and ex situ mass propagation and restoration of rhododendron population in the wild.

Baseline information

RHODODENDRONS cover a vast section of southeastern Asia between the northwestern Himalaya through Nepal, Sikkim, eastern Tibet, Bhutan, Arunachal Pradesh, and upper Burma, western and central China. More than 90% of the world's natural population of rhododendrons is from this region. The genus *Rhododendron*, having about 50 species in India, is mainly distributed in the Himalayan region[1] (one species, *R. nilagiricum* in southern India), and is one of the most neglected group of plants in terms of scientific inquiry in India. The first of many rhododendrons which were to come from southeastern Asia was the tree species, *R. arboreum* with blood-red flowers, which was discovered by Captain Hardwicke in 1799. *R. campanulatum* was introduced in England in 1825 and the beautiful *R. barbatum* was introduced in 1849. In 1849 and 1850, Sir Joseph Hooker's[2] expedition to Sikkim in the eastern Himalayas discovered forty-five new species, including the yellow-flowered *R. campylocarpum* and *R. wighti*; the red-flowered *R. thomsoni*; the small trees *R. falconeri*, *R. grande* and *R. hodgsoni* with their enormous leaves; the epiphytes *R. dalhousiae* and *R. maddeni*; the large vigorous *R. griffithianum* with massive white flowers; and the interesting *R. triflorum*, *R. edgeworthi*, *R. fulgens*, *R. niveum*, *R. wallichi*, *R. lanatum*, *R. glaucophyllum*, *R. cinnabarinum* and *R. lepidotum*. Both the explorers found *R. hookeri* and *R. nuttalli* from Bhutan in 1852. There has been no concerted effort on the estimation of total number of species, sub-species and varieties of rhododendrons.

Rhododendrons, which are the denizens of high-altitude environment, have a characteristic slow growth rate. Ranging in size from small mat-like growths in alpine region to giants having heights of over 25 m is another characteristic feature of the genus. On record, 98% of the Indian species is found in the Himalayan region among which 72% is found in Sikkim. Distribution of rhododendrons in the Sikkim Himalaya is shown in Figure 1. In the light of the above, Sikkim may be considered as the most appropriate location for conservation and propagation studies of rhododendrons in India.

The 36 species of rhododendrons in the Sikkim Himalaya[3] showed barrel-shaped altitudinal distribution (Figure 2). Nine different altitudinal distribution ranges were categorized between 1500 and 6000 m. *R. arboreum* is a common species that showed distribution from 1500 m elevation up to 4000 m, while other species of wide ecological amplitude ranging from 2500 m up to 6000 m were *R. anthopogon* and *R. setosum* (Figure 2). Highest species occurrence was recorded between 3000 and 3500 m. The species availability decreases drastically from 4500 m upwards and 2500 m downwards. Species concentration increases with the latitudinal progression from south to north. Rhododendron forests are attractive

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destination for a large number of visitors (Figure 3), the increasing influx always exerting more pressure regarding its survival. It provides habitat for a variety of wildlife, including avifauna.

The rhododendron flowers have a great range of colours, shapes and sizes in their wild form (Figure 4 *a* and *b*). Horticulturists in the Western countries have further worked over it to produce quite a good range of beautiful hybrids, some of which have earned awards and merit citations at the Royal Horticultural Show, UK in the past. As the rhododendrons are conducive to inter-generic crosses a new scope of hybridization is open to be tapped. Revolution in the horticultural aspect of the genus is needed to fully convert the aesthetics of rhododendrons into commercial advantage.

Existing threats

Conservation of biotic resources remains one of the basic needs in terms of preserving biotic diversity which, in time, translates into the richness of biotic wealth. Plant-hunting expeditions by the earlier explorers from Europe into interior Asia since the turn of the century, had set the trend for removal of rhododendrons in large scale from the region. In Britain, Pacific Northwest and northeastern United States, these rhododendrons found way to many gardens both in natural forms and hybrids. However, little importance was given to the genus in its homeland, that has gradually led to the present conservation threat on many species.

With the shrinking of green cover almost everywhere, the rhododendrons are also experiencing the impact of disturbed ecological systems. This is clearly visible in the Himalaya, where the ecological systems and land physiography are understood to be fragile and found to be easily disturbed (Figure 5). In the context of Sikkim Himalaya, though heavy degradation is not evident, it surely shows certain signs of the menace entering the region. The rise in population with demand on land for farming, increased animal husbandry practices, construction of roadways, hydel power stations and allied works, army personnel garrisoned at alpine locations and lately the tourist influx have collectively resulted in the building up of considerable pressure on the availability of rhododendron species. The major threats to rhododendrons are deforestation and unsustainable extraction for firewood and incense by local people[4]. Due to the presence of polyphenols and flavonoids, rhododendrons make excellent firewood that burns even under wet conditions. Rhododendron firewood is also being used in the high-altitude trekking corridor for the purpose of tourism. A total of eight rhododendron species recorded out of 36 species (with 45 different forms, including sub-species and varieties) in the Sikkim Himalaya, are now in definite danger of elimination unless immediate resurrection measures are taken (Table 1). Some of the species have already become

Figure 1. Distribution of rhododendrons in the Sikkim Himalaya.

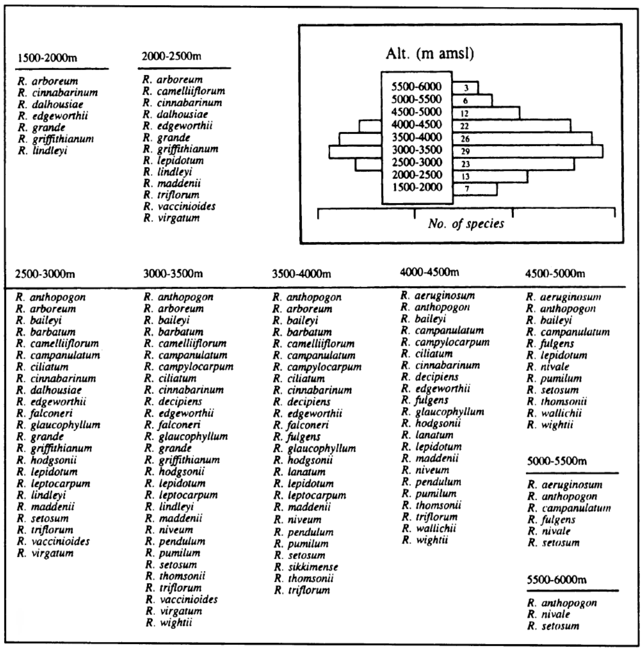

Figure 2. Altitudinal distribution of rhododendron species in the Sikkim Himalaya.

scarce, for example, *R. leptocarpum* is endangered and reported to have only 16 surviving individuals at present in the region. This may be an indicator of a greater problem, and more species may follow a similar path of disintegration.

Why rhododendrons for biodiversity conservation?

Rhododendrons form dominating species all along the cool temperate, subalpine and alpine zones in the Sikkim Himalaya (Figure 6). It supports a wide range of plants and animals and, if disturbed, can degrade habitats that threaten associated biodiversity. Restoration of rhododendrons and their conservation in nature promotes the existence of other biodiversity components. It provides food preserve for a wide range of birds at an altitudinal gradient. The subalpine to alpine transition zone that includes timberline is the most fragile ecosystem in the Himalaya. Rhododendron is the only group of plants that has continuum in the aforesaid ecotone and beyond doubt, maintains the biological sustenance in this fragile zone.

Figure 3. Rhododendrons in habitat.

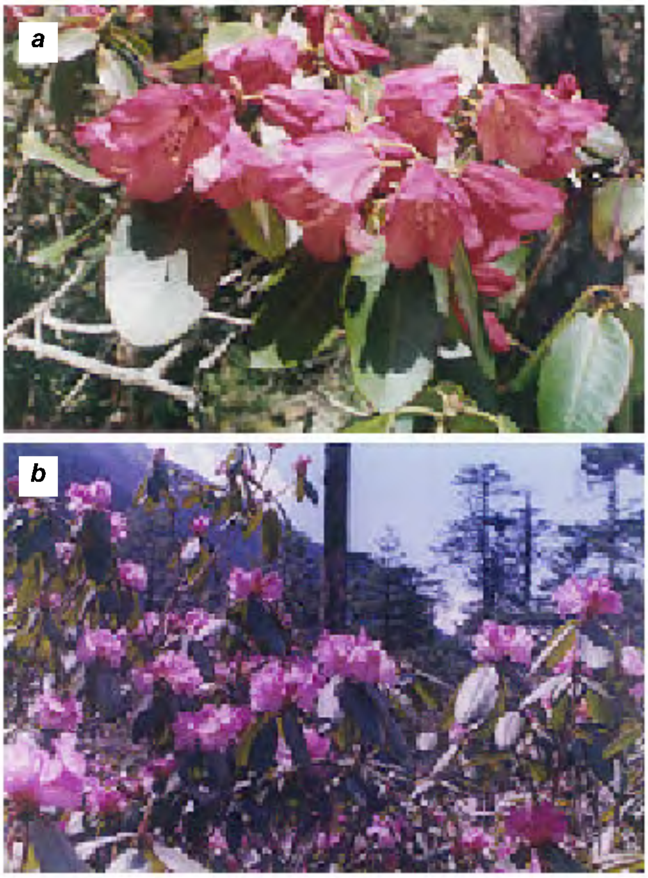

Figure 4. ***a***, *Rhododendron thomsoni*; ***b***, *Rhododendron campanulatum* from north Sikkim.

Conservation initiatives

The Sikkim Himalaya, one of the hot spots of biodiversity, has rhododendrons forming a major dominating group. The conservation initiatives of the government are highly commendable, where large areas have been set aside as protected areas (Table 2). There is one biosphere reserve, two national parks and six wildlife sanctuaries, where the 36 species of rhododendrons of the region are found. The Shingba Rhododendron and the Barsey Rhododendron sanctuaries are exclusively declared as protected areas, keeping in view the conservation of rhododendron species. Simple protection from grazing and human interference allows some of the rhododendrons to naturally regenerate. Other than this, not much has been done. The G.B. Pant Institute of Himalayan Environment and Development (GBPIHED) Sikkim Unit has been evaluating the status of these rhododendrons in nature for the past eight years, and *ex situ* regeneration work is in progress (Figure 7).

Some of the rare and endangered species are now under *in vitro* research procedures. In *R. maddeni*, from five-week-old seedlings, cotyledonary nodal parts were used for shoots multiplication on Anderson[5] medium with antioxidants (PVP, ascorbic acid and citric acid) or without antioxidants, containing 2iP (1.0–15.0 $mg l^{-1}$) along

Figure 5. Degraded rhododendron forest in west Sikkim.

Table 1. Rare and endangered species of rhododendrons in the Sikkim Himalaya

	Availability		Space		
Species	Few	Extremely few	Localized	Extremely localized	Status
R. fulgens		*	*		Rare
*R. leptocarpum**		*		*	Endangered
R. maddeni		*		*	Endangered
R. niveum		*		*	Endangered
R. pendulum		*	*		Rare
R. pumilum		*		*	Endangered
R. sikkimense		*		*	Endangered
R. wighti	*		*		Rare

*Also called *R. micromeres*.

Figure 6. Dominating understorey rhododendrons in cool temperate zone of Sikkim Himalaya.

Figure 7. Germination of rhododendrons under *ex situ* condition (two-year-old seedlings).

Table 2. Protected areas of the Sikkim Himalaya where rhododendrons are commonly found

Place/Name	Area (km^2)
Sikkim	
Khangchendzonga Biosphere Reserve	2619.92
Shingba Rhododendron Sanctuary	43.00
Kyongnosla Alpine Sanctuary	31.00
Fambonglho Wildlife Sanctuary	51.76
Barsey Rhododendron Sanctuary	104.00
Maenam Wildlife Sanctuary	35.34
Darjeeling	
Singalila National Park	78.60
Neora Valley National Park	88.00
Sinchel Wildlife Sanctuary	39.45

with IAA (0.01–1.0 mgl^{-1}). Anderson medium with antioxidants, containing low concentration of 2iP and IAA, was used as maintenance culture. Multiplication of shoots was induced from 3 to 5 nodes of the isolated shoots on Anderson medium containing 2iP with antioxidants within a period of 5–6 weeks. At higher concentration of 2iP, the shoots induced were small and compact, with difference in nodes and internodes. Decreasing the level of cytokinins was suitable for multiplication and elongation of shoots on liquid medium with filter-paper bridge. Shoot production in liquid filter-paper bridge medium was 10-fold higher than that with agar and activated charcoal solidified medium (Figure 8).

Immature leaves and stem explants were transferred on to various concentrations of media containing growth regulators and antioxidants after sterilization. After 4–5 weeks, callus is obtained from 20% stem explants on B5 medium containing BA (0.1–2.0 mgl^{-1}) and 2,4-D (0.1–5.0 mgl^{-1}). Texture of the callus was white-brown, soft and compact; all explants were transferred on to B5 medium containing NAA (0.1–2.5 mgl^{-1}), 2,4,5-T (0.1–2.5 mgl^{-1}) and BA (0.1–5.0 mgl^{-1}). Non-embryogenic callus could be obtained directly on the above medium (Figure 9). Studies on the variation in basal salt medium,

Figure 8. Multiplication of shoots on Anderson medium containing 2iP (7.0 mg l^{-1}) with antioxidants on filter-paper bridge, with agar and activated charcoal.

Figure 9. Callus induction from young stem on B5 medium containing 2,4-D (0.50 mg l^{-1}) and BA (0.25 mg l^{-1}).

salt strength, auxins and cytokinins types or treatments gave no conclusive correlation among salt medium, plant growth regulators and per cent response. There are several problems associated with this plant system before it can be used in biotechnology for mass propagation[6,7].

Douglas[8] described a method for propagation of eight cultivars of *Rhododendron in vitro* using agar-solidified and liquid media. Direct rooting of shoots *in vivo* using expanding bud explants were grown on media used for propagation containing macronutrients, sucrose and adenine sulphate as per Anderson[5], and the micronutrients of Murashige and Skoog medium[9], and vitamins according to Gamborg's[10] B5 formulation (containing 2iP and IAA). Yield of shoots on agar-solidified medium varied with each cultivar; yield in the liquid medium was 10-fold higher than that in the agar-solidified medium. Rooting was increased by 80–90% on pre-treating shoots in a solution of minerals, vitamins and sucrose. Pre-treatment for at least 10 days in a medium supplemented with IBA (5–10 mgl^{-1}) increased the number of roots produced per shoot.

Biotechnological research on *R. maddeni* has been supported by central funding agencies since 1999, but further work is required to reach the next level of *in vitro* programme, i.e. complete establishment of proliferation in culture. In nature, the plant is a slow-growing woody shrub. Higher polyphenols and flavonoids exudation from the explants makes the sterilization and proper establishment of explants difficult. An *ex situ* conservation initiative of the GBPIHED has been the establishment of a rhododendron arboretum at Pangthang (c. 1800 m amsl) near Gangtok in Sikkim. Twenty-four species of rhododendrons are housed here and some of them have started flowering. This is the only *ex situ* conservation initiative on rhododendrons in India. The rare and endangered species mentioned in Table 1 are in process of mass propagation by the GBPIHED for their restoration in nature in the near future.

It may be summarized that the threat to about a quarter of species of rhododendrons from Sikkim Himalaya is an eye-opener towards anthropogenic interference over natural biota and its consequence in the Himalayan region. In a relative sense, it may be argued that more interference would bring about greater disturbances to the rhododendron species and its habitat. Research activities may mitigate the impasse to a certain degree, but overall success depends on the conservation concept engrained in and championed by the local inhabitants and campaigning/ monitoring by government institutions.

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