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Preliminary results on the influence of growth hormones on the *in vitro* regeneration of Phalaenopsis flower stalks

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Abstract This paper follows the propagation of *Phalaenopsis* by *in vitro* technique, starting with floral - stalk fragments inoculated in Murashige and Skoog medium supplemented with different concentrations of growth hormones. The aim is obtaining a large number of plants in a short period of time. The biggest advantages of this propagation method are genetic preservation of the mother plant and the fact that parent plant itself remains unharmed in the tissue harvesting process.

Key words

orchid, Phalaenopsis, propagation, modern, flower-stalk

Phalaenopsis is one of the most popular orchids on the Horticulture Market, due to the beauty of its blooms, the wide variety of colors and the availability of a large number of resistant hybrids.

The evolution of orchid cultivation in the course of time was a very slow one. If in the beginning orchid propagation outside of their natural habitat was impossible, reason why they ware very expensive at that point in time; today the orchid culture is being practiced on an industrial level through tissue culture. The nowadays low production cost and the ease of this plants maintenance are the reasons that Phalaenopsis is the most bought and loved orchid (6).

The species name derivates from two Greek terms ("phalaina" = butterfly and "opsisi" = resemblance) and they are referring to the elegant shape of the flowers, that look like the wings of a butterfly. The approximately 60 species of this genus are originally from the thick, moist and warm forests of India, Indonesia and the Philippines. They can be found at altitudes of 200-400m (7).

Being a monopodial orchid species, Phalaenopsis does not branch nor dose it produces new shoots. The plant exhibits a monopodal form of growth with a single vertical main stem, which produces a series of thick, fleshy and distichous leaves. One spray of flower develops from each leaf axel. The lower part of the plant consists in the root system made out of green fleshy roots that can be found in the growing medium or they can grow outside of the pot up into the air (5).

The first clonal propagation in vitro of orchids was achieved by Dr. Gavino Rotor while he was a graduate student of Lawrence McDaniels at Cornell University. He employed sections of Phalaenopsis flower stalks each containing a lateral bud, which he cultured on Knudson C medium (1). Rotor obtained active growth (swelling of the bud and appearance of the first leaf) after 2 weeks. This method is for relatively limited production of plantlets. Rotor's method has been ignored at first, but after 10 years others devised new procedures (2).

The development of Knudson's asymbiotic method has vastly improved the germination of orchid seeds and paved the way for orchid tissue culture. To date, improved tissue culture methods using orchid roots, leaves, flower buds, stems and inflorescences have been adopted, making orchid cultivation faster and easier. There is an active market for micropropagated orchid plantlets. However, there are also many problems associated with the commercial production orchid plantlets: slow growth of orchid plantlets, low multiplication rate, vitrification, poor rooting and high mortality during acclimatisation. Among others, the shortage of high quality planting materials further constrains the full expansion of the orchid industry. It is therefore important to formulate economically viable strategies to improve the quality and production rate of micropropagated orchid plantlets (4).

The growing cycle of Phalaenopsis orchids is long, a cycle being 2–3 years. Using the traditional hybridization to transmit useful traits into the commercial varieties is a long process which takes years to achieve. In addition, some species of orchids are cross-incompatible, thereby limiting the work of variety improvement. Hence, new approaches and techniques are needed in order to produce superior *Phalaenopsis* varieties for the fast growing and highly competitive markets (3).

Material and Methods

The material used for this paper consists out of 15 adult Phalaenopsis amabilis of the same hybrid (2 years), from which the material, in this case the flower stalks have been harvested after all the flowers have passed.

Plant material used: clean, healthy, vigorous flower stalks were segmented in sections leaving fragments of about 2 centimeters, each one of them

having one node. The first and the second nodes from the stalk are removed (they do not have buds) and also the top where the flowers were displayed. The stalks on which only a few flowers have bloomed are the best choice for this propagation method. Each stalk shows a total of 4 to 6 nodes that can be used.

Sterilizing the plant material: The obtained fragments were washed with water and mild detergent so that all impurities were removed from the surface. 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 ($HgCl_2$) 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, 10 vessels / variation. As such there are a total of 30 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	V_1	V_2	V_3
Base medium	MS	MS	MS
NAA quantity	0.5 mg/l	1.5 mg/l	1 mg/l
BAP quantity	1 mg/l	2 mg/l	2.5 mg/l

Results and Discussions

The experiment started with a number of 10 Phalaenopsis orchids for each of the 3 variations (V1, V2 and V3).

After approximately 4 weeks from inoculation, dormant buds started to grow. A slight swelling of the bud indicates the fact that the culture can be a successful one (Fig. 2, a).

In this first stage the biggest success is represented by the inoculants from the vessels of the V2 variation, where all 10 buds became active and started vegetation. A part of the inoculants from the V1 and V3 variations have yellowed or have remained dormant (Table 2). At the end of the first phase the percentage of healthy plants decreased from 100% to 70% for V1 and 80% for V3. The percentage of the remaining healthy plants for V2 remained at the initial level of 100% (Fig. 1)

A transfer will be carried out in which all obtained fragments will be moved in a fresh growth medium with the same recipe as the one they were initially placed on.

The next data collection took place after another 4 weeks when significant changes in the bud could be seen. In this stage the emerged shoot is fully developed; it has an elongated stalk and the first leaves make their appearance (Fig. 2, b).

The collected data for the second stage has shown a pronounced decrease in healthy specimens, as follows: for V1 the percentage decreased to 30% from the beginning of the experiment; for V2 it decreased to 80% and for V3 to 50% (Fig. 1).

The collection of data for the third stage occurred after 9 weeks when the development of multiple shots from each flower stalk fragment was visible. (Fig. 2, c).

Because the stalk fragments were maintained on a nutrition medium treated with NAA and BAP hormones, the shoots formed numerous new branchings. Every flower stalk fragment has 4-5 new shoots, which in subsequent phases of the experiment will yield new plantlets. After 2 or 3 leaves have been produced, roots will also appear.

If these new shoots are separated from their floral stems fragment and exposed to an environment that will contain rooting hormones, they will form a root system appropriate for healthy new plants. Since these plants were produced from "cuttings", they will have identical features with the mother plant.

Table 2

Experimental results regarding the number of plants

Variation	V_1	V_2	V_3
Number of explants	10	10	10
Stage I	7	10	8
Stage II	3	8	5
Stage III	3	8	5

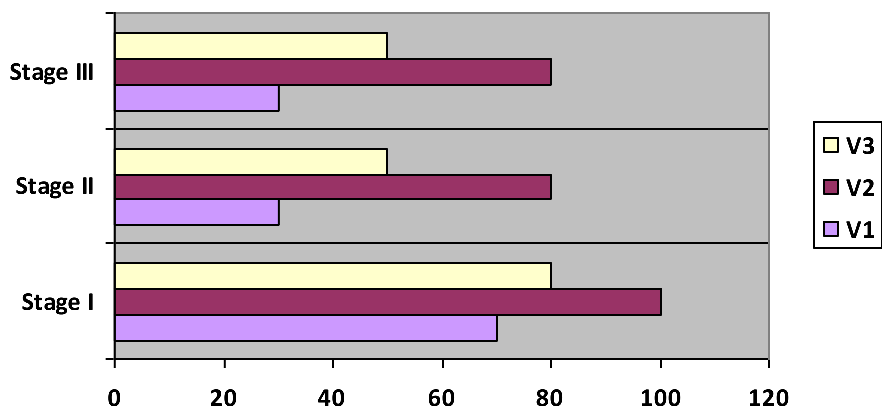

Fig. 1 Results concerning regeneration

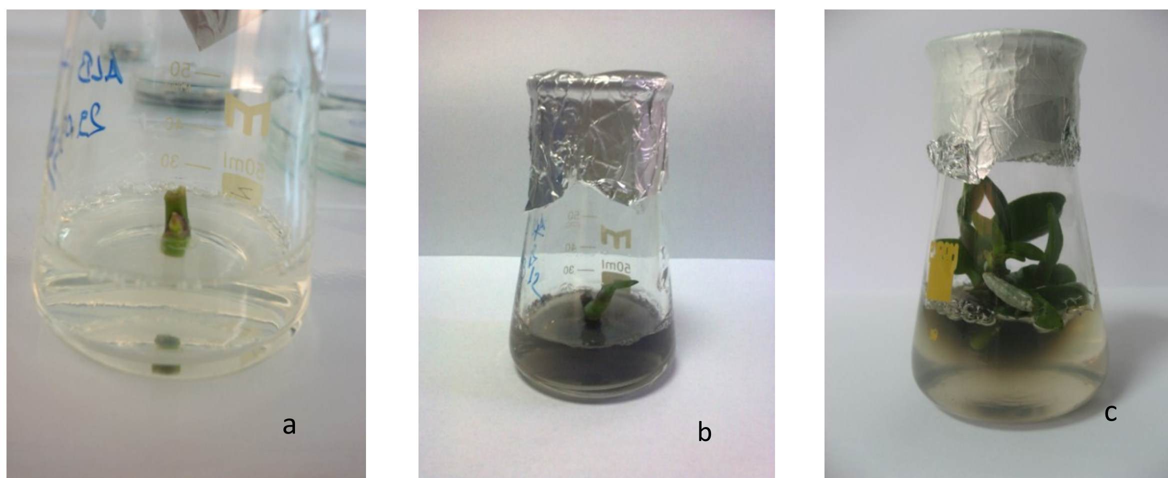

Fig. 2. Growth phases. a. stage I, b. stage II, c. stage III

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 80%, each inoculum generating a total of 4-5 shoots.

In the next stage the regenerated stalk fragments will be placed in a rooting medium and then be subjected to acclimatization.

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

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