



***In vitro* Propagation of *Dendrobium jerdonianum* Wight Through Flower Stalk, Leaf, Nodes/ Internodes as Explants**

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Abstract: *Dendrobium jerdonianum* Wight is an Epiphytic tufted orchid at higher elevations. The maximum percentage establishment of Flower stalk, leaf, node and internode explants was observed on MS, VW and KC Medium. However, MS media fortified with activated charcoal exhibited maximum multiplication rate for *in vitro* rooting. MS, VW and KC media supplemented with various concentrations of auxins and cytokinins were used in flower stalk, leaf, nodes and internodes for plantlet formation. In the evaluation of the media MS basal medium fortified with 0.5mg 2, 4, D-dichloro phenoxy acetic acid + 5mg BAP + 50ml suitable for flower stalk culture. KC medium equipped with 0.5mg 2, 4, D-dichloro phenoxy acetic acid was found to be suitable for Leaf and internodes Culture. VW medium equipped with 0.5mg 2, 4, D-dichloro phenoxy acetic acid + 3mg BAP + 50ml CM, was found to be suitable for nodal segments. The entire above medium used with different composition gives highest percentage of 80-95 percent results for plantlet formation. *In vitro* rooting was successful with MS medium supplemented with 0.5mg 2, 4, D-dichloro phenoxy acetic acid + 5mg IAA + 50ml CM and 500mg of activated charcoal. 90 days old *in vitro* plantlets were hardened and transferred to green house after *ex vitro* rooting technique. Significance of the present work is discussed here.

Keywords: Plantlet Formation, Explants, *Dendrobium jerdonianum*, Propagation, Establishment

1. Introduction

The Western Ghats is one of the well-known wildlife centres in India, for its many protected areas, wild locations and beautiful scenery (Mohan, M. and Balakrishna, N. P. 1991) [12]. The Western Ghats is also known as one of the richest areas in the world in terms of biodiversity, making it the one-among-25 Hotspots of the world (Mittermeier et al., 2000) [11] along with Sri Lanka. Amongst the various components the bio geographical area can boast are high endemism, taxonomic uniqueness, possibly yet to be discovered flora and fauna, ca. 1,500 endemic angiosperm taxa (Nayar, 1996) [13]. *Dendrobium jerdonianum* Wight belongs to the family Orchidaceae which comprises the genus *Dendrobium*. The endemism in the flora of a country or geographical region provides an important insight into the biogeography of that region and also to the centres of diversity and adaptive evolution of the floristic components

of that region. *Dendrobium jerdonianum* is a threatened orchid of Western Ghats (Govaerts et al., 2012) [8]. A large concentration of endemic species is found in the tropical moist deciduous and tropical semi evergreen patches of Western Ghats and to a much lesser degree in Eastern Ghats. For the present analysis information on the endemic orchids of peninsular region was collected from literature such as (Hooker 1888-1890) [9], (Blatter, 1928) [5]. The present work is our modest attempt to give an up-to date account of the endemic orchids of the peninsular region and to include nomenclature changes, new distributional records and new species records (Ahmedullah, M. & Nayar, M. P. 1987) [1].

In vitro studies through stimulation of flower stalk, nodes, internodes, and leaves as explants using various additives (Park, S. Y., Murthy, H. N & Paek, K. Y. 2002) [15]. In propagation method of Plant tissue culture technique, only small pieces of plant tissue are required to regenerate on plant tissue culture medium under sterile conditions (CSS451 Plant Tissue Culture, 2010) [6]. The plant tissues used are

flower stalk, internodes, nodes, leaves etc. The most suitable Medium rate for the *Dendrobium jerdonianum* Wight is Murashige and Skoog (MS) medium. Efficient *in vitro* regeneration of *Dendrobium jerdonianum* Wight was achieved from flower stalk, node, internode and leaf explants on MS, VW and KC medium with different concentrations and combinations like BAP, IAA and 2,4-D. The maximum numbers of multiple shoots were achieved from flower stalk, leaves, nodal and inter nodal explants. Rooted plantlets were successfully acclimatized (Arditti, J. and Ernst, R. 1993) [4].

Dendrobium jerdonianum Wight

Dendrobium jerdonianum Wight, Stem ribbed, Sickle shaped and grooved, sheathed with minute black hair. Flowers 2 to 3 on considered peduncle, leaf opposed, flowers yellow on long pedicel, lip 3 lobed marked. Found in southern India and Sri Lanka at elevations of 800 to 2000 meters as a miniature sized (Fay, M. E 1994) [7], warm to cool growing epiphyte with grooved, swollen towards the apex, zigzag with few too many angles, yellowish, black hairy stems carrying 3 to 6, distichous, succulent, stiffly leathery, dark green, linear-lanceolate to linear-oblong, apically bilobed leaves that bloom in the later spring, summer and earlier fall on 2 to 3, short, from opposite and above the leaf axils 2 to 4 flowered inflorescence. (W3 Tropicos, Kew Monocot list, International Plant Names Index (IPNI)) [17].

Flowers are delicate, orange, in leaf opposed 3-4-flowered, short racemes. Petals are 14 x 2-3 mm, oblong-lance-shaped, pointed, 5-veined, lip 17 x 4.6 mm, obovate-lance-shaped, 3-lobed, lateral lobes oblong, rounded; midlobe ovate-lance-shaped, pointed, disc 4-lamellate. Flower-stalks are 1.3 cm long. Dorsal sepal is 15.5 x 2-3 mm, oblong-lance-shaped, pointed, 5-veined, lateral sepals 13 x 2-3, linear-lance-shaped, pointed, 5-veined. Stem 20-25 cm long, terete, tufted. Leaves 3.5-4 x 0.9-1 cm, sessile, ovate-lanceolate, obliquely lobed at the tip. (Orange Kerala *Dendrobium*, <http://www.flowersofindia.net/>) [14].

2. Materials and Methods

It is distributed in Kodagu Hassan, Chickmangalur, and Kudremukh in Karnataka, Kerala, Tamil Nadu, and Sri Lanka. Stem is not straight, but slender and rigid. Flower Orange not spreading fully ovary with pedicel. Flowering and fruiting is in the month of April to November. The healthy plants of *Dendrobium jerdonianum* Wight were collected from Kodagu (Arditti, J. 1977) [3] and raised in pots containing soil and farm yard manure in greenhouse in the Department of Botany St. Joseph's college for Research centre, Bangalore, Karnataka.

2.1. Surface Sterilization of Explants

Surface sterilization of explants, treatment involving HgCl₂ (0.1%) for 3 min gave better Results. sterilization of flower stalk, leaf, Single node explants (1-2 cm), internodes (0.5-1.0cm) were dissected out, washed once in sterile distilled water and taken to the inoculation room for *in vitro* culturing using following protocols.

2.2. Protocols for Flower Stalk, Leaf, Nodes and Internodes Culture

2.2.1. Flower Stalk Culture

Flower stalk segments of *Dendrobium jerdonianum* Lindl were collected from Kodagu and plants grown in the greenhouse of the St. Joseph's College. Limitations of availability of explants were encountered in other two species. Explants were washed under running tap water and followed by a 5% liquid detergent for 5 min. After thorough wash in double distilled water, the flower stalk segments were sterilized using 0.1% HgCl₂ solution for 12 min. They were then washed thoroughly with sterile double distilled water. Sterilized flower stalk segments were cut into a size of 10-15 mm with one bud were cultured on various media.

2.2.2. Leaf Culture

Leaf of *Dendrobium jerdonianum* Lindl were surface sterilized by cleaning thoroughly under running tap water and washed with a 5% tepol for 5 minutes and rinsed with sterile distilled water for 3 minutes. The cleaned explants were finally treated with HgCl₂ for 3 minutes under aseptic conditions and washed three times with sterile distilled water to remove traces of HgCl₂.

After surface sterilization, leaf explants were trimmed and inoculated on MS, B5, VW and KC basal medium supplemented with various concentrations of phytohormones and coconut milk. KC medium gave 90% of plantlets compared to other medium.

2.2.3. Nodes / Internodes

Nodes / Internodes segments of *Dendrobium jerdonianum* were used to initiate shoot cultures. These explants were cut into pieces of about 2 – 3 cm and kept in a conical flask. They were then washed in a detergent (Tepol) solution for 20 min before being rinsed in distilled water. Next, the explants were transferred to a laminar air flow chamber, and sterilized with 20% of HgCl₂ for 5 - 20 min. They were then washed thoroughly with sterile double distilled water to remove the traces of HgCl₂ and blotted over sterile filter paper discs before transfer to nutrient medium. The explants were implanted vertically on the nutrient medium (Aktat, S. Nasiruddin, K. M. & Huq, H. 2007) [2].

2.2.4. In vitro Rooting

A protocol was developed for *in vitro* rooting of *Dendrobium jerdonianum* Wight for the plantlets from Flower, Leaf, Node and Internode. MS medium with the composition of BAP and IAA were evaluated in the presence of activated charcoal. A combination of MS medium supplemented with 0.5mg BAP + 5mg IAA + 50ml CM and 500 mg of activated charcoal were proved excellent for *in vitro* rooting.

2.2.5. Ex vitro Rooting

In vitro rooted healthy shoots from the shoot multiplication medium were separated from were dipped in appropriate concentrations of IAA for 10 minutes.

2.2.6. Hardening Technique

Plants treated with IAA for *ex vitro* rooting were further subjected to Bavistin treatment. Plants were dipped in Bavistin solution (0.05%) for 5 minutes and then were planted in small thumb pots containing solrite (potting mix) to avoid fungal infection. These were transferred to plastic trays which can hold 50pots together and further covered with perforated plastic cups individually to each thumb pot to maintain humidity. This condition is maintained for 15 days

under open laboratory conditions of light and temperature before transferring to the green house conditions.

2.2.7. Acclimatization

Standard protocols have been developed to acclimatize the plants under greenhouse conditions.

3. Observations

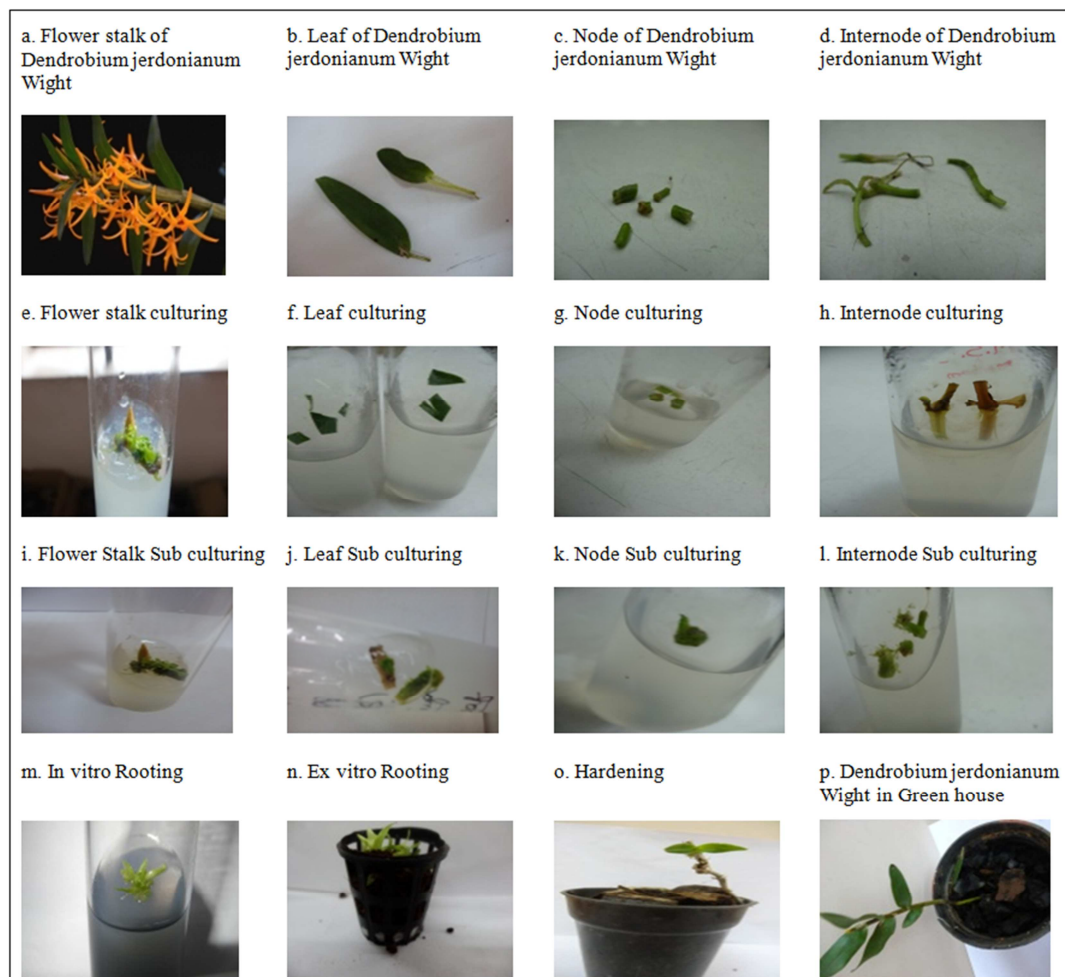


Figure 1. Stages of growth of explants of *Dendrobium jerdonianum*.

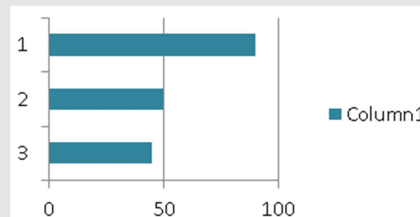
4. Results and Discussion

The present analysis resulted in the multiplication of plantlets from *Dendrobium jerdonianum* Wight using Flower stalk, nodes, internodes and leaves as explants. The plantlets were made to grow healthily in the Tissue Culture bottles using various additives with each different medium for the explants as Figure 1 shows clearly the growth as various stages. Table 1 - Table 5 shows the medium used and the best results obtained for a particular composition clearly showing the growth is maximum in certain composition giving 90-95% results. In Table 1 the best results is from MS medium with media composition of 0.5mg 2,4,D-dichloro phenoxy acetic acid +5 mg BAP +50 ml CM, Table 2 KC medium

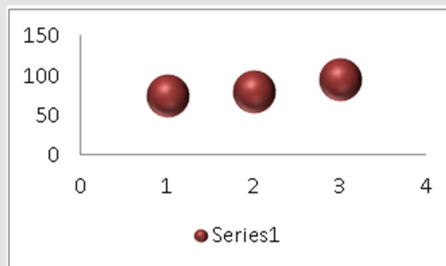
with media composition of 0.5mg 2,4,D-dichloro phenoxy acetic acid +3 mg BAP +50 ml CM gave the best results. From Table 3 VW medium with media composition of 0.5mg 2,4,D-dichloro phenoxy acetic acid +3 mg BAP +50 ml CM gave the best results. From Table 4 KC medium with media composition of 0.5mg 2,4,D-dichloro phenoxy acetic acid +5 mg BAP + 50ml CM gave the best results. From Table 5 MS medium with media composition of 0.5mg 2,4,D-dichloro phenoxy acetic acid +5 mg IAA + 500mg of activated charcoal and 50ml CM gave the best results for *in vitro* rooting. Figure 2 gives a clear picture of the comparative study of the medium used. According to the results VW medium supplemented with 0.5mg 2,4,D-dichloro phenoxy acetic acid +3 mg BAP +50 ml CM gave the best results for the plantlet formation.

Table 1. Flower Stalk Culture.

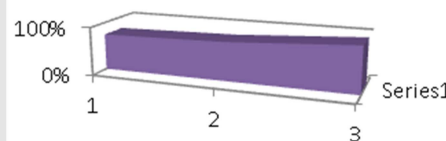
Media used	Media composition	Average plantlet formation (percentage)
MS	0.5mg 2,4,D-dichloro phenoxy acetic acid +5 mg BAP +50 ml CM	90%
KC	1.5mg 2,4,D-dichloro phenoxy acetic acid +3 mg BAP + 50 ml CM	50%
VW	1mg 2,4,D-dichloro phenoxy acetic acid +2 mg BAP + 50ml CM	45%

**Table 2.** Leaf Culture.

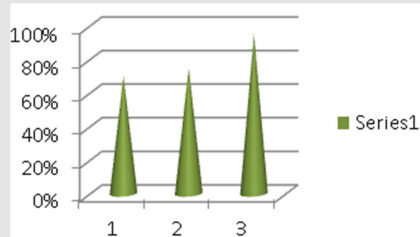
Media used	Media composition	Average plantlet formation (percentage)
MS	1mg 2,4,D-dichloro phenoxy acetic acid +1mg BAP + 50 ml CM	75%
KC	0.5mg 2,4,D-dichloro phenoxy acetic acid +3 mg BAP +50 ml CM	90%
VW	1.5mg 2,4,D-dichloro phenoxy acetic acid +2 mg BAP + 50ml CM	80%

**Table 3.** For Nodes.

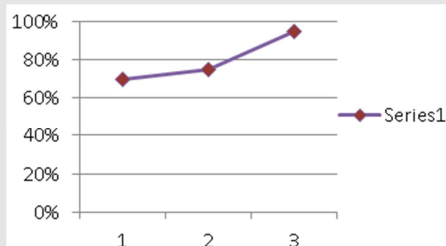
Media used	Media composition	Average plantlet formation (percentage)
MS	2mg 2,4,D-dichloro phenoxy acetic acid +1 mg BAP + 50ml CM	60%
KC	1mg 2,4,D-dichloro phenoxy acetic acid +2mg BAP + 50 ml CM	70%
VW	0.5mg 2,4,D-dichloro phenoxy acetic acid +3 mg BAP +50 ml CM	95%

**Table 4.** For Internodes.

Media Used	Media Composition	Average plantlet formation (percentage)
MS	1.5mg 2,4,D-dichloro phenoxy acetic acid +2 mg BAP + 50ml CM	75%
VW	1mg 2,4,D-dichloro phenoxy acetic acid +3 mg BAP + 50ml Cm.	80%
KC	0.5mg 2,4,D-dichloro phenoxy acetic acid +5 mg BAP + 50ml CM.	90%

**Table 5.** For the In vitro Rooting.

Media used	Media composition	Average plantlet formation (percentage)
	1.5mg 2,4,D-dichloro phenoxy acetic acid +2 mg IAA + 50ml CM	70%
MS	1mg 2,4,D-dichloro phenoxy acetic acid +3 mg IAA + 50ml CM	75%
	0.5mg 2,4,D-dichloro phenoxy acetic acid +5 mg IAA + 500mg of activated charcoal and 50ml CM	90%



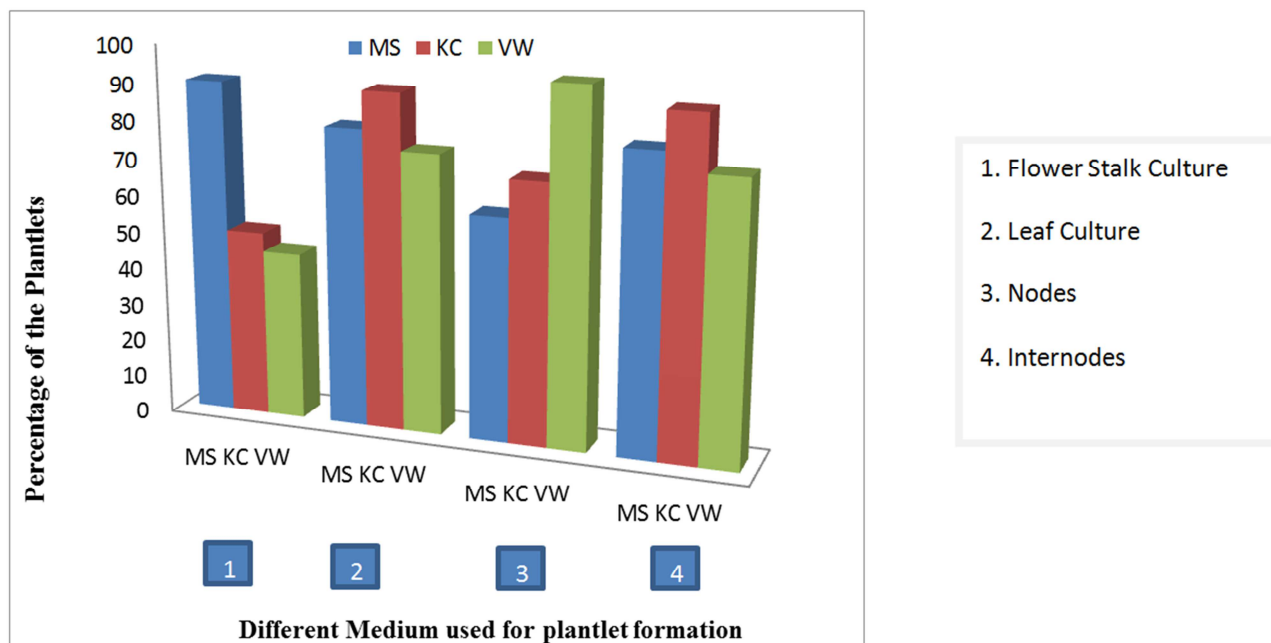


Figure 2. Comparative study of the medium used.

5. Conclusion

The present investigation on *in vitro* mass multiplication of propagation of *Dendrobium jerdonianum* Wight through flower stalk, nodes, internodes and leaves as explants clearly demonstrated its potentiality for rapid propagation with (Figure 1) clearly explains the various stages of growth of explants. And finally being shifted to the green house. It is estimated that using the present protocol of *in vitro* propagation, large number of plantlets can be produced from flower stalk, nodes, internodes and leaf as explants using MS medium with composition of 0.5mg 2,4,D-dichloro phenoxy acetic acid +5 mg BAP +50 ml CM (Table 1), KC medium with 0.5mg 2,4,D-dichloro phenoxy acetic acid +3 mg BAP +50 ml CM (Table 2), VW medium with 0.5mg 2,4,D-dichloro phenoxy acetic acid +3 mg BAP +50 ml CM (Table 3), KC medium with 0.5mg 2,4,D-dichloro phenoxy acetic acid +5 mg BAP + 50ml CM (Table 4) The above tables shows the results for plantlet formation. MS medium with composition of 0.5mg 2,4,D-dichloro phenoxy acetic acid +5 mg IAA + 500mg of activated charcoal and 50ml CM (Table 5) was suitable for *in vitro* rooting for *Dendrobium jerdonianum* Wight. According to the results obtained VW medium for nodal segment as explants gave 95% of plantlets compared to MS and KC medium with 90% plantlet formation. Therefore VW medium is suitable for orchid propagation which has given 95% results (Figure 2). The major concentrations of endemic orchid species are found in the Western Ghats (Subramanian, & Nayar, 1974) [16] are Agasthyamalai Hills, Anamalai-High Ranges, Nilgiris-Silent and Valley-Wynad-Kodagu region. 95 endemic orchid species are particularly restricted to these areas. Eastern Ghats have geological antiquity with isolated mountain ranges. *Dendrobium jerdonianum* (Wight) have very wide

distribution in the peninsular region. The endemic orchids of the peninsular region are facing various kinds of localized threats like livestock grazing and forest fires as well as landscape-level threats such as mining, construction of roads, large as well as micro-hydral power projects, wind farms, large-scale agricultural expansion and creation of monoculture plantation. To cite an example *Dendrobium jerdonianum* which was once found in plenty in southern India is now difficult to locate (Jeewan Singh Jalal, & Jayanthi, J. 2012) [10].

ABBREVIATIONS: VW - Vacin and Went medium, MS- Murashige and Skoog medium, KC – Knudson C, NAA – Naphthalene Acetic Acid, IAA – Indole Acetic Acid, BAP – Benzyl Amino Purine, 2,4,D-dichloro phenoxy acetic acid, AC – Activated Charcoal & CM–Coconut Milk.

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