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Research Article

EFFECT OF GROWTH REGULATORS, LIGHT, HUMIDITY AND GROWTH STIMULANTS ON PHYSIOLOGICAL AND MORPHOLOGICAL PARAMETERS OF TISSUE CULTURED ORCHID *PHALAENOPSIS* SP. DURING *EX VITRO* ESTABLISHMENT

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Abstract: A pot culture experiment was conducted during 2018 November to 2019 January at the Department of Plant Physiology, College of Agriculture, Vellayani to study the physiological and morphological changes that occur during *ex vitro* establishment of orchid (*Phalaenopsis* sp.) and to find out measures to overcome the field mortality rate and improve the propagation efficiency. The orchid used for the experiment was *Phalaenopsis* sp. The results revealed that physiological parameters like specific leaf area and photosynthetic rate were significantly higher at all the four stages of observation, in the treatment provided with 40-50% light intensity and 80-90% humidity (T6) and the transpiration rate was lowest in T3 (plantlet dip with triazole @ 5 ppm + foliar application of triazole @ 5ppm, after 15 days of planting). At 45 and 60 days after planting (DAP), the plantlets which were provided with 40-50% light intensity and 80-90% humidity (T6) recorded the highest plant height. Number of leaves per plantlet and survival percentage were also found higher in T6. But in the treatment, T3 (plantlet dip with triazole @ 5 ppm + foliar application of triazole @ 5ppm) maximum number of roots was observed at all the four stages of observation. Among the different treatments, T6 (plantlets provided with 40-50% light intensity and 80-90% humidity) recorded the highest plantlet survival percentage at 15,30,45 and 60th day of observation (80, 76, 72, 66 percentage respectively) compared to control.

Keywords: *Phalaenopsis, Ex vitro establishment, Physiological, Morphological characters*

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Introduction

Orchids are the most valuable group in the nature of flowering plants distributed throughout the world from the tropics to the high alpine mountains. They exhibit an incredible variety of diversity in terms of the size, shape and colour of their flowers. Among orchids, *Phalaenopsis* or moth orchids are the most common ones due to the ease of production and availability of flowering plants throughout the year. *Phalaenopsis* orchids are monopodial type with long, thick roots and short stems.

Since the conventional methods of orchid propagation are time consuming and laborious, tissue culture is being carried out to produce a greater number of plants in a short period of time. In many countries, *Phalaenopsis* plantlets are being produced using tissue culture techniques for commercial production.

During *ex vitro* establishment, tissue cultured *Phalaenopsis* plantlets usually show high rate of mortality due to sudden shock of environmental changes. So, there is a need to standardise the *ex vitro* establishment of tissue cultured *Phalaenopsis* orchids. While transferring the in vitro grown plantlets to field conditions they are unable to compete with soil microbes and to survive in the environmental conditions. During hardening stage, efforts are focused on the control of both physical and chemical environment and biohardening of plantlets in order to increase growth and reduce mortality. Studies regarding the physiological changes that occur during *ex vitro* establishment of orchids and how they influence the plant growth and survival in the new environment are very much limited. With, this back ground the present study was carried out with an objective to study the physiological and morphological changes that occur during *ex vitro* establishment of orchid (*Phalaenopsis* sp.) and to find out measures to overcome the field mortality rate and improve propagation efficiency.

Materials and methods

The experiment was conducted at College of Agriculture, Vellayani and the experimental site was located at 8.5° North latitude and 76.9° East longitude and at an altitude of 29 m above mean sea level. The orchid cultivar chosen for the experiment was *Phalaenopsis* sp. In vitro derived plantlets from shoot meristem culture were used for this study, and they were obtained from Biotechnology and Model Floriculture Centre (BMFC) Kazhakuttam.

The experiment was laid out in completely randomized block design with three replications of ten treatments as well as a control. The ten treatments were T1 and T2 (plantlet dip with triazole @ 5ppm and 10 ppm respectively), T3 and T4 (plantlet dip with triazole @ 5 ppm + foliar application of triazole @ 5ppm after 15 days of planting and plantlet dip with triazole @ 10 ppm + foliar application of triazole @ 5ppm after 15 days of planting respectively) , T5 and T6 (plantlets providing with 40-50% light intensity & 60-70% humidity and 40-50% light intensity & 80-90% humidity respectively) T7 and T8 (plantlets providing with 70-80% light intensity & 60-70% humidity and plantlets providing with 70-80% light intensity and 80-90% humidity respectively), T9 (plantlet dip of PGPR mix I -5%), T10 (root zone application of arbuscular mycorrhizal fungi -5g/plantlet) and a control (C). The plants were maintained in the hardening chamber for 70 days and observations were taken at 15, 30, 45 and 60 days of *ex vitro* transfer. The physiological observations *viz*., specific leaf area photosynthetic rate, transpiration rate, stomatal index, stomatal frequency and morphological characters *viz*., plant height, number of leaves, number of roots and survival percentage were taken. The data were statistically analysed and the treatment means were compared at 5 percent probability.

Results and Discussion

Effect on physiological parameters

Growth regulators, growth stimulants, light and humidity treatments significantly influenced physiological characters viz., specific leaf area (SLA), photosynthetic rate, transpiration rate, stomatal index and stomatal frequency (at 15,30,45 and 60 days after planting). In general, growth regulators, growth stimulants, light and humidity treatments recorded higher specific leaf area than control. Specific leaf area is the ratio of leaf area to leaf weight. Higher SLA indicates, more leaf area per unit biomass which result in higher photosynthetic rate. In this experiment, the treatment T6 (plantlets provided with 40-50% light intensity and 80-90% humidity) recorded significantly higher specific leaf area at all the four stages of observation. Matsoukis *et al.* [11] has reported similar result in *Lantana camara* L. (subsp. camara) wherein specific leaf area was found highest with 60% shading. This might be due to the reduction in leaf thickness of plants grown under shade nets and as the leaf thickness reduces, the leaf dry weight also reduces. In the plants grown under shaded condition, there was an expansion in the leaf area per unit of leaf biomass. Juraimi *et al*. [9] also observed that in *Cynodon dactylon*, 65% shading enhanced the specific leaf area. Generally, triazole treatments are reported to reduce the specific leaf area and this might be due to the reduction of leaf area due to triazoles [1].

Table-1 *Effect of growth regulators, light and humidity, growth stimulants on specific leaf area (cm^2 g^{-1}) of tissue cultured orchid plants Phalaenopsis sp.*

Treatments	Specific leaf area, cm^2 g^{-1}			
	15 DAP	30 DAP	45 DAP	60 DAP
T_1	448.20	460.80	500.52	518.15
T_2	443.25	454.50	478.59	496.22
T_3	428.40	455.85	474.72	493.21
T_4	400.50	449.10	469.99	482.46
T_5	504.90	515.25	531.48	538.79
T_6	519.30	530.10	542.66	550.83
T_7	462.60	482.85	505.68	513.85
T_8	450.90	474.30	500.09	509.12
T_9	438.30	449.10	475.58	488.48
T_{10}	474.30	495.90	525.46	541.37
C	456.30	474.75	492.35	502.24
SE m (±)	3.67	5.37	5.86	3.67
CD (0.05)	1.244	1.818	1.985	1.242

In the present study, growth regulators, growth stimulants, light and humidity treatments had significant effect on photosynthetic rate at 15, 30, 45 and 60 days of *ex vitro* transfer. Also, the photosynthetic rate was found significantly higher in plantlets provided with 40-50% light intensity and 80-90% humidity(T6). This might be due to the impact of high humidity. High relative humidity was reported to reduce the extent of leaf damage and chlorophyll damage during the acclimatization of eucalyptus plants [8]. A significantly higher photosynthetic rate was also reported during the *ex vitro* establishment of orchid plantlets (Dendrobium sp.) provided with 50 percent light intensity and 70 to 90 percent humidity [14].

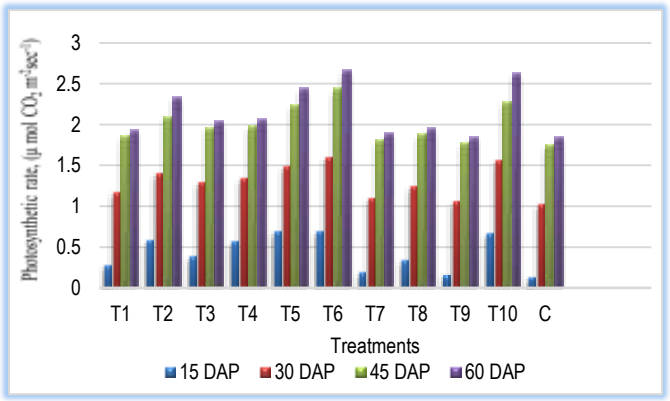

Fig-1 Effect of growth regulators, light and humidity, growth stimulants on photosynthetic rate of tissue cultured orchid plants (*Phalaenopsis* sp.)

Transpiration is an important physiological process that affects the growth and establishment of tissue cultured orchids. Among the different treatments studied, transpiration rate was found the lowest in triazole treated plantlets. Fila *et al*., [2] suggested that the development of cuticle, epicuticular wax and effective stomatal regulation of water loss causes gradual reduction in cuticular transpiration rates during acclimatization to *ex vitro* conditions. This might be the reason for the lowest transpiration rate in triazole treated plantlets, since cuticle thickness was the highest in these plantlets. Similar result was reported by Gopi *et al*., [5] who stated that at all stages of growth, the rate of transpiration was lowered in triazole treated elephant foot yam plants.

Table-2 *Effect of growth regulators, light and humidity, growth stimulants on transpiration rate of tissue cultured orchid plants (Phalaenopsis sp.)*

Treatments	Transpiration rate (m.mole H_2O m^{-2} s^{-1})			
	15 DAP	30 DAP	45 DAP	60 DAP
T_1	0.424	0.384	0.432	0.476
T_2	0.510	0.484	0.530	0.551
T_3	0.418	0.351	0.304	0.364
T_4	0.559	0.528	0.469	0.499
T_5	0.576	0.535	0.495	0.445
T_6	0.594	0.553	0.513	0.463
T_7	0.650	0.594	0.554	0.504
T_8	0.714	0.641	0.601	0.551
T_9	0.750	0.705	0.665	0.615
T_{10}	0.830	0.788	0.766	0.748
C	0.816	0.766	0.716	0.666
SE m (±)	0.012	0.011	0.012	0.014
CD (0.05)	0.037	0.032	0.036	0.041

Stomatal frequency is defined as the number of stomata per unit leaf area [13]. Stomatal frequency was found higher in plants with triazole treatment. Triazole compounds influenced an increase in the number of stomata per unit area when compared to the control plants. The structure and ontogeny of stomata in different plants will vary with the application of different growth regulators [6]. This might be due to the effect of plant growth regulating properties of triazoles which are mediated by their inference with isoprenoid pathway and shift in the balance of plant hormones [3].The result of the present study is in conformity with the findings of Gao *et al*. [4] who reported that triadimefon (TDM) treatment increased the stomatal number per unit area in wheat leaves.

Effect on morphological parameters

Growth regulators, growth stimulants, light and humidity treatments significantly influenced the morphological characters. Plantlets treated with AMF showed the highest plant height at earlier stages. This might be due to the effect of AMF symbiosis formed with the root system of *in vitro* derived plantlets and which in turn result in vigorous plants and improved absorption of nutrients and water [7]. Also the result obtained in this study reinforces those findings by Yano-Melo *et al*. [18] who pointed out that height of the banana plantlets inoculated with AMF was approximately 32% higher, than the non-inoculated plants.

In the present study, highest number of leaves were observed in plantlets provided with 40-50% light intensity and 80-90% humidity (T6). This result is in conformity with the findings of Sun *et al*., [17] who reported that there was positive linear correlation between the relative humidity and the number of shoots in red fuji apple trees. Also, in mandarin orange, number of leaves, shoot length and dry matter were reported to increase with increase in humidity level [10].

Table-3 *Effect of growth regulators, light and humidity, growth stimulants on number of leaves of tissue cultured orchid plants (Phalaenopsis sp.)*

Treatments	Number of leaves			
	15 DAP	30 DAP	45 DAP	60 DAP
T_1	2.67	4.00	4.33	5.67
T_2	2.77	3.44	3.78	5.11
T_3	3.22	4.22	4.56	5.89
T_4	2.18	2.89	3.22	4.56
T_5	3.11	4.44	4.78	6.11
T_6	4.22	5.22	5.56	6.89
T_7	2.44	3.11	3.45	4.78
T_8	2.33	3.67	4.00	5.33
T_9	2.89	4.11	4.44	5.78
T_{10}	3.22	4.56	4.89	6.22
C	2.10	2.78	3.11	4.44
SE m (±)	0.39	0.49	0.48	0.49
CD (0.05)	1.175	1.462	1.465	1.463

Samasya, [14] pointed out that, in the *in vitro* derived dendrobium orchid plantlets subjected to the treatment with 5 mg L-1 of triazole, there were a greater number of roots than control plants. In the present study, the treatment T3(plantlets dipped with triazole @ 5 ppm + foliar application of triazole @ 5ppm after 15 days of *ex vitro* transfer) recorded the highest root number. This was owing to the fact of shift in partitioning of assimilates from the leaves to the roots due to the action of triazoles [15]. In the present investigation, maximum survival percentage was noted in the plantlets subjected to 40-50% light intensity and 80-90% humidity (T6). This might be attributed to the high leaf area, rooting, a greater number of leaves, high photosynthetic rate and more dry matter accumulation. Improvement in survival percentage was also observed by Moraes *et al.* [12] who reported that when tissue cultured *Dendrobium nobile* plantlets were grown under a light intensity of 50%, they showed a survival rate ranging from 77.8 to 95.2%. Also, more than 90% survival rate was reported when *Dendrobium transparens* plantlets were acclimatized by providing 50% shading [16]

Table-4 *Effect of growth regulators, light and humidity, growth stimulants on survival percentage of tissue cultured orchid plants (Phalaenopsis sp.)*

Treatments	Survival percentage, %			
	15 DAP	30 DAP	45 DAP	60 DAP
T_1	79.66	74.00	68.66	63.66
T_2	73.33	69.00	64.00	59.66
T_3	80.00	73.66	69.33	66.00
T_4	76.33	70.33	64.66	60.67
T_5	76.00	71.33	67.66	62.67
T_6	80.66	76.00	72.33	66.33
T_7	59.00	54.00	50.00	45.33
T_8	62.66	58.66	53.33	48.00
T_9	47.66	42.33	37.33	33.33
T_{10}	57.33	53.33	48.66	45.33
C	44.33	40.33	36.00	32.66
SE m (±)	1.87	1.79	1.61	1.31
CD (0.05)	5.526	5.299	4.756	3.879

Conclusion

From this experiment it is concluded that, considering the physiological and morphological characters, treatment T6 (plantlets provided with 40-50% light intensity and 80-90% humidity) is adjudged as the best physiological approach to overcome field mortality and improve propagation efficiency of tissue cultured orchid *Phalaenopsis* sp. during *ex vitro* establishment.

Application of research: Understanding the physiological changes that occur during *ex vitro* establishment of orchids and how they influence the plant growth and survival in the new environment to find out measures to overcome the field mortality rate and improve propagation efficiency.

Research Category: Plant physiology

Abbreviations: TDM- triadimefon, SLA -specific leaf area, AMF-Arbuscular Mycorrhizal fungi, PGPR-Plant growth promoting rhizo bacteria, DAP- days after planting.

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