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STANDARDIZATION OF MEDIA MIXTURE FOR *EX-VITRO* HARDENING OF *IN VITRO* ROOTED PLANTLETS OF *SPATHIPHYLLUM*

K. Karthik[1*], A.V.D. Dorajee Rao[2], K. Ravindra Kumar[3], G. Ramanandam[4], A. Snehalatha Rani[3] and D.R. Salomi Suneetha[6]

[1]Department of Floriculture and Landscaping, Dr YSRHU, Venkataramannagudem, West Godavari - 534 101, A. P., India.

[2]College of Horticulture, Dr YSRHU, Venkataramannagudem, West Godavari – 534 101, Andhra Pradesh, India.

[3]Dr YSRHU-HRS, Kovvur, East Godavari – 534 350, Andhra Pradesh, India.

[4]Department of Horticulture, Dr. YSRHU, Venkataramannagudem, West Godavari - 534 101, Andhra Pradesh, India.

[5]Department of Biochemistry, Dr. YSRHU, Chinalataripi, S.P.S.R. Nellore - 523 105, Andhra Pradesh, India.

*Corresponding author E-mail : karthik777y@gmail.com

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ABSTRACT

Spathiphyllum, commonly known as the peace lily, is a widely cultivated ornamental plant valued for its aesthetic appeal and air-purifying properties in indoor environment under low light conditions. While micro-propagation is an efficient method for large-scale production, the successful *ex vitro* acclimatization of tissue-cultured plantlets remains a challenge due to weak root systems, underdeveloped cuticles, and susceptibility to environmental stress. This study evaluated the influence of various potting media on the survival and growth performance of *in vitro* raised plantlets. The experiments were conducted at Dr. YSRHU-Horticulture Research Station, Kovvur during the year 2023–24. The rooted tissue culture plantlets were hardened in different media combinations comprising of red earth, FYM, cocopeat, sand, vermicompost, perlite and vermiculite under a controlled shade net environment. Among the media, the combination of Sand: Cocopeat: Vermicompost (1:1:2) resulted in the highest survival rate (96.7%), swift leaf emergence (8.50 days) and other superior growth parameters, including plant height (20.16 cm), number of leaves per plant (9.35), leaf area (6.44 cm^2), chlorophyll content (34.13), number of roots per plant (16.75) and root length (12.19 cm). The improved growth performance was attributed to the enhanced water retention, aeration and nutrient availability provided by the selected medium. These findings highlight the significance of optimized potting media in improving plantlet establishment, ensuring successful acclimatization, and enhancing commercial viability. This study provides valuable insights into the standardization of potting media for the efficient *ex vitro* hardening of Spathiphyllum plantlets.

Key words : Acclimatization, Cocopeat, Hardening, Potting media, Vermicompost.

Introduction

Spathiphyllum belongs to the family Araceae and is indigenous to tropical regions of Central America. It is commonly known as peace lily, snow flower and spathe flower. It is sold as cut flower and potted plants in ornamental industry. It is one of the attractive pot plants, characterised by distinctive spathe and highly suitable for maintaining under indoor shade conditions. The NASA clean air study found that *Spathiphyllum* cleans indoor air of certain environmental contaminants, including benzene and formaldehyde. The plant is in great demand both in local and global markets (Rout *et al.*, 2006). *Spathiphyllum* can be propagated through sexual and asexual means. Mostly accomplished through division of clumps. However, conventional propagation may not be viable in order to meet rising need for large scale, high-quality and uniform plant material. In these conditions, micro-propagation is the order of the day for rapid production of quality plant material in limited space (Atak and Çelik, 2012).

The introduction of micro propagated plantlets to the *ex-vitro* environment can be a delicate process, as the plants must adapt to changes in temperature, humidity, light intensity, and nutrient availability. Proper *ex vitro* hardening is most crucial for ensuring the survival and healthy growth of the plantlets, as it allows them to develop the necessary physiological and morphological characteristics to thrive in the natural environment (Shekhawat *et al*., 2015). Susceptibility of the plants to environmental stress, the presence of poorly developed stomata, thin cuticles and a partly heterotrophic nature are the common challenges of the hardening process as reported by Hazarika *et al*. (2006), Chandra *et al*. (2010). Weak root system and poorly developed cuticle are the principal reasons for poor survival of tissue culture plants in harsh *ex vitro* conditions when they were transferred from controlled *in vitro* environment (Mathur *et al*., 2008). The plantlets at this stage are vulnerable to high percentage of damage and loss (Ortega-loeza *et al*., 2011).

Tissue cultured plants are susceptible to transplantation shock leading to high mortality during the final stage of micro-propagation i.e., hardening (Kumar and Rao, 2012). Successful micro-propagation depends on the ability to transfer *in vitro* raised plantlets to potting mixture and acclimatize them successfully to the field conditions. Studies on the hardening of *in vitro* grown plantlets reveal that, media plays an important role on hardening success as well as in producing plantlets of the intrinsic quality. Vermicompost, an organic nutrient-rich substrate, plays a pivotal role in fostering acclimatization and robust growth of plants (Sharma *et al*., 2023). The application of tissue culture for regeneration and commercial propagation of whole plants is an established technique. However, the successful transition of *invitro* raised plantlets to *ex-vitro* conditions remains a crucial challenge. Hence, this study aims to investigate the acclimatization by using different media and their composition for survival and growth of spathiphyllum plantlets in a shade net environment.

Materials and Methods

The experiment regarding Standardization of growing media for *ex-vitro* hardening of rooted micro-shoots in spathiphyllum plantlets was conducted at the Dr. YSRHU - Horticultural Research Station, Kovvur during the period of 2024 - 25.

Rooted plants with a minimum of 3-4 roots and height of 3-4 cm were removed from culture bottles. The agar sticked to the roots was washed under running tap water, followed by immersing them into Bavistin @1 g/L solution for 30 sec. Then, they were transplanted in 40 celled protrays containing a mixture of five different treatment compositions of red earth, FYM, Cocopeat, Sand, Vermicompost, Perlite and Vermiculite. Four replications per treatment and 25 plants per each replication was arranged in CRD design. After transplanting the tissue culture plants, the protrays were placed under a tunnel structure covered with transparent polythene sheet in a 50% shade net house, for proper maintenance of relative humidity (RH) in primary hardening. The plantlets were constantly monitored and as per the requirement watering was done. During the second week, the polythene cover was slightly opened for 1 to 2 hrs every day to accustom the tissue culture plants for reduced levels of relative humidity inside the polythene tunnel. During the third week of acclimatization, the transparent polythene cover was kept open for 5-6 hrs daily. Subsequently, the plantlets were completely uncovered at the end of fourth week of the transplantation. After four weeks of pre-acclimatization, only survival and well-developed plantlets were maintained.

After six weeks, acclimatized plantlets were watered, as and when required, fertilized weekly with 19:19:19 (1 mg L^{-1}) and followed other regular cultural activities followed in nursery. During the experimentation, observations were recorded on Per cent survival, Number of days taken for new leaf emergence, Plant height (cm), Number of leaves per plant, Leaf area (cm^2), SPAD value, Number of roots per plant, Root girth (mm), Length of root (cm). The replicated data is statistically analysed using OPSTAT software.

Results and Discussion

Studies on the hardening of *in vitro* grown plantlets reveal that, potting media play an important role on survival, growth and hardening of tissue culture plants.

The present study is about the use of proper media for hardening as well as potting media to increase the survival rate and sustain healthy growth of spathiphyllum plants. The selection of a proper potting medium with high aeration, permeability and stable pH is the major prerequisite for successful ex vitro growth of the plantlets (Diaz *et al*., 2010).

Survival of *in vitro* plantlets and days taken for first leaf emergence

The result of the experiment showed that, there was a significant difference in the survival (%) of plantlets among the different media compositions. Out of various media combinations used for hardening, significantly highest survival percentage was found in sand + vermicompost + cocopeat in 1:1:2 ratio (T_4) (96.7%) followed by sand + vermicompost + cocopeat in 2:1:1 ratio (T_3) (90.0%) and Cocopeat + Perlite + Vermiculite

Table 1 : Effect of different growing media on Percentage survival and Number of days taken for new leaf emergence.

Treatments		Percentage survival	Number of days taken for new leaf emergence
T_1	Red earth + FYM + Cocopeat (2:1:1)	71.7	11.05
T_2	Red earth + FYM + Cocopeat (1:1:2)	76.7	10.55
T_3	Sand + Vermicompost + Cocopeat (2:1:1)	90.0	9.75
T_4	Sand + Vermicompost + Cocopeat (1:1:2)	96.7	8.50
T_5	Cocopeat + Perlite + Vermiculite (1:1:1)	86.7	9.10
SE(m)		4.01	0.37
CD		12.21	1.14
CV		9.52	7.65

Table 2 : Effect of different growing media on Plant Height at 45, 90, 120 days after planting.

Treatments		Plant height (cm)		
		45 DAP	90 DAP	120 DAP
T_1	Red earth + FYM + Cocopeat (2:1:1)	5.57	8.48	12.34
T_2	Red earth + FYM + Cocopeat (1:1:2)	6.99	9.39	15.79
T_3	Sand + Vermicompost + Cocopeat (2:1:1)	7.62	11.85	18.05
T_4	Sand + Vermicompost + Cocopeat (1:1:2)	7.99	14.28	20.16
T_5	Cocopeat + Perlite + Vermiculite (1:1:1)	7.09	12.37	16.65
SE(m)		0.17	0.52	0.43
CD		0.55	1.59	1.29
CV		4.81	9.29	5.12

1:1:1 ratio (T_5) (86.7%) after six weeks of transplanting in *ex vitro* conditions. The T_4 media had also shown the least number of days taken for new leaf emergence (8.50 days) (Table 1) compared to other treatments.

The results showed that the potting medium having high water holding capacity and moderate aeration was the best for acclimatization of *in vitro* grown spathiphyllum plantlets. In this, Coco peat is suitable to improve water holding capacity of the substrate, Vermicompost as mineral source and sand provide good aeration at the initial stage. These results are in colloborative with Nazki *et al*. (2015) in *Gerbera jamesonii*. Similar results were also reported in Aglaonema by Sawardekar and Sherkar (2024) where he obtained 90.83% survival percentage while using cocopeat as medium and Gautam *et al*. (2019) in carnation recorded 100% survival of *in vitro* raised plantlets when cocopeat, sand and perlite (2:1:1, v/v) were used as hardening media. Patra and Buera (2016) noticed minimum number of days for new leaf formation (7.67) in Gerbera using (soil: sand: FYM: coco peat in ½ :1:1:2). Rajesh *et al*. (2011) in Syngonium noticed a dramatic increase in the survival percentage (88%) with the inclusion of cocopeat and vermicompost in the hardening media. Khanchana *et al*. (2018) recorded the highest survival of 97.17 per cent followed by (92.79%) in tuberose when vermicompost+ sand+ cocopeat in the ratio 1:1:1 was used as potting mix.

Plant height (cm)

The data regarding the shoot height at 45, 90 and 120 days was presented in Table 2. The data showed that, the maximum plant height of 7.99 cm, 14.28 cm, 20.16 cm was noticed in media having cocopeat + vermicompost + sand in the ratio 2:1:1 (T_4) at 45, 90 and 120 days, respectively (Fig. 1). The next best treatment was sand + vermicompost + cocopeat in the ratio 2:1:1 (T3) which recorded the plant height of 18.05 cm at 120 days. The vigorous growth increase in the shoot is primarily because of the enhanced aeration, high water-holding and nutrient holding capacity of cocopeat and vermicompost at the initial stages of plant growth which provided excellent conditions for the establishment of hardened plantlets in the field. Gautam *et al*. (2019). Similar results were observed by Singh *et al*. (2022) in *Chrysanthemum morifolium* Ramat. in which the

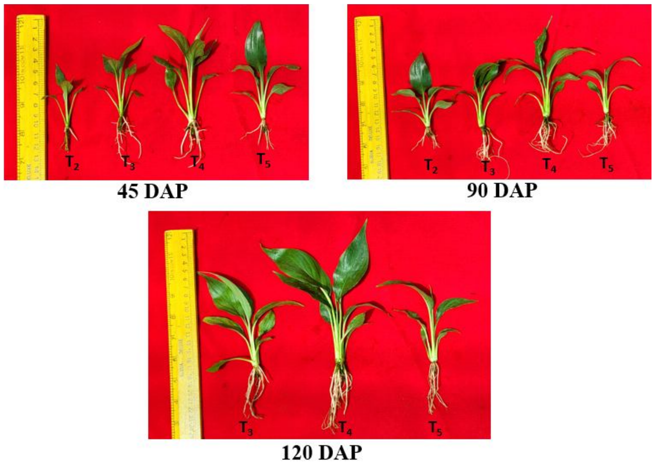

Fig. 1 : Growth Dynamics of plants in different treatments at 45, 90 and 120 DAP.

Fig. 2 : Representative plant from the best treatment (T_4) with maximum number of leaves and roots.

Table 3 : Effect of different growing media on Leaf parameters at 45, 90, 120 days after planting.

Treatments		No of leaves per plant			Leaf area (cm²)			SPAD value		
		45 DAP	90 DAP	120 DAP	45 DAP	90 DAP	120 DAP	45 DAP	90 DAP	120 DAP
T_1	Red earth + FYM + Cocopeat (2:1:1)	4.10	5.45	6.85	2.01	3.04	4.43	23.68	26.98	27.80
T_2	Red earth + FYM + Cocopeat (1:1:2)	4.65	5.95	7.20	2.15	3.36	5.49	26.78	27.90	28.40
T_3	Sand + Vermicompost + Cocopeat (2:1:1)	5.60	7.65	8.45	2.35	4.04	6.35	28.65	29.70	32.85
T_4	Sand + Vermicompost + Cocopeat (1:1:2)	6.20	8.20	9.35	2.42	4.40	6.44	31.18	31.98	34.13
T_5	Cocopeat + Perlite + Vermiculite (1:1:1)	5.90	7.25	7.90	2.23	3.98	6.22	28.53	29.33	31.83
SE(m)		0.19	0.17	0.22	0.05	0.08	0.09	0.93	1.65	1.15
CD		0.61	0.54	0.66	0.17	0.27	0.32	2.84	N/A	3.48
CV		7.48	5.13	5.48	4.92	4.71	3.41	6.73	11.31	7.40

medium comprising cocopeat, vermicompost, and leaf mold in a 2:1:1 ratio resulted in the highest plant height (30.72 cm) and Gautam *et al.* (2019) in carnation where he recorded maximum increase in height of plantlets when cocopeat, sand and perlite in the ratio 2:1:1, v/v were used as hardening media.

Leaf parameters

The data regarding the number of leaves per plant, leaf area, chlorophyll content was presented in Table 3.

The results showed that at 120 days the maximum number of leaves (9.35), highest leaf area (6.44 cm^2) and SPAD value (34.13) were noticed in media having Sand + Vermicompost + Cocopeat in the ratio 1:1:2 (T_4) (Fig. 2) and the next best results were seen in media having Sand + Vermicompost + Cocopeat in the ratio 2:1:1 (T_3). N is a chief constituent of protoplasm providing metabolic energy to the cell division and cell enlargement. This substrate mixture may have provided nutrient directly to the plant causing stimulation of new leaves. Chlorophyll content is a vital indicator of a plant's photosynthetic capacity and overall health. The integration of vermicompost into the potting medium has been shown to enhance chlorophyll levels. Research indicates that vermicompost supplies essential nutrients in readily available forms, thereby boosting chlorophyll synthesis (Singh *et al.*, 2017).

The increased leaf area gradually from 30 DAP to 120 DAP may be attributed by higher carbohydrate accumulation in leaves facilitated by favourable nutrients has led to higher photosynthetic activities resulting in an increased leaf area. An expansive leaf area enhances a plant's photosynthetic efficiency, contributing to better

Table 4 : Effect of different growing media on root parameters at 45, 90, 120 days after planting.

Treatments		No of roots per plant			Length of root (cm)			Root girth (mm)		
		45 DAP	90 DAP	120 DAP	45 DAP	90 DAP	120 DAP	45 DAP	90 DAP	120 DAP
T_1	Red earth + FYM + Cocopeat (2:1:1)	8.75	8.95	9.95	1.85	6.12	9.22	0.81	1.15	1.94
T_2	Red earth + FYM + Cocopeat (1:1:2)	9.20	10.05	11.75	1.98	6.46	10.51	0.90	1.62	2.16
T_3	Sand + Vermicompost + Cocopeat (2:1:1)	11.35	12.65	15.2	2.61	8.09	11.77	1.17	1.97	2.85
T_4	Sand + Vermicompost + Cocopeat (1:1:2)	13.05	15.55	16.75	2.81	8.47	12.19	1.29	2.13	3.37
T_5	Cocopeat + Perlite + Vermiculite (1:1:1)	10.80	12.05	13.45	2.56	7.68	11.35	1.03	1.74	2.52
SE(m)		0.31	0.22	0.22	0.05	0.08	0.09	0.02	0.02	0.02
CD		0.94	0.68	0.67	0.15	0.25	0.28	0.06	0.07	0.07
CV		5.83	3.79	3.29	4.24	2.25	1.68	4.11	2.81	1.88

growth and yield. Similar results were observed by Khilari *et al*. (2023) in orchids, where she recorded the increase in number of leaves per plant (6.43) when Chopped Coconut Husk + Brick + Charcoal + Cocopeat were used in the ratio of (1:1:1:1).

Root parameters

The data regarding the number of roots per shoot, length of the root (cm), root girth (mm) was presented in Table 4.

The results showed that highest number of roots per plant (16.75), highest length of the root (12.19 cm) and root girth (3.37 mm) were obtained at 120 days in media having Sand + Vermicompost + Cocopeat in the ratio 1:1:2 (T_4). The development of the root system (root number and length) is vital to anchor the plant and also to ensure water and nutrients absorption. This may be due to optimum water holding capacity, porosity, better aeration and drainage in the medium, which provides suitable condition for further growth and development of the plants. This overall increase in the root parameters was attributed to cocopeat's high cation exchange capacity, which enhances nutrient availability, and its ability to improve the physical structure of the substrate by reducing weight and increasing water-holding properties which leads to improved root proliferation and overall increase in the root parameters which is helpful in successful acclimatization (Gautam *et al.*, 2019). A robust root system also supports enhanced vegetative growth. Similar results were reported in Aglaonema by Sawardekar and Sherkar (2024), where he obtained maximum number of roots (6) and maximum length of the root (8cm), while using cocopeat as medium.

Conclusion

In conclusion, the incorporation of cocopeat and vermicompost into potting media offers substantial benefits for the *ex-vitro* hardening of tissue-cultured Spathiphyllum plants. These substrates collectively enhance shoot height, root and leaf development, chlorophyll content and leaf area, thereby promoting vigorous plant growth and successful acclimatization. Among all treatments tested, T_4 (cocopeat: vermicompost: sand in the ratio 2:1:1) was the most effective, showing the highest survival percentage, early leaf emergence, and superior shoot, leaf and root parameters. The combination of cocopeat, vermicompost and sand provided optimal aeration, water retention and nutrient availability, enhancing plantlet establishment. Improved chlorophyll content and leaf area in T_4 further supported its role in promoting photosynthetic efficiency. These findings highlight the importance of selecting an appropriate potting medium for successful acclimatization and growth of tissue-cultured plants.

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