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TISSUE CULTURE MEDIA : SELECTION AND REFINEMENT FOR A MONOPODIAL ORCHID HYBRID

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Abstract

The green capsules in *Mokara* Walter Oumae White x *Aranthera* Annie Black were harvested and surface sterilized prior to extraction of seeds. The seeds were inoculated in five different media compositions and incubated. Coconut water 200ml l^{-1} was used as organic additive in all the cases. Activated charcoal was also added at a concentration of 1g l^{-1}, to all the nutrient media. Observations on height of seedlings, number of leaves, length of the longest leaf, number of roots and length of the longest root were recorded after six months of inoculation and the data were statistically analysed. MS half strength was selected as suitable medium for monopodial hybrid seeds. To improve the *in vitro* growth of hybrid seedlings in monopodial orchids, different combinations of growth hormones were added to this identified best culture medium and their effect was studied. The various treatments included, T_1 - Control (no growth regulators); T_2 - 8 mg l^{-1} BA + 2 mg l^{-1} IAA; T_3 - 8 mg l^{-1} BA + 2 mg l^{-1} NAA; T_4 - 2 mg l^{-1} IAA + 8 mg l^{-1} NAA; T_5 - 8 mg l^{-1} IAA + 2 mg l^{-1} NAA; T_6 - 10 mg l^{-1} IAA, and T_7 - 10 mg l^{-1} NAA. Six months after inoculation, observations on height of seedling, number of leaves, length of the longest leaf, number of roots and length of the longest root were recorded and the data were subjected to statistical analysis. To promote seedling growth, 8 mg l^{-1} IAA + 2 mg l^{-1} NAA was identified as the most suitable treatment.

Introduction

MONOPODIAL ORCHIDS have longer vase-life and are easier to maintain than their sympodial counterparts. They are even better priced in the international floriculture trade because of their spectacular flowers of numerous colour combinations and patterns (Beena Thomas and Lekha Rani, 2008). Monogeneric and bigeneric hybrids as well as multigeneric grexes have been developed in these. The multigeneric hybrids can adapt to a veriety of environmental conditions, and have improved floral attributes (inflorescence and flower size, colour, pattern) as compared to the individual species or hybrids of the parental genera (Mercy and Dale,1997). For their commercial production, an appropriate culture medium needs to be selected for scientific refinement. Present studies are a step further in this direction. The aim has been to develop a monopodial hybrid, using a multigeneric combination.

Material and Methods

The hybrid combination used for the study was *Mokara* Walter Oumae White x *Aranthera* Annie Black. The pods from this successful hybrid combination, harvested at the green capsule stage, formed the experimental material.

Stage of Green Capsule Harvest

From the successful combinations green capsules were harvested at 70-90 per cent maturity. The best time to harvest a capsule is when the tip of capsule starts to turn yellow.

Surface Sterilization of Capsule

The freshly harvested green capsules were excised to remove extra length of pedicel and the wilted perianth parts. They were subsequently washed in running tap water. For proper cleaning of intact capsules soaking in one percent solution of laboline detergent in distilled water was practised for 20 minutes. Then the capsules were rinsed thoroughly three to four times with distilled water.

Inoculation and Incubation

As the immature seeds cannot be stored, all seeds must be inoculated immediately after harvest. The capsule was surface sterilized inside a laminar air flow chamber, first by immersing in 0.1% mereuric chloride solution for 10 min. Then it is dipped in 70 per cent ethyl alcohol for three to five min prior to 'flaming' and then is 'flamed' by passing through the flame of a spirit lamp. Then it is cut open under sterile conditions. The immature embryos are scrapped out and sown in sterile flasks containing the culture medium.

The first sown flask is the master flask; further it was they may be subcultured to distribute the crowded seedlings to a larger number of flasks.

Selection of Culture Media

Effect of various culture media on the *in vitro* growth of monopodial orchid seedlings was studied. The seeds were inoculated on five different media compositions, viz., KC (Knudson, 1946) full strength, MS (Murashige and Skoog, 1962) quarter strength (25 % concentration of inorganic salts), MS half strength (50 % concentration of inorganic salts), MS full strength and VW (Vacin and Went, 1949) full strength and incubated. Coconut water 200ml l^{-1} was used as organic additive in all the cases. Activated charcoal was also added at a

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concentration of 1g l^{-1}, to all the media studied. Observations on height of seedlings, number of leaves, length of the longest leaf, number of roots and length of the longest root were recorded after six months of inoculation and the data were statistically analysed (Panse and Sukhatme, 1967).

Refinement of Culture Media

To improve the *in vitro* growth of hybrid seedlings in monopodial orchids, different combinations of growth hormones were added to the identified best culture medium and their effect was studied. The basal medium selected was MS (half strength). The various treatments included in this research work were as listed below.

T_1 - Control (no growth regulators); T_2 - 8 mg l^{-1} BA + 2 mg l^{-1} IAA; T_3 - 8 mg l^{-1} BA + 2 mg l^{-1} NAA; T_4 - 2 mg l^{-1} IAA + 8 mg l^{-1} NAA; T_5 - 8 mg l^{-1} IAA + 2 mg l^{-1} NAA; T_6 - 10 mg l^{-1} IAA; T_7 - 10 mg l^{-1} NAA.

After proper sterilization techniques, hybrid seeds were inoculated in these various treatments and incubated properly as described above. Six months after inoculation, observations on height of seedlings, number of leaves, length of the longest leaf, number of roots and length of the longest root were recorded and the data were subjected to statistical analysis (Panse and Sukhatme, 1967).

Results

Effect of Culture Media

Effect of different media on *in vitro* growth of seedlings in monopodial orchid hybrids was studied using the hybrid combination *Mokara* Walter Oumae White x *Aranthera* Annie Black. Observations were taken at six months after inoculation and the data were analysed statistically. The results are presented in Table 1.

Seedling Height

Significantly high value for height of seedling (3.76 cm) was recorded in MS half strength medium followed by MS quarter strength medium (3.44 cm) which was on par with MS full strength medium (3.12 cm). The value for this trait was lowest in KC medium (2.48 cm).

Number of Leaves

The highest number of leaves of 3.6 was recorded in MS half strength medium which was on par with MS quarter strength medium (3.2) and MS full strength medium (2.8). This was the lowest in KC medium.

Length of Leaves

Maximum length of leaves was observed in MS half strength medium with a significantly high value of 2.16 cm. This was minimum in KC medium.

Number of Roots

The highest number of roots were produced when the hybrid seedlings were inoculated in MS half strength medium with a significantly high value of 3.4, followed by MS quarter strength medium (3.0).

Length of Root

Mean length of roots were significantly high (2.08 cm) in MS half strength medium. Shortest root was observed in MS full strength medium with a value of 0.86 cm.

Table 1. Effect of media on *in vitro* growth of seedlings in a monopodial hybrid.

Sl. No.	Medium	Seedling Height (cm)	Leaves		Roots	
			Number	Length (cm)	Number	Length (cm)
1.	KC (full strength)	2.48	2.20	1.18	2.00	1.44
2.	MS (1/4 strength)	3.44	3.20	1.78	3.00	1.80
3.	MS (1/2 strength)	3.76	3.60	2.16	3.40	2.08
4.	MS (full strength)	3.12	2.80	1.62	2.80	0.86
5.	VW (full strength)	2.78	2.40	1.36	2.00	1.26
SE_m		0.043	0.219	0.038	0.244	0.032
CD (0.05)		0.128	0.646	0.113	0.723	0.095

Combination : *Mokara* Walter Oumae White x *Aranthera* Annie Black
Culture period : six months

Table 2. Effect of BA, IAA and NAA on *in vitro* seedling growth in a monopodial hybrid.

Treatments	Concentration (mgl^{-1}) of BA	IAA	NAA	Height of seedling (cm)	No. of leaves	Length of leaves (cm)	No. of roots	Length of root (cm)
T_1	0	0	0	3.20	2.80	1.58	2.60	1.50
T_2	8	2	0	4.04	3.80	2.30	4.80	2.32
T_3	8	0	2	2.80	2.00	1.26	2.20	1.32
T_4	0	2	8	3.86	3.60	2.04	3.80	2.04
T_5	0	8	2	4.24	4.60	2.44	4.20	2.20
T_6	0	10	0	3.56	3.00	1.80	3.00	1.78
T_7	0	0	10	3.10	2.40	1.46	2.60	1.08
SE_m				0.033	0.227	0.031	0.262	0.032
CD (0.05)				0.094	0.657	0.090	0.758	0.095

Medium, MS half strength; Combination, *Mokara* Walter Oumae White x *Aranthera* Annie Black; Culture period, six months .

Refinement of Culture Media

Effect of BA, IAA and NAA on *in vitro* growth of seedlings in monopodial orchid hybrids was evaluated in detail using the hybrid combination Mokara Walter Oumae White x Aranthera Annie Black. Observations on various morphological characters were recorded six months after inoculation and statistical analysis was carried out. The results of this media refinement study are presented below (Table 2).

Height of Seedlings

Significantly high value (4.24 cm) of seedling height was recorded in T_5 followed by T_2. Shortest seedlings (2.80 cm) were developed by T_5 .

Number of Leaves

Maximum number of leaves *i.e.*, 3.8 was produced in T_2 which was on par with T_4 with a value of 3.6. Number of leaves (2.0) was minimum in T_2.

Length of Leaves

The longest leaves were formed when hybrid seeds were cultured in T_5 with a length of 2.44 cm which was observed to be statistically on par with T_2 (2.30 cm).

Number of Roots

The highest number of roots were found in T_2 (4.8). This was on par with T_5 (4.2) and T_4 (3.8). Number of roots was the lowest in T_3 (2.2).

Length of Root

Significantly high values for root length were registered in T_2 (2.32 cm) followed by T_5 (2.20 cm) and T_4 (2.04 cm). This was lowest in T_3 (2.32 cm).

Discussion

As orchid seeds lack endosperm, they are to be cultured *in vitro*. Green capsule culture was a major advancement in increasing the germination of orchid seeds in culture media. Withner (1959) was of opinion that very young as well as fully mature ovules did not form good explants *in vitro* due to dormancy, pH, inhibitory and other metabolic factors. Sauleda (1976) found that the pistillate parent was mainly responsible for determining the correct capsule maturity stage. Earler, immature seeds from green capsuls were succesfully culture in a large number of species by various authors (Hazarika and Sarma, 1995; Hossain *et al*., 2008, 2009; Krishnan *et al*., 1993; Pathak and Vij, 2007; Pathak *et al*., 2001, 2011; Piri *et al*., 2013; Sobhana, 2000; Vij and Pathak, 1988; Vij *et al.,* 1981) harvested green capsules of *Dendrobium* at 75-90 per cent maturity, *i.e.*, 90-140 days after pollination. In the present study, capsules were harvested at 74-135 days after pollination with very high success in terms of *in vitro* germination.

Hybrid seeds were *in vitro* cultured, after selecting suitable medium for monopodials *i.e.*, MS (1/2 strength), in the present research work. Refinement of culture medium to promote seedling growth was done, identifying 8 mg l^{-1} IAA + 2 mg l^{-1} NAA as the best treatment. Coconut water 200 ml/l was also added to improve the *in vitro* growth of seedlings in all situations. In earlier studies also, coconut water has proved very useful in orchids (Sivamani, 2004. Activated charcoal was added at a concentration of 1g l^{-1} to all the media

studied due to its beneficial effect of adsorption of inhibitory phenolic and carboxylic compounds produced by the tissues in culture.

Generally, when complex bigenerics and trigenerics are included in the parentage the hybrid combinations exhibited more number of days for all the *in vitro* developmental stages, till deflasking. Ninitha (2003) revealed similar findings in monopodials. A wide range in duration from 15.50 to 40.50 days was found for in vitro germination. The highly bred bigeneric hybrid varieties included in the parentage required more number of days. The same general trend of slow growth was followed by the complex hybrids throughout *in vitro* growth, till deflasking.

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