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# Auxin induced germination and plantlet regeneration via Rhizome section culture in Spiranthes sinensis (Pers.) Ames: A Vulnerable medicinal Orchid of Kashmir Himalaya

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***Abstract:*** *For the conservation of temperate orchids of Kashmir Himalaya various steps were carried out and a number of orchid speciesgrowing in the Kashmir Himalaya were subjected to ex-situ conservation strategies using different plant parts like pseudobulbs, adventitious roots, tubers and rhizome sections etc. Auxins particularly IBA have been found to induce germination and plantlet regeneration in rhizome sections of Spiranthes sinensis (Pers.) Ames. The apical bud elongated and developed into shoot in pots. The shoots grew well and flowered in 2-3 months. The results obtained suggest that this vulnerable medicinal orchid can be propagated easily through rhizome sectionsso as to achieve its mass multiplication.*

***Keywords:*** *Orchids, vegetative propagation; Spiranthes sinensis (Pers.) Ames; Rhizome sections*

## I. Introduction

Orchids have a great importance in the field of floriculture because of their multi-coloured curious shaped flowers with long lasting quality. Some of the species exhibit lot of variation in the wild populations which are very much useful for the breeding programmes to develop hybrids of commercial value in the International market. The use of orchids in herbal medicines has also a very long history. There is not a single ailment for which, therapeutic and curative elements are not found in one or the other orchid species. Orchids also hold great repute as medicine with strengthening and demulcent qualities in 'Ayurveda'. Orchids are also grown as a cash crop in several countries including Thailand, Malaysia, Singapore South Korea and Sri Lanka (Vij and Pathak, 2012). Kashmir Himalaya harbors a rich diversity of valuable Orchids, and attempts are being made at different levels for sustainable utilization of this resource in order to develop the medicinal plants sector. Majority of the species collected are terrestrial and mostly growing in shady coniferous forests of sub-alpines. The orchid species inhabit three types of habitats viz: marshy lands, shady coniferous forests of sub-alpines and open moist land of alpines. The maximum representation of orchid species occurs between altitudinal gradient of 2000-3000m asl. This zone provides all the suitable condition for the growth and development of orchids (Jalal, 2012). The low species richness of orchids at lower altitudes may be attributed to little rain fall and higher temperature because soils with less moisture and high temperature does not favour orchid growth. Terrestrial orchids usually grow at the places where sufficient shade and moisture is available (Jalal et al., 2010). Some orchids prefer the places with high moisture, shade and humus rich soils (Jalal et al., 2010).

*Spiranthes sinensis* (Pers.) Ames is a terrestrial orchid species found in open grassy slopes of submountain to mountain regions of Kashmir Himalaya. *Spiranthes sinensis* (Pers.) Ames plants are perennial short-lived, terrestrial, autotrophic or rarely mycotrophic herbs, with rhizomes, tubers, or rootstocks with mycorrhizal fungi in roots. The plants usually flower in the month of May. The flowers are arranged in a helix around the spike, and flower sequentially. The flowers are small, no more than 6mm across. Seed formation is rapid, probably taking just a few weeks. It has many medicinal values like decoction of plant is given in intermittent fever. Fresh tubers are edible and used as tonic.

Orchid seeds commonly require four to five years or more to reach flowering stage. Also it has been reported that they possess only 0.3% seed germination ratio in nature. It is also one of the reasons that this family Orchidaceae consists of most number of the threatened taxa in nature. So far, no work has been done on micropropagation of *Spiranthes* species using tissues other than seeds and protocorms. It is in this context that the root tips, rhizome tips or segments, divided protocorms and leaves (bases, tips) are some tissues which were explored as explants in the present *in vitro* studies and out of these explants only rhizome sections responded. Hence a successful protocol was developed for the regeneration of *Spiranthes sinensis*(Pers.) Ames via rhizome section culture.

## II. Materials And Methods

Field trips were organized to collect fresh plants from the natural habitat. Explants include leaves, stems and rhizome were washed under running water for 30min. and then immersed in an aqueous solution of 5% (1:1 v/v) liquid detergent (Laboline, Qualigens, India) for 5–6 min followed by rinsing five to six times with distilled water.The rhizomes were cut longitudinally into smaller sections each having a bud scar. These rhizome sections were then surface-sterilized with an aqueous solution of 0.1% Mercuric chloride solution and rinsed five to six times with sterile double distilled water and were used for regeneration studies. Rhizome sections were cultured on different concentrations of growth hormones like Indole Acetic Acid (IAA), Indole Butyric Acid (IBA), Napthylene Acetic Acid (NAA), Gibberellic Acid ($GA_3$), and Cytokinin. 5ml of medium were dispensed into sterilized petri dishes and thereafter sealed with parafilm. After 72hours of culture, the percentage of surviving explants, the percentage of bud formation, the number of plantlets and length of plantlets was recorded.

## III. Results And Disscussion

The number of orchid species is rapidly and steadily declining because of their low rate of propagation in nature and the ongoing collection from nature. Careless collection of these species has led to serious genetic and ecological erosion; many have already been listed as endangered species (Ozhatay, 2000; Clements, 2003; Machaka-Houri et al., 2012). Therefore, *in vitro* propagation could be useful for the mass propagation of orchids for commercial purposes. Tissue culture techniques have been widely used for the *in vitro* mass propagation of several commercially important orchids over the past few decades (Chen and Chang, 2000, 2004). Use of fresh flowers in everyday life represents a quality lifestyle and of late, the orchids have come to play a lead role among the much valued cut flowers. Bud production on the roots has been observed in many temperate terrestrials, which would make propagation by division successful (Rasmussen, 1995). In some temperate terrestrial orchid species a root tip meristem can even transform directly into a shoot meristem (Rasmussen, 1986; Rasmussen, 1995). Since the propagation of orchids in nature is slow as only 0.3% of germination of seeds has been reported, hence propagation of orchids from seeds is a complicated process.The rhizome was cut longitudinally with sharp surgical blade into many sections each having one or more than one upto three buds under aseptic conditions. Therefore in the present study clonal propagation via rhizome section culture was tested for the threatened orchid *Spiranthes sinensis* (Persoon) Ames. The explants (rhizome sections) were cultured on different concentrations of growth hormones like Indole Acetic Acid(IAA), Indole Butyric Acid (IBA), Naphthalene Acetic Acid (NAA), Gibberellic Acid ($GA_3$) and Cytokinin. Nearly 5ml of medium were dispensed into sterilized petri dishes and thereafter sealed with parafilm. Afterwards the following observations were recorded.

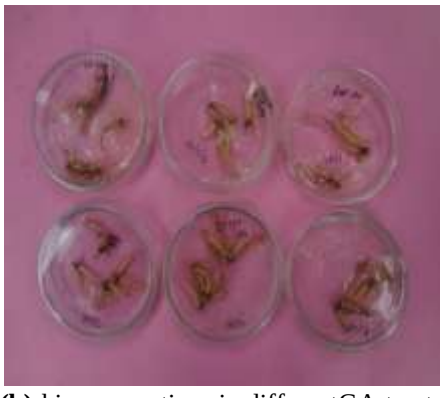

**Fig.(a)**Habit of Spiranthes sinensis**Fig.(b)**rhizome sections in different$GA_3$treatments

**Fig.(c)** rhizome sections in different IB Atreatments**Fig.(d)** rhizome sections in different BA Ptreatments

**Fig.(e**) rhizome sections in different IB Atreatments

**Fig.(f)** regenerated plantlets in pots**Fig.(g)** regenerated plantlets in pots

**Fig.(h)** regenerated plantlets in pots **Fig.(i)** regenerated plantlets in pots **Fig.(j)**flowering in regenerated plantlets in pots

**Table: 1 Effects of different hormones on rhizome germination and plantlet development in *Spiranthes sinensis* (Pers.) Ames (Data obtained after 72 hours of culture.)**

| Hormone | Hormone Concentration (ppm) | Percentage of bud formation/ (%) | Germination | Survival (%) | Length of plantlets (cm) |
|---|---|---|---|---|---|
| IBA | 50 | - | - | - | - |
| | 100 | 100 | Good | 100 | 0.6±0.02 |
| | 200 | 40 | Little | 100 | 0.1±0.001 |
| IAA | 50 | 100 | Good | 100 | 0.4±0.02 |
| | 100 | 40 | Little | 100 | 0.2±0.001 |
| | 200 | - | - | - | - |
| BAP | 50 | - | - | - | - |
| | 100 | 40 | Little | 100 | 0.2±0.001 |
| | 200 | - | - | - | - |
| $GA_3$ | 250 | - | - | - | - |
| | 500 | 90 | Good | 90 | 1.0±0.04 |
| | 750 | 40 | Little | 100 | 0.2±0.001 |
| Distilled Water | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| (Control) | - | - | - | - | - |

30 replicates per treatment

From the above table it is clear that IBA at a concentration of 100ppm favoured good germination of rhizome explants with cent percent of survival rate. The length of shoot measured was 0.6cm.Also $GA_3$ at a concentration of 500ppm favoured good germination of rhizome explants with 90% percent of survival rate. The length of shoot measured 1.0cm. Similarly IAA and BAP at a concentration of 100ppm favoure dlittle germination of rhizome explants with cent percent of survival rate. The length of shoo tmeasured was 0.2cm.

The highest plantlet length (1.0cm) was observed in case of 500ppm of $GA_3$ treatment followed by the 100ppm IBA concentration treatment (0.6)with 90% of both germination and survival rate. However in case of 100ppm of IBA treatment there was cent percent of germination as well as survival rate. So it was concluded that auxins particularly IBA at 100ppm was responsible for the development of plantlets and the protocol can be used for the mass multiplication of this orchid.

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