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In vitro propagation of Kashmir Himalayan Rhododendron (*Rhododendron anthopogon* D.Don)

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ABSTRACT

Rhododendron anthopogon D.Don (Family:Ericaceae), an ornamental and medicinal plant commonly known as 'Talisfer' in Kashmir, is one of the smallest of Rhododendrons. It grows almost 2-3ft high. The flowers are white or yellow, tinged with pink, grow in small compact clusters of 4-6 and each flower is 2 cm across. The leaves are dark green and oval shape and strongly aromatic and densely scaly underneath. The leaves of Rhododendron anthopogon are mixed with Juniper and used as incense in Buddhist monasteries as well as in Hindu religious ceremonies. The species of Rhododendron anthopogon is globally distributed. It grows in the Himalayan range across Pakistan, India, Nepal, Bhutan and SE Tibet between an altitudinal range of 3000-4800m asl. Within India, it has been recorded in Jammu & Kashmir, Himachal Pradesh, Uttar Pradesh, Sikkim and Arunachal Pradesh. During the present study the In vitro propagation of Rhododendron anthopogon has been achieved using seed explants. The seeds were inoculated on Woody Plant basal Medium, M.S basal Medium and Rhododendron Anderson basal Medium. The seeds responded only on Woody Plant basal Medium. Callus was regenerated within 4 weeks of culture from which plantlets regenerated simultaneously.

Keywords: conservation, In vitro, Micropropagation, Rhododendron, Woody plant Medium, .

I. INTRODUCTION

The term 'Rhododendron' has been derived from two Greek words "Rhodo" and "dendron" meaning "rose–tree" (Hora, 1981)[1]. Rhododendron belongs to family Ericaceae and was described for the first time by Carl Linnaeus in 1753 (Cox and Cox, 1997)[2]. The history of Himalayan *Rhododendron* commences with the visit of Captain Hardwick of the Siwalik Mountains of Srinagar, Kashmir in 1796, where he encountered *R. arboreum*. It was described by Sir James Smith in *Exotic Botany* in 1805 (Paul*et.al*2005)[3].

Rhododendrons have a characteristics slow growth rate, ranging in size from tiny mat-like growth few cm in height in alpine region (*R.pumilum*, *R.setosum*) to giants 2.5 m in height (*R.arboreum*). The beautiful, magnificent flowers and evergreen foliage of Rhododendrons have attracted the attention of botanists and horticultural enthusiasts throughout the world. Today, nearly 50% of the species are under cultivation world wide and about 5000 to 6000

hybrids of Rhododendrons have already been developed. These hybrids are sold in the market at high cost. They are mainly grown in gardens, parks and other important places for their showy and attractive flowers. In addition to that it has medicinal and aromatic importance as well as fuel wood values (Paul*et.al.*2005)[3].

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 fire wood 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 (Sekar and Srivastava, 2010)[4].

The present study has been carried out to develop micropropagation protocol for *Rhododendron anthopogon* through *in vitro* seed germination for its large scale propagation.

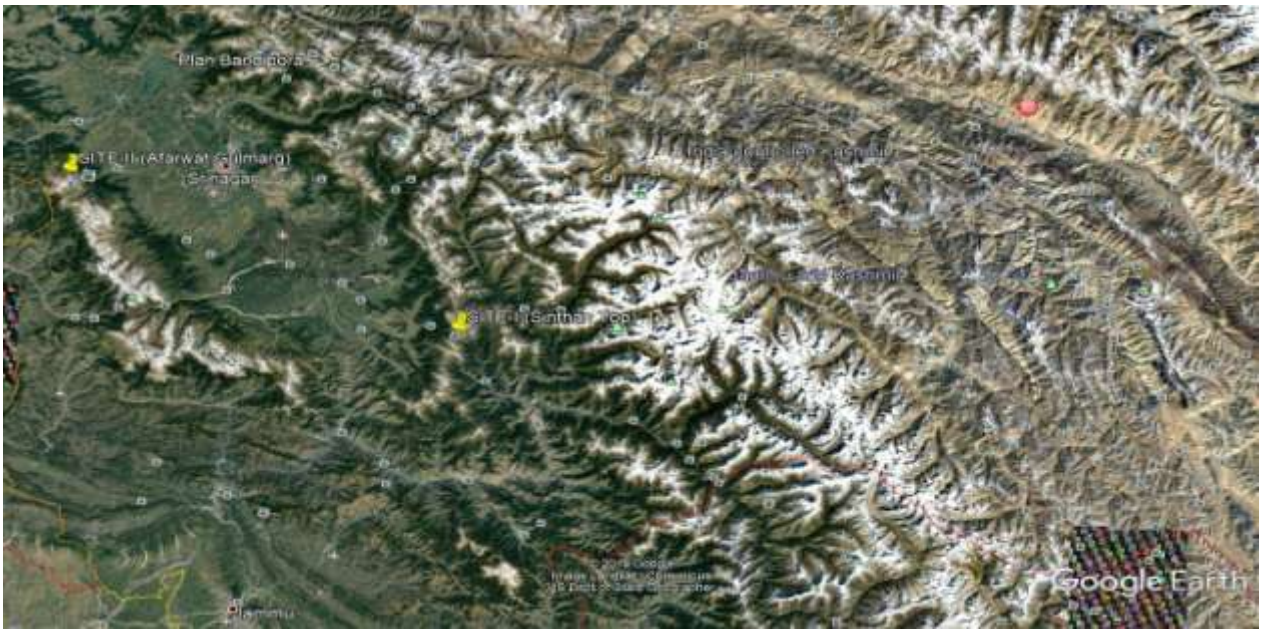
Rhododendron anthopogon D.Don (Fig:2) is an ornamental and medicinal plant commonly known as '*Talisfer'* in Kashmir. The species of *Rhododendron anthopogon* was discovered by David Don, a British botanist. This plant is found in the Himalayan region from Kashmir to Bhutan at an altitude of 3000-520m asl. The leaves are crowded towards the end of branches, shortly stalked, leathery, entire and are 2.5-4 cm long. The flowers are yellow and in dense, terminal corymbs. *Rhododendron anthopogon* is highly aromatic shrub and can easily be identified by its sulphur coloured flowers and salver-shaped corolla as well as by its aromatic odour. The leaves of this species possess stimulant properties and generally both the flowers and the leaves of this plant are used as medicine. This plant is used for cold treatment. The flowers are anti-tussive, febrifuge and tonic, mostly used in the treatment of inflammations, lung disorders and general weakness of the body. *Rhododendron anthopogon* is commonly known as dwarf Rhododendron and mainly used in making an essential oil. Anthopogon oil, is obtained by steam distillation of the aerial part of this shrub. *Rhododendron anthopogon* can be used in gouty rheumatic conditions.

II. MATERIAL AND METHOD

1. Collection of seeds:

The seeds of *R.anthopogon* were collected from natural alpine habitats of Kashmir Himalayan region. The alpine areas of Kashmir Himalayan region from where collections were made included Site–I – Sinthan Top (Kokernag) and Site-II –Afarwat (Gulmarg). (Fig: 1)

The freshly collected seeds were first tested for seed viability. Seed viability was determined by tetrazolium test .Seeds were soaked in distilled water for 24 hours and later incubated in dark in 1% aqueous solution of 2,3,5 tri phenyl tetrazolium chloride for 24 hours. Seeds showing strongly stained red embryos were considered viable and unstained seeds were considered non-viable. The stained seeds were then examined under sterio zoom microscope (Fig:3)



(Fig 1: Map showing collection sites.) Site -1: Sinthan top (Kokernag),

Site -2: Afarwat (Gulmarg)

2. *In vitro* seed germination:

The seeds of *Rhododendron anthopogon* are small in size(fig:4) The viable seeds were kept in falcon tubes for washing using tap water. With the help of 5ml syringes seed washing procedure was done as the seeds were minute. This was followed by washing with 2% labolene (lab detergent) solution and surfactant (Tween-20). After this procedure, seeds were washed with double distilled water and there after the seeds were treated with chemical sterilant (2% sodium hypochlorite) for 10 minutes. This was followed by washing with autoclaved double distilled water under laminar air flow hood. Rhododendron Anderson basal medium, M S basal Medium and Woody Plant basal Medium were used for *in vitro* seed germination studies. The pH of the media were adjusted to 5.8 and were gelled with 0.8% agar. After boiling the media about 30 ml of media were dispensed in 100ml conical flasks and autoclaved at 121 ºC for 25 minutes. Inoculation of the seeds in the culture flasks was carried out in aseptic environmental conditions inside laminar air flow hood. The cultures were incubated in the culture room, in artificial light provided by fluorescent tubes of 500 lux, 16-hour photoperiod and the temperature was maintained at 24-26ºC. The humidity was maintained between 50-60%. Observations were made from the seventh day of inoculation. The parameters recorded include formation of the callus, callus characteristics like colour, texture etc. and regeneration of plantlets from callus.

III. RESULTS

In the beginning the response of seeds on all three media was very slow and finally seeds responded on basal Woody Plant Medium by forming callus and plantlets simultaneously after four weeks of culture (fig.5). The callus formed was friable, creamish in colour and embryogenic in nature. Large number of plantlets regenerated after six weeks of culture (fig: 6). The mean length of shoots was approximately 1cm. The regenerated plantlets were transferred to plastic pots containing potting mix like coco peat and perlite in1:1 ratio. These pots were kept in greenhouse where these plantlets got hardened after five weeks of transplantation (fig:7).

Fig 2:Rhododendron anthopogon (mother plant).

Fig3:Viable seeds under steriozoom microscope.

Fig 4: Seeds of Rhododendron anthopogon.

Fig 5: Seed Germination and formation of callus and plantlets on Basal Woody Plant medium after 4 weeks of culture.

Fig 6: Regeneration of plantlets from callus after six weeks of culture on Basal Woody Plant medium.

Fig7: pots containing plantlets kept in green house for hardening.

IV. CONLUSION

The field of tissue culture is based on the fact that plants can be separated into their component parts which can be manipulated *in vitro* and then grown back into complete plants .An efficient in vitro propagation protocol has been developed using seeds of *Rhododendron anthopogon* as explants. Among the different basal media used, Woody Plant basal medium is best medium for callus formation and regeneration of plantlets. The regenerated plants has

been transferred to pots containing potting mix coco peat and perlite were kept in green house .The plantlets gets hardened after five weeks of transplantation.

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