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Original Article

In Vitro Rooting of *Dendrobium nobile* Orchid: Multiple Responses to Auxin Combinations

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Abstract

Orchids are the most adorable in flowering plants, cultivated as the cut flower and potted plants throughout the world at different occasions. For their commercial exploitation and conservation of endangered species, micropropagation has been extensively practiced, which may be affected by several factors at each step. *In vitro* rooting is the most important stage that may ultimately be responsible for successful transplantation of the plantlets. Auxins play a vital role for *in vitro* rooting. In present study NAA and IBA treatments to *in vitro* developed microshoots produced multiple responses. Findings show that NAA concentrations alone were better and vigorous than IBA alone in terms of number, length and root thickness. Increasing the NAA concentration from 0.1 to 3.0 mg l^{-1} proved progressive. The highest significant value in root development was obtained with NAA at 3.0 mg l^{-1}. In case of IBA alone, the root number was increased by increasing its concentration up to 1.0 mg l^{-1} but, root number decreased when the concentration of IBA was increased to 3. 0 mg l^{-1}. However, the combined effect of both the PGRs over root formation indicated a considerable decline in root formation as well as callus formation at microshoot bases.

Keywords: Acclimatization, callus, microshoots, micropropagation, orchids, rooting

Introduction

Orchids are considered very fascinating flowering plants due to their long lasting blooms, various shapes, forms and colours. Their resemblance to butterflies (*Oncidium papilio*), slipper or "moccasins" (*Paphiopedilum* and *Cypripedium*) spiders (*Arachnis*), moths (*Phalaenopsis*), or dancing ladies (*Oncidium*) led to their beautiful names and attraction to peoples (Adhikari and Fischer, 2012). Their modern use as the cut flower and potted plants are increased globally. Chen and Henny (2008) reported that more than 507 million foliage plantlets are annually produced worldwide including 150 million orchids.

The genus *Dendrobium* bears a great diversity among the sympodial orchids, containing about 1100 species, scattered from Australia to Southeast Asia and New Guinea (Pant and Thapa, 2016). Due to its attractive flowers, *D. nobile* holds a major position in cut flower industry (Bhattacharyya *et al.*, 2014). *D. nobile* is one of the slow grower orchids and their propagation through conventional methods is also very slow. Moreover, they require a combination of various factors in nature for continued production. The increasing demand of orchids as the cut flower and potted plants throughout the world on various events has invited the growers to exploit plant tissue culture technology for their mass propagation to reach the market demands.

In vitro rooting and acclimatization are very important stages in micropropagation of orchids. The successful transplantation of the *in vitro* plantlets to *ex vitro* conditions during acclimatization primarily depends upon the *in vitro* developed roots. This may be accomplished with different culture media compositions, culture conditions and plant growth regulators (PGR) depending upon the type of species and explant. Mostly, auxins, either alone or in combination with other auxins or cytokinins, have been used in culture media for *in vitro* rooting of microshoots. Fukaki and Tasaka (2009) have reported that auxins stimulate lateral root initiation and development by activating quiescent pericycle cells.

In current study two different auxins namely IBA (Indole Butyric Acid) and NAA (Naphthalene Acetic Acid) were used alone and in combinations for *in vitro* root development of *D. nobile* orchid, and their impact on *in vitro* rooting and overall growth during acclimatization is discussed.

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Materials and Methods

The current study was carried out at the Biotech. Lab., DPRI, Shah Abdul Latif University, Khairpur, Sindh, Pakistan during the period from 2011 to 2012.

Plant material

The plantlets with uniform growth (2-4 leaves) from the *in vitro* shooting medium, involved different concentrations of BA in combination with Kinetin, were selected for the current study.

Media preparation

The culture media used for *in vitro* cultures were the basal nutrient medium (MS medium) supplemented with mgl^{-1}: 0.5 pyridoxine-HCL; 0.5 nicotinic acid; 0.1 thiamine-HCL; 100.0 myo-inositol; 2.0 glycine; 400.0 glutamine; 2200.0 agar (winlap); 1400.0 gel; 30000.0 sucrose and 500.0 activated charcoal (AC). Before autoclaving the pH of the medium was set to 5.7 ± 0.1. Media was dispensed into small jars (150 ml) in aliquots of 35 ml per jar and was capped with polypropylene closures. Subsequently the media was autoclaved for 20 minutes at 1.11 kg/cm^2 and 121 °C. The concentrations and combinations of NAA and IBA used are tabulated in Table (1). All the primary roots of the selected microshoots were trimmed to the minimum length of 1-5 mm before starting the experiment.

Evaluation of data

Each treatment contained 5 replicates and each replicate (Jar) comprised 1 to 3 microshoots. The experiment was designed as factorial Randomized Complete Block and the data was transformed to arithmetic means prior to analysis of variance (ANOVA). The differences in means among the treatments was determined using LSD test at 5% according to Stell *et al.* (1980).

Results and Discussion

The rooting is the most important step in micropropagation of orchids. It has a very significant impact on plantlet survival and growth during the acclimatization stage. The central goal of the current study was to improve *in vitro* root development and growth of microshoots, which may have enhanced the overall growth and development of the orchid plantlets at their subsequent stages.

Table 1. Different combinations of NAA and IBA ($mg\ l^{-1}$) used for rooting experiments

Treatments	NAA	IBA
0	0.0	0.0
1	0.0	0.1
2	0.0	1.0
3	0.0	3.0
4	0.1	0.0
5	1.0	0.0
6	3.0	0.0
7	0.1	0.1
8	1.0	1.0
9	3.0	3.0

Treatment 0 is control (without any hormone).
Treatments from 7 to 9 are the combinations of both PGRs.

In vitro rooting response to NAA alone

In current study NAA was found better than IBA for root formation and development. The roots formed on NAA concentrations were better and vigorous than IBA in terms of root number, length and thickness (Table 2). Increasing the NAA concentration from 0.1 to 3.0 mg l^{-1} was proved progressive for *in vitro* rooting. The highest significant values in root number, length and thickness were obtained with NAA at 3.0 mg l^{-1} (Fig. 1).

It is reported that auxin treatments to *in vitro* microshoots strengthen the adventitious roots by inducing the internal contents of enzymes which induce cell division, elongation and differentiation of tissues (Husen and Pal, 2007; Pant *et al.*, 2011). The results in the present study are in agreement with the findings of Parvin *et al.* (2009), who used two different concentrations of NAA (0.1 and 0.2 mg l^{-1}) for *in vitro* growth and development of the *Dendrobium* orchid, found that NAA at its higher concentration (0.2 mg l^{-1}) produced maximum roots. They suggested that increasing the concentrations of NAA might give better results. Basker and Bai (2006) have reported similar results for NAA. They observed that MS medium added with different PGRs; NAA and BA individually and in combinations showed different results for *in vitro* root induction of *Coelogyne stricta*. Among all combinations used the root number was better increased with NAA alone (1.0 and 2.0 mg l^{-1}).

In vitro rooting response to IBA alone

In case of IBA used for rooting, root number was increased by increasing IBA concentration up to 1.0 mg l^{-1} but, root number decreased when IBA concentration was increased to 3.0 mg l^{-1} (Fig. 2). In addition, root length and thickness was also decreased with higher IBA concentrations (Table 2). Pant and Swar (2012) has reported the similar inhibitory impact of IBA on root formation at higher concentration. According to them, the increasing in concentration of IBA to 2.0 mg l^{-1} resulted in inhibition of root formation. In addition, Mohanty *et al.* (2012) also found parallel results during *in vitro* rooting of *Cymbidium mastersii*. Their findings show that MS medium added with 2.0 mg l^{-1} IBA produced the highest roots (7.46±0.09) per plantlet as compared to the medium comprising 3.0 mg l^{-1} IBA. Ozel *et al.* (2006) reported that IBA at its higher levels inhibits shoot bud formation, which may stopover root production as the auxins from the shoot apex moves to root primordial.

Moreover, according to Asghar *et al.* (2013), the medium containing IBA (2.0 mg l^{-1}) gave better roots (97.55%) for *D. nobile*, whereas the medium containing NAA (1.5 mg l^{-1}) produced 85.0% roots. They also reported that increasing the auxin concentration gave a gradual increase in rooting percentage, but there was a declining trend in rooting percentage with higher levels (3.0 mg l^{-1}) as compare to the optimum levels. The highest root length (3.47 cm) and number (4.70) were achieved with IBA (2 mg l^{-1}) as compared to NAA, where the highest root length (2.25 cm) and number (3.30) were obtained at 1.5 mg l^{-1}.

In vitro rooting response to NAA and IBA combinations

Regarding the combined effect of both PGRs on root formation, there was a considerable decline in root number, thickness and length (Table 2). The treatment number 9 (T9)

Table 2. Effect of different concentrations of NAA and IBA (mg l^{-1}) on root number, length and width of *in vitro* orchid plantlets

Treatments	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	LSD at 0.05
NAA/IBA (mg l^{-1})	0.0/0.0	0.0/0.1	0.0/1.0	0.0/3.0	0.1/0.0	1.0/0.0	3.0/0.0	0.1/0.1	1.0/1.0	3.0/3.0	
Root number	6.1e	7.9bc	8.1b	7.1cd	7.2bcd	9.8a	12.0a	6.5de	5.8e	4.6f	0.000***
Root length (cm)	3.8d	4.6c	4.7bc	4.6cd	4.6c	5.3ab	5.4a	4.4c	4.0cd	3.7d	0.000***
Root thickness (cm)	2.0de	2.0cd	2.1cd	2.0de	2.2bc	2.4b	2.7a	2.0de	1.9de	1.8e	0.000***

Means followed by the same letter(s) in each row are insignificantly different at 5% level

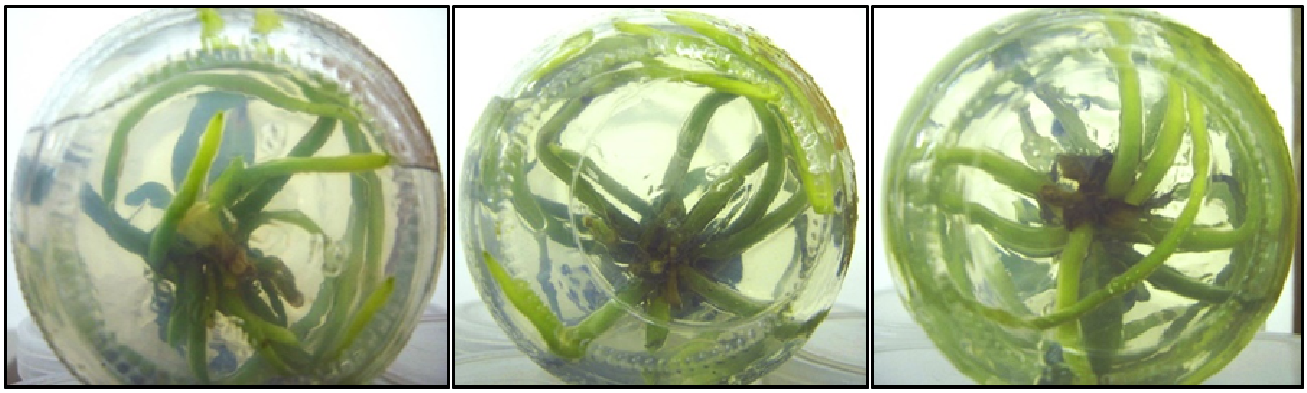

Fig. 1. *In vitro* better root formation of orchid plantlets on 3.0 mg l^{-1} NAA (right jar) compared to 0.1 and 1.0 mg l^{-1} NAA (left to right two jar)

Fig. 2. *In vitro* better root formation of orchid plantlets on 0.1 and 1.0 mg l^{-1} IBA (from left to right two jars), compared to 3.0 mg l^{-1} IBA (right jar)

Fig. 3. *In vitro* inhibitory impact of increasing NAA and IBA from 0.1/0.1 and 1.0/1.0 mg l^{-1} to 3.0/ 3.0 mg l^{-1}, jars from left to right

containing NAA (3.0 mg l^{-1}) in combination with IBA (3.0 mg l^{-1}) showed the poorest rates in root formation. Moreover, in T9, there was callus formation at microshoot base with much reduced root number and length (Fig. 3). The plant growth regulator IBA is considered as a physiologically more active auxin than NAA (Liu *et al.,* 2002). In present study, IBA at its higher concentration in combination with NAA gave less number of roots than NAA and IBA alone, probably due to the inhibitory effect produced by IBA and NAA accumulations at higher concentration.

Impact of NAA and IBA during ex vitro acclimatization

The results obtained during acclimatization were similar to the *in vitro* results. The treatment containing 3.0 mg l^{-1} NAA

Fig. 4. Dying-off of *in vitro* poorly developed roots (left) and survival of *in vitro* well developed roots (right) of orchid plantlets during acclimatization stage in net house

Table 3. Effect of NAA and IBA (mg l^{-1}) on different growth parameter of orchid plantlets under net house conditions

Treatments	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	LSD at 0.05
NAA/IBA (mg l^{-1})	0.0/0.0	0.0/0.1	0.0/1.0	0.0/3.0	0.1/0.0	1.0/0.0	3.0/0.0	0.1/0.1	1.0/1.0	3.0/3.0	
Shoot number	3.15bc	3.17bc	3.17bc	3.27b	3.33b	3.50ab	3.72a	3.40ab	3.27b	2.90c	0.004**
Leaf number	4.40cde	5.25abc	5.49a	5.30ab	4.83abcd	4.50bcde	4.17de	4.95abcd	4.67abcd	3.75e	0.003**
Leaf length (cm)	5.24d	6.23c	6.42bc	6.01c	6.46bc	6.81ab	7.13a	6.00c	4.83de	4.48e	0.000***
Leaf width (cm)	2.08cde	2.10cde	2.19bcde	2.29bcd	2.40bc	2.45b	2.83a	2.01def	1.90ef	1.74f	0.000***

Means followed by the same letter(s) in each row are insignificantly different at 5% level

followed by 1.0 mg l^{-1} NAA, was better among all the treatments in terms of increase in shoot number, length and width.

The data show in the Table 3 that the maximum rate of shoot formation was found on T6 (3.0 mg l^{-1} NAA) where the root formation, length and thickness was also better during *in vitro* phase (Fig. 1). Whereas, the lowest rate in shoot formation was found on T9 (3.0 mg l^{-1} NAA and 3.0 mg l^{-1} IBA). Similar results were obtained in respect to leaf length. The highest rate of leaf length was recorded on the same treatment (T6). The lowest rate in leaf length was found on T9, where the inhibitory impact of NAA and IBA combinations were observed during *in vitro* phase. The leaf width was also statistically significant on T6 followed by the T5. The lowest value among all treatments was again found on T9.

In general, the plantlets grown in NAA treatments were found better during *ex vitro* phase as compared to the plantlets grown in treatments containing IBA alone or in combination. The rooting quality of *in vitro* grown orchid plantlets was the most important factor that increased the overall growth in *ex vitro* phase. During *ex vitro* phase, it was found that the plantlets with well-developed *in vitro* roots, formed with NAA treatments (3.0 and 1.0 mg l^{-1}), were better adaptable to *ex vitro* conditions, as compared to *in vitro* poorly developed roots, formed with IBA alone or in combination (Fig. 4). It is reported by Hegazy *et al.* (2006) that the optimum growth rate of *ex vitro* plantlets did not occur until new roots and leaves developed in the green house. In the present study it was observed that the inferior quality of root led to extend the required time for an early growth of *ex vitro* plantlets from 2 to 3 months. During which the plantlets may be exposed to fungal infection under the humid conditions at early phase of acclimatization (Abul-Soad and Jatoi, 2014).

Conclusions

The plant growth hormones NAA and IBA are very vital for *in vitro* plantlets rooting. Generally, NAA concentrations alone were better and vigorous than IBA alone. The highest significant value in root development was obtained with NAA alone. However, In case of IBA alone, root number decreased when the concentration of IBA was increased to 3. 0 mg l^{-1}. The combined effect of both the PGRs over root formation indicated a considerable decline in root formation as well as callus formation at microshoot bases.

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