www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(7): 2306-2309

www.thepharmajournal.com
Received: 08-04-2023
Accepted: 12-05-2023

Madhavi Khilari
Department of Floriculture and Landscape Architecture, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Heera Lal
Department of Floriculture and Landscape Architecture, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Megha Kashyap
Department of Floriculture and Landscape Architecture, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Shashi Kiran Minj
Department of Floriculture and Landscape Architecture, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Lilagar Singh Verma
Professor, Department of Floriculture and Landscape Architecture, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Corresponding Author:
Madhavi Khilari
Department of Floriculture and Landscape Architecture, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Effect of different hardening media on survival percentage and root growth performance of *in vitro* grown plantlets of orchids (*Dendrobium nobile*)

Madhavi Khilari, Heera Lal, Megha Kashyap, Shashi Kiran Minj and Lilagar Singh Verma

Abstract
The present research was done on "Effect of different hardening media on survival percentage and root growth performance of *in vitro* grown plantlets of orchids (*Dendrobium nobile*)." with objective to standardize hardening media for acclimatization of orchid plantlets. The research was laid out in Completely Randomized Design (CRD) in combination of 16 treatments containing charcoal, brick, cocopeat, bark, rice husk, groundnut shell, chopped coconut husk and chopped paddy straw in different combination all 16 treatments replicated thrice for analyzing the effect of media under poly house condition. Among the treatments, T_8 (chopped coconut husk + brick + charcoal + cocopeat) had superior result in almost all considerable aspects of study than all other treatments including control. The results revealed that combination of chopped coconut husk, brick, charcoal and cocopeat had been beneficial to significantly increase the root growth and can be recommended for hardening media of orchids.

Keywords: Hardening, orchids, chopped coconut husk, cocopeat, charcoal, *in vitro*, rice husk, media

Introduction
Orchids are the most beautiful flowers in gift of God to earth. Orchid's are symbol of beauty, luxury, love and mystery. Because of their long spikes, numerous colors and flower shapes and longevity orchids have earned sixth place globally. The remarkable range of orchids that make up the biggest family of flowering plants on the planet comprising 800 genera, 20,000 species and at least 200,000 hybrids. However, there are numerous orchid species, including *Dendrobium*, *Epidendrum*, *Phalaenopsis*, *Onicidium*, *Vanda*, and *Cattleya*. The *Dendrobium* orchid is the most famous flower in the world market. Despite the spectacular growth of orchid micro-propagation over the past 45 years, its widespread use is still thought to be constrained by issues like the exudation of phenolics from explants and a high rate of plant loss or damage when shifted to *ex vitro* condition. High levels of irradiance, low humidity, and water scarcity are problems brought on by the hydraulic conductivity of roots and root stem links. Additionally, direct exposure results in wilting of plants and charring of leaves. The final success of *in vitro* propagation is determined by how well the plants respond to the growing medium.

Micropropagation has primarily been used to quickly multiply disease-free plants under *in vitro* conditions. However, when *in vitro* plantlets are transferred to *ex vitro* circumstances, there could be plant damage because cultured plants grown *in vitro* have a weak root system and an under developed cuticle, plant mortality is high when *in vitro* micro shoots are transferred to *ex vitro* conditions. At this point, the plantlets are highly susceptible to loss and damage. Therefore, during the acclimatization stage, it is crucial to choose an appropriate growing medium and to also provide optimal environmental factors to achieve good *in vitro* plantlet survival rates under *ex vitro* conditions.

Materials and Methods
The experiment was conducted at the Poly house of Commercial Tissue Culture Laboratory, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh during the academic year 2019–2020 to identify the best potting material for orchid plantlets that were grown *in vitro*. Combination of different media such as charcoal, brick, cocopeat, bark, rice husk, groundnut shell, chopped coconut husk, chopped paddy straw are used as hardening media.

Collection of hardening media

Substrates which are available locally such as charcoal, brick, cocopeat, rice husk, bark, groundnut shell, coconut husk, paddy straw are selected for the acclimatization of micropropagated orchid plantlets. The substrates are collected from different place to prepare the different potting media for acclimatization of *in vitro* plantlets. The substrate pieces having 1.0-3.0 cm long were taken.

Sterilization of hardening media

The charcoal, bark, rice husk, groundnut shell, chopped coconut husk and chopped paddy straw are soaked in water for three days to remove condition of those substrates. All the substrate pieces were put separately into the clean cloth bags and they were then placed inside the water bath for 30 minutes after that all the materials of hardening media were sterilized by using 0.1% fungicide (Bavistin) for 30 minutes to reduce the contamination before making potting mixture.

***In vitro* plantlets**

Collection of *in vitro* plantlets

In vitro raised plantlets of orchid *Dendrobium* cv. Sonia, obtained from COE-AIB Tissue Culture Lab College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, (C.G.) for hardening of plantlets under *ex vitro* conditions. The well developed plantlets measuring 3.0- 4.0 cm height and having 2-3 leaves are taken out carefully from the *in virto* culture vessels.

Sterilization of *in vitro* plantlets

In vitro plantlets are taken outside carefully from the culture vessels and placed in a plastic tray. They are then thoroughly washed in tap water to remove any culture medium adhered to their roots without causing damage to the roots.

Results and Discussion

The collected observational data for the current research were statistically analyzed using the analysis of variance method at a 5% level of significance. ANOVA showed that the various treatments had a noticeable effect on survival and growth at 120 DAT.

1. Shoot girth (mm)

The maximum shoot girth (3.46 mm) was observed with treatment T_2 (Bark + Brick + Charcoal + Cocopeat) at 30, 60, 90 and 120 DAP respectively which was noticed *at par* with T_8 (Chopped Coconut Husk + Brick + Charcoal + Cocopeat), T_{14} (Rice Husk + Charcol + Brick + Cocopeat) and T_5 (Groundnut Shell + Brick + Charcoal + Cocopeat) at 120 DAP. The minimum shoot girth (2.04 mm) was noted in treatment T_{10} (Chopped paddy straw + Brick + Charcoal) at 120 DAP respectively

Treatment Details

S. No	Notation	Treatments
1	T_0	Charcoal + Brick + Cocopeat (1:1:1)
2	T_1	Bark + Brick + Charcoal (1:1:1)
3	T_2	Bark + Brick + Charcoal + Cocopeat (1:1:1:1)
4	T_3	Bark + Brick + Charcoal + Rice Husk(1:1:1:1)
5	T_4	Groundnut Shell + Brick + Charcoal (1:1:1)
6	T_5	Groundnut Shell + Brick + Charcoal + Cocopeat (1:1:1:1)
7	T_6	Groundnut Shell + Brick + Charcoal + Rice Husk (1:1:1:1)
8	T_7	Chopped Coconut Husk + Brick + Charcoal (1:1:1)
9	T_8	Chopped Coconut Husk + Brick + Charcoal + Cocopeat (1:1:1:1)
10	T_9	Chopped Coconut Husk + Brick + Charcoal +Rice Husk(1:1:1:1)
11	T_{10}	Chopped Paddy Straw + Brick + Charcoal(1:1:1)
12	T_{11}	Chopped Paddy Straw + Brick + Charcoal + Cocopeat (1:1:1:1)
13	T_{12}	Chopped Paddy Straw + Brick + Charcoal + Rice Husk(1:1:1:1)
14	T_{13}	Rice Husk + Brick + Charcoal (1:1:1)
15	T_{14}	Rice Husk + Brick + Charcoal + Cocopeat (1:1:1:1)
16	T_{15}	Charcoal + Brick + Cocopeat + Rice Husk + Bark + Groundnut Shell + Coconut Husk +Paddy Straw (1:1:1:1:1:1:1:1)

The media combination T_2 (Bark + Brick + Charcoal + Cocopeat) might be accredited to its richer nutritional status which enhanced photosynthetic activity resulted in more plant stored material, thereby increasing shoot girth. A similar result was found in Lakshanthi and Seran (2019) [3] and Sabreeswaran *et al*. (2019) [7] in orchids.

Root parameters

1. Number of root plant^{-1}

Maximum number of root (14.44) was observed with treatment T_9 (Chopped coconut husk + Brick + Charcoal + Rice Husk) which were noticed *at par* with treatment T_6 (Groundnut Shell + Brick + Charcoal + Rice Husk) and T_3 (Bark + Brick + Charcoal + Rice Husk) but significantly better over rest of the treatments. Minimum number of root (6.00) was noted in treatment T_{10} (Chopped Paddy Straw + Brick + Charcoal).

The maximum value attained for number of root plant^{-1} in T_9 containing Chopped coconut husk + Brick +Charcoal + Rice husk may be due to their combined beneficial effects. Superior performance with the root parameters may be attributed to the presence of coconut husk, charcoal, brick and rice husk in the media which might have played a role in improving the physical parameters of the media such as porosity, water retention capacity and drainage. Similar findings have been observed by Deb and Imchen (2010) [8], Muna *et al*. (2016) [4] and Sanghamitra *et al*. (2019) [9].

2. Root length

Maximum root length (7.77 cm) was observed with treatment T_9 (Chopped coconut husk + Brick + Charcoal + Rice Husk) which were noticed varied significant from other treatments. Minimum root length (4.37 cm) was noted in treatment T_{10} (Chopped Paddy Straw + Brick + Charcoal). Maximum root length in T_9 (Chopped Coconut Husk + Brick + Charcoal + Rice Husk) might be due to the fact that the media in not

compact, provide good aeration and allowed free growth of roots which resulted in increase of length of roots. Mirani *et al*. (2016) [10] reported identical outcome.

3. Root volume

Maximum root volume (6.60 cm^3) was observed with treatment T_9 (Chopped coconut husk + Brick + Charcoal + Rice Husk) which were noticed varied significant from other treatments. Minimum root volume (1.53 cm^3) was noted in treatment T_{10} (Chopped Paddy Straw + Brick + Charcoal). The increase in the root volume may be attributed from the increase in number of root $plant^{-1}$ and root length in plant. The results have been in contradiction to the findings of Sabreeswaran *et al*. (2019) [7] and Sanghamitra *et al*. (2019) [9].

Table 2: Effect of hardening media on survival and root parameters of orchid plants at 120 DAP

Treatments	Survival Percent	Number of roots $plant^{-1}$	Length of roots $plant^{-1}$	Root volume (cm^3)	Shoot girth (mm)
		120 DAT	120 DAT	120 DAT	120 DAT
T_0	68.04	12.67	6.37	4.20	2.87
T_1	49.86	7.78	5.97	1.86	2.20
T_2	83.42	10.56	6.37	3.00	3.46
T_3	62.40	13.89	6.92	4.80	2.82
T_4	48.62	7.56	5.71	1.80	2.16
T_5	78.57	10.00	6.33	2.83	3.08
T_6	60.00	13.33	6.60	4.62	2.21
T_7	56.02	8.00	5.90	0.93	2.18
T_8	92.48	10.89	6.29	3.61	3.24
T_9	66.80	14.44	7.77	6.60	2.80
T_{10}	38.02	6.00	4.37	1.53	2.04
T_{11}	38.50	6.33	4.43	1.65	2.04
T_{12}	60.02	9.00	6.27	2.22	2.40
T_{13}	40.67	7.33	5.10	1.74	2.08
T_{14}	73.07	12.22	6.48	3.61	3.17
T_{15}	57.18	9.56	6.18	2.40	2.34
$SE_{m\pm}$	4.24	0.44	0.25	0.05	0.13
CD	12.22	1.27	0.72	0.13	0.39

Survival percent

Maximum survival percent (92.48%) was observed with treatment T_8 (Chopped coconut husk + Brick + Charcoal + Rice Husk) which were noticed *at par* with treatment T_2 (Bark + Brick + Charcoal + Cocopeat) but significantly better over rest of the treatments. Minimum survival percent (38.02) was noted in treatment T_{10} (Chopped Paddy Straw + Brick + Charcoal).

The maximum survival percent caused by treatment T_8 (Chopped coconut husk + Brick + Charcoal + Rice Husk) might be due to the reason that substrate containing Coconut husk, Brick, Charcoal and Cocopeat provide enhanced moisture and nutrient holding capacity at initial stage of plant growth. Further provide good support to the plant rescued in increased survival percent. Identical results have been also reported by Sharma and Chauhan (1995) [6], Muna *et al*. (2016) [4], Sabareeswaran *et al*. (2018) and Lakshanthi and Seran (2019) [3].

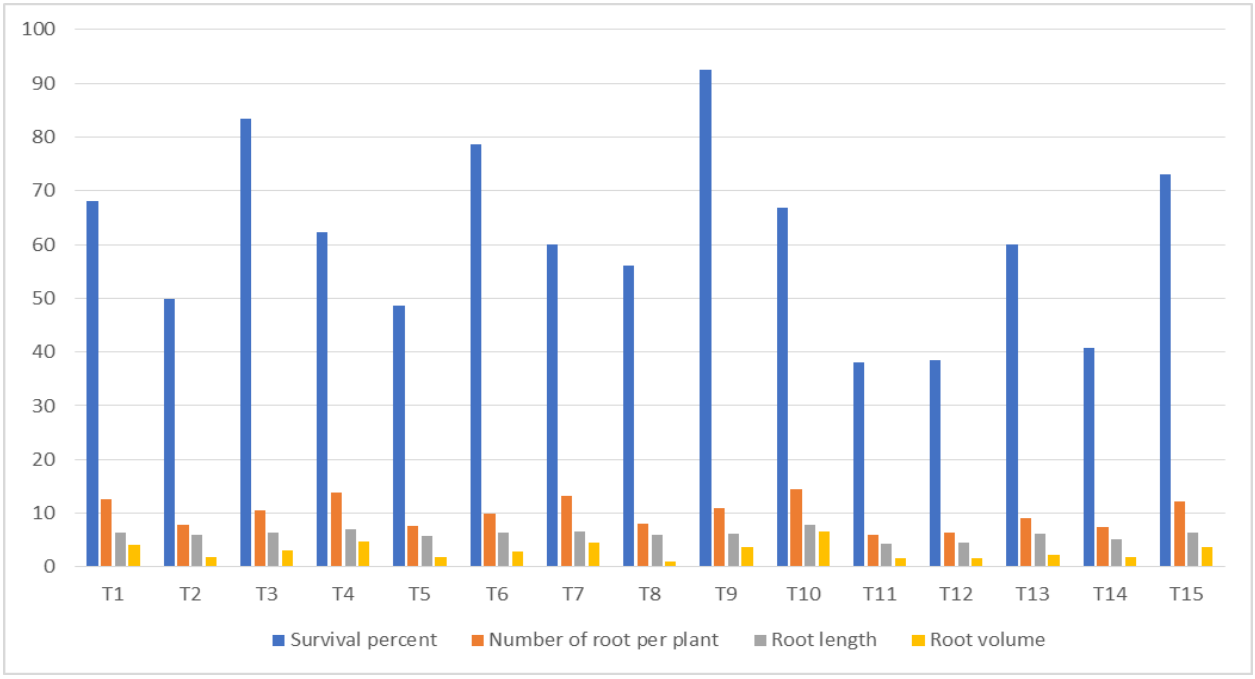

Fig 1: Graphical representation of effect of different hardening media on survival percent and root growth parameters of Orchids plants.

Conclusion

A thorough analysis of the research data revealed that the hardening media combination T_8 (chopped coconut husk, brick, charcoal, and cocopeat) produced better results in every significant area of the study.

The outcomes showed that the optimal media for acclimatizing *in vitro* propagated orchids would be hardening medium with moderate aeration and high water holding capacity. Because it comprised coconut husk, brick pieces, charcoal, and cocopeat, treatment T8 produced better outcomes. Brick pieces aid in giving plants excellent aeration and mechanical support, while coconut husk is best suitable for enhancing the substrate's capacity to hold water and provide nutrient content in the beginning. charcoal is also helpful in providing aeration and retaining fertilizer when pouring fertilizer solution and it steadily transfer nutrients to developing plants on subsequent watering, Treatment T_{10} produces the lowest results in almost all parameters, which may be because the medium which contains chopped paddy straw, brick, and charcoal could not provide the growth with the necessary nutrients and support. Therefore the media combination T_8 (chopped coconut husk + brick + charcoal + cocopeat) may be recommended for hardening media of orchids.

Reference

1. Bose TT, Bhattacharjee SK. Orchids of India. Naya Prokash, India; c1980.
2. Indhumati K, Kannan M, Jawaharlal M, Amaranath V. Standardization of prehardening and hardening techniques for *in vitro* derived plantlets of *Dendrobium* Orchid Hybrid Sonia-17. Journal of Ornamental Horticulture. 2003;6(3):212-216.
3. Lakshanthi JMT, Seran TH. Survival rate and growth performance of *in vitro* raised plantlets of orchid (*Dendrobium* sp.) in different hardening substrates. International Journal of Advanced Research and Review, 2019;4(3):01-09.
4. Muna S, Beura SK, Biswal M, Taria B, Sahu P. Standardization of media combinations for hardening of orchid *Dendrobium* Spp Cv. Sonia-17. International Journal of Agriculture Science, 2016;8(6):1042-1044.
5. Pathania NS, Sehgal OP, Debojit P, Dilta BS, Paul D. Studies on micropropagation in Dendrobium cv. Sonia. Journal of Orchid Society India. 1998;12:35-38.
6. Sharma J, Chauhan YS. Establishment of *in vitro* raised seedling of *Dendrobium chrysanthum* and *Paphiopedilum spicerianum.* Journal of Orchid Society India, 1995;9:37-41.
7. Cherian RS, Anju S, Paul W, Sabareeswaran A, Mohanan PV. Organ distribution and biological compatibility of surface-functionalized reduced graphene oxide. Nanotechnology. 2019 Nov 20;31(7):075303.
8. Deb CR, Imchen T. An efficient in vitro hardening technique of tissue culture raised plants. Biotechnology. 2010;9(1):79-83.
9. Iragavarapu T, Radhakrishna T, Babu KJ, Sanghamitra R. Acute coronary syndrome in young-A tertiary care centre experience with reference to coronary angiogram. Journal of Primary Care Specialties. 2019 Jan 1;5(1):18-25.
10. Patel PL, Suram A, Mirani N, Bischof O, Herbig U. Derepression of hTERT gene expression promotes escape from oncogene-induced cellular senescence. Proceedings of the National Academy of Sciences. 2016 Aug 23;113(34):E5024-5033.