

In vitro plant growth and rooting of *Dendrobium nobile* using different growth hormones concentration

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Abstract Orchid culture is a widely debated topic among scientists. Along the time, experiments were made on different parts of the orchid like the shoot tip, flower stalk, root and the leaf. The *Dendrobium* is currently one of the most studied species, achieving the best results in vitro cultures. This paper follows *Dendrobium nobile* multiplication in vitro by culturing canes on Murashige-Skoog medium supplemented with various growth hormones for the purpose of obtaining a large quantity of plants in a short period of time.

Key words

orchid, *Dendrobium*, propagation, modern, steams

Dendrobium is the second largest genus in the family Orchidaceae. It exhibits a vast diversity in vegetative and floral characteristics and is of considerable interest due to its broad geographic distribution and high value of hybrids as a floricultural commodity (2).

Dendrobium nobile is an immensely popular ornamental orchid; plant breeders have raised numerous cultivars with showy inflorescences (6). It is also valued in traditional Chinese medicine. It is used to nourish and stimulate the stomach. A tea or a tonic ar made from the stems of this and other *Dendrobium* species and is taken for complaints such as fever, sunstroke and excessive perspiration and for promoting the production of body fluid (3).

The name of the species derives from two greek terms: "dendros" = tree and "bios" = life, which would mean "plant that lives on trees", referring to the fact that the plants are epiphytic and root themselves in tree hollows from tropical forests (5).

The pseudobulbs of *Dendrobium nobile* are swollen at the nodes, giving a jointed appearance, and grow up to 60 cm long in upright clusters. The leaves are bright green, strap-shaped and slightly leathery. Leaves are borne alternately at nodes along most of the length of the stem. The fragrant, waxy flowers are 5-7 cm in diameter and are variable in colour. The flowers normally appear in late winter to spring and can last up to six weeks (4).

The first effort to culture any part of a *Dendrobium* (except seeds) in vitro by a „partially sterile” method was by Itsuhiko Ito at the Laboratory of Olericulture, Kyoto Prefectural University, Japan. The procedure was not used for clonal propagation, but fertile seeds were produced from pollinated ovaries cultured in test tubes (1).

Materials and Methods

For this project, 5 mature *Dendrobium nobile* specimens were used, from which young and vigorous stems have been harvested for further experiments. The best time to perform this experiment proved to be spring, when the plants are in full growth.

Vegetal material used: long cane-shaped stems were cut in sections, each having 2-3 cm with a node on each fragment. Out of the 19 stems harvested from the 5 specimens resulted in a total of 42 segments. The first two nodes on the bottom of the stem and the last two on the top were not used, thus each stem resulting in 2-3 segments with 1 node.

Sterilizing the plant material: leaves and dried pods were removed from the segments, which were afterwards washed with water and detergent to remove any remaining impurities from the surface of the segments. Sterilization was carried out in several stages: for 10 minute the material had been kept in a solution based on 5% sodium hypochlorite (NaOCl). Afterwards it was washed with sterile distilled water and moved for 10 seconds in alcohol with a 70% concentration - which is the best to use and 10 minutes in mercuric chloride (HgCl₂) of 0.1% concentration. At the end of the procedure the material is cleansed off with sterile distilled water 5-6 times, 5 seconds at a time.

Growth medium: as a base a Murashige and Skoog medium has been used (Murashige and Skoog, 1962) with an addition of Naphthaleneacetic acid (NAA) and 6-Benzylaminopurine (BAP) in different concentrations, resulting in 3 different growth medium variations (Table 1). The pH value of the nutrient medium was adjusted to 5.4.

Culture vessels: the vessels used were Erlenmeyer flask, in which about 20 ml of growth medium was introduced. After that they were sealed with aluminum foil. The inoculated amount is as

follows: 1 fragment / vessel, 9 vessels / variation. As such there are a total of 27 vessels.

Growing conditions: the incubation of cultures was made in the growth room where artificial light was

provided with fluorescent tubes. The room temperature was maintained at 24° C and a relative humidity of 70%. The photoperiod was set to be at 16 hours of light and 8 hours dark.

Table 1

Experimental variations

Variation	V1	V2	V3
Basic medium	MS	MS	MS
NAA quantity	0.5 mg/l	1.5 mg/l	1 mg/l
BAP quantity	1 mg/l	2mg/l	2.5 mg/l

Results and Discussions

This experiment began with a total of 42 stem fragments, assigning 14 fragments to each of the 3 experimental variations (V1, V2 and V3). After an inoculation period of roughly 4 weeks the dormant buds started to grow. A significant development of the buds can be noticed in the next 2 weeks, therefore after 45 days the buds reaching a height of 0.8 – 2.5 cm with a healthy green color (Fig. 1).

In the first phase of this experiment the V2 variation showed the best results, with all of the 14 buds becoming active and an observable elongation of the bud.

In the second phase, 70 days after the inoculation period has begun, data showed that the sprouts undergoing hormonal treatment with the 3 variants, grew to form roots and rosette leaves. In the case of V2 and V3 variants new shoots can be observed forming at the base of its fragment. The success rate remained the same for V2 (100%), all the shoots growing in length and forming leaves. In the V1 and V3 variants the success rate has changed, thus for V1 variant only 9 buds remained (64,2%) and for V3 a remaining number of 12 buds (85,2%).

In the third phase, which took place over a period of 95 days after inoculation, a significant change could be observed in the growth and

development of the *Dendrobium* fragments. The shoots obtained by the V2 variant grew roots and a significant number of additional shoots emerged at the base. Likewise 1st and 2nd phase the V1 variant success rate kept its par, while the V2 and V3 suffer minor changes in their success rates, thus 2 shoots out of the 9 remaining stopped their development process in the V1 case and 1 shoot out of 12 remaining in the V3 variant turned yellow (Table 2). Again we notice a difference between the two sprout/shoot versions. Hence the V1 shoots further elongated and increased the number of roots, while in the V3 case the roots grew to form new shoots at the base (Fig. 2).

Due to the large number of shoots obtained in the culture dish it was necessary to separate and place them in other vessels with the same medium, but only using the concentration of hormones that gave the best results, while the large shoots being submitted to acclimatization process. Thusly a number of 32 shoots were obtained in pots and additionally 58 pots with shoots newly rooted.

Table 2

Experimental results regarding the number of plants

Variation	Number of explants	After 45 days	After 70 days	After 95 days	Number of resulting plants
V1	14	14	9	7	12
V2	14	14	14	14	22
V3	14	14	12	11	24

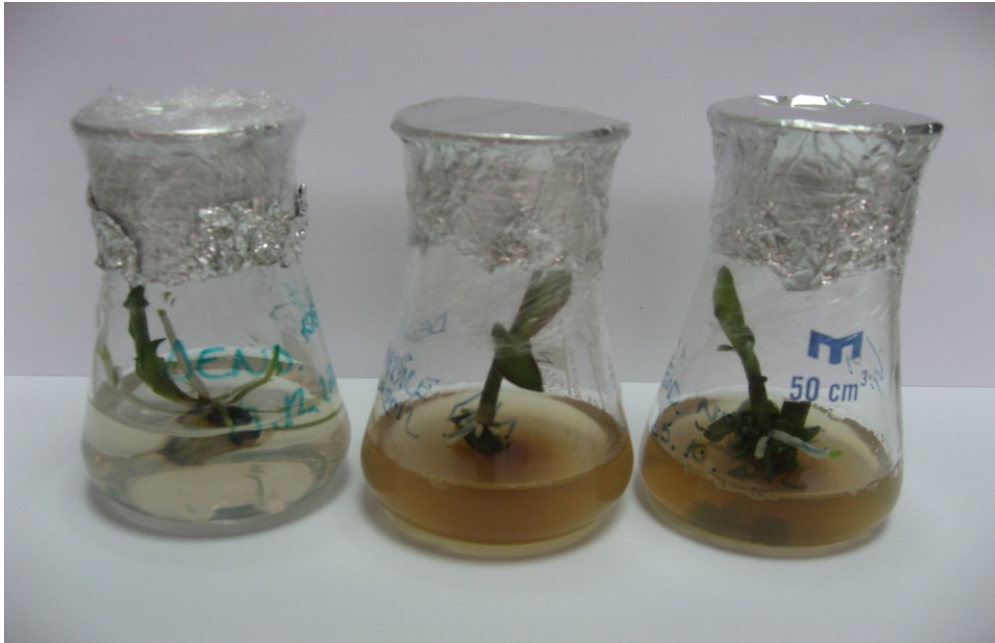


Fig. 1 Explants after 45 days



Fig. 2 Explants after 95 days

Conclusions

The presented results in this paper indicate that the percentage of regeneration, the number of shoots per explant and the percentage of rooting plantlets was affected by the different amount of hormones in the growing medium.

The best propagating rate has been observed in the experimental version V2 where the highest number of shoots was obtained. The percentage of healthy regenerated stalk fragments on V2 was 100%, each inoculum generating a total of 2-3 shoots.

This method of treatment is of interest because it combines the breaking of dormancy with the induction of multiple plantlets. Using stem sections of

in vitro grown plant as explant source could be efficiently for rapid micropropagation to get multiple shoots and plantlets of Dendrobium orchid.

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