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Research Article

Effects of plant preservative mixture™ on in vitro germination of *Dendrobium thyrsiflorum* Rchb.f. and its application in orchid conservation

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Abstract In vitro conservation is one of the most effective strategies for rare plant protection, especially for orchid species. To maximize the success rates of in vitro explant establishment (stage I) in conservation programs, the application of tissue culture additives such as Plant Preservative Mixture™ (PPM™) should be emphasized. In this study, we used *Dendrobium thyrsiflorum* Rchb.f. (1875) seeds and seedlings as a model for the evaluation of PPM™'s phytotoxicity in the meristematic tissues of epiphytic orchids. PPM™ had no observable inhibitory effect on protocorm, shoot, or root development when it was supplemented at 0.1%. PPM™ supplementation caused adverse effects on *D. thyrsiflorum* explants at concentrations > 0.2%. At high concentrations, young in vitro seedlings showed damage, especially at the root tissue level. Based on this model, supplementation of 0.1-0.2% PPM™ to culture media was successfully implemented to establish in vitro cultures of other rare orchid species in our conservation program.

Keywords protocorm, micropropagation, agriculture, production, *Nervilia*

Introduction

Anthropogenic climate change and over-exploitation are significant causes of species extinction, including rare members of Orchidaceae (Wraith and Pickering 2018). In a Southeast Asian country, such as Vietnam, there are 68 orchid species listed on the IUCN Red List of Threatened Species Vietnam (2007). Among those, 22 *Dendrobium* species are endangered (Ban 2007). The demand for rare orchid specimens can sometimes become extreme. For example, *Dendrobium anosmum* var alba was traded for thousands of US dollars at its peak, 'fueled' both orchid smugglers and local collectors. Over-collecting, thus, threatened many tropical orchid species, while reintroduction efforts are still a not common conservation strategy in underdeveloped countries (Seaton et al. 2013).

To date, in vitro shoot propagation or asymbiotic seed germination is considered a reliable method for the conservation of orchids (Hoang et al. 2016; Pujasatria et al. 2020; Santos-Díaz et al. 2022). Especially the number of explants that are available for in vitro conservation projects can be seasonal or very limited in quantity (Sarasan 2010). During field surveys, specimens of endangered species may take days to locate and approach; severe damage and microbial contamination may prevent conservation efforts (Arab et al. 2014; Hoang et al. 2016; Izarra et al. 2020). Hence, implementing low-cost and innovative solutions to improve explant survival could facilitate conservation projects in under-development countries (Agrawal et al. 2010).

The Plant Preservative Mixture™ has antimicrobial activity that can eliminate a wide range of microbial organisms and prevent biofilm formation (Compton and Koch 2001). Its main component is a thermostable isothiazolone compound, includes 5 - chloro - 2 - methyl - 3(2H) - isothiazolone (MCI) and 2 - methyl - 3(2H) - isothiazolone (MI) (Patent No. 5,750,402). Due to its thermostability, the compound can be added directly to the culture medium before autoclave (Guri 1998). PPM™ is commonly used as a biocide in plant tissue culture (Faizy et al. 2017; Givnish et al. 2016; Leão et al. 2020; Rihan et al. 2012; Romadanova et al. 2022). We believe the full potential of PPM™ is not limited to commercial micropropagation, but it can be a

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powerful solution for in vitro conservation. Unfortunately, no literature could adequately address the value of PPM™ for rare plant protection.

In this research, we focused on the potential use of PPM™ for in vitro orchid conservation. We evaluated the effect of PPM™ on the germination, growth, and development of *Dendrobium thyrsiflorum* Rchb.f. seeds and seedlings. As a model, *Dendrobium thyrsiflorum* Rchb.f. is affected and threatened in its natural habitat due to its ornamental value and over-exploitation (Zhu et al. 2019). We successfully applied these results in the culture establishment and conservation of other rare orchids, such as *Dendrobium officinale* and *Nervilia* spp. To our knowledge, this study is the first one to promote and emphasize the unique value of commercially available formulations, such as PPM™, for in vitro rare plant conservation.

Material and Method

The *Dendrobium thyrsiflorum* Rchb.f. seeds and seedlings were used as model explants to evaluate the effectiveness of PPM™ on *Dendrobium* germination and in vitro development. Ripen unopened capsules were collected from Cat Tien National Park (Dong Nai, Vietnam). Capsules were covered in plastic bags, then transferred to the laboratory for experimenting. The sterilization process was performed in a bio-safety cabinet. The capsules were surface sterilized in 5% NaClO solution for 15 minutes, followed by flaming twice with 96% ethanol. After surface sterilization, the capsule was slit, and seeds were collected. Seeds were dried in 10 mL scintillation jars using silica beads for 24 hours. After that, the vials are sealed and stored in a refrigerator at 4°C (Shao et al. 2020; Zi et al. 2014).

Effects of PPM™ concentrations on *Dendrobium thyrsiflorum* Rchb.f. in vitro seed germination

The *D. thyrsiflorum* Rchb.f. seed vials were transferred from the refrigerator to room temperature before use. Seeds were weighed in sterilized containers. The orchid seed sterilization solution contained 5% NaClO, 96% C_2H_5OH, and sterilized distilled water with 1:1:18 ratio, respectively (Hoang et al. 2016). The seed sterilization solution was added to the seed containing jar and shaken for one minute. The seeds were washed three times with sterilized distilled water. After that, the seeds were suspended with 2 mL of sterilized distilled water. 50 µL of suspension solution was transferred on sterilized filter paper (1 cm × 2 cm), and seed numbers were counted. We transferred the filter paper to culture tubes containing 10 mL ½ MS (Murashige and Skoog 1962) supplemented with 30 g/L sucrose, 7 g/L agar, and 0.0, 0.1, 0.2, 0.4, 0.8, and 1.6% PPM™, respectively. PPM™ was added into the medium before pH adjusting to 5.8 and autoclaving at 121°C/1 atm. Each culture tube was considered a replicate. Each treatment had totally of 6 replicates, and each replicate contained 40-50 seeds as sub-replicates (260-383 seeds per treatment). The chamber temperature was 25 ± 2°C, 40 - 50%, humidity, and illuminated with 30 $\mu Mm^{-2}s^{-1}$ growth light LED. During the 10-week experiment, seed germination and seedling development stages are categorized using the description from Hoang et al. (2016).

Effects of PPM™ concentrations on *D. thyrsiflorum* Rchb.f. in vitro seedlings growth

Three-month old seedlings *D. thyrsiflorum* Rchb.f. (approximately 1 cm height) on ½ MS medium supplemented with 30 g/L sucrose, 7 g/L agar culture were used as material. The seedlings' weight and height were measured before transferring to culture tubes containing 10 mL solidified ½ MS culture medium supplemented with 0.0, 0.1, 0.2, 0.4, 0.8, and 1.6% PPM™, respectively. The experiment had six treatments, each with 10 culture tubes (n=10), and each tube had three seedlings (30 sub-replicates per treatment). Every 10 days, seedling fresh weight and dried weights were collected. Growth changes (height, fresh, and dried increases) were measured by deducing parameters between each growth interval. The culture chamber condition was described above.

Application of low PPM™ concentration on other orchids' explant establishment

To evaluate the potential use of PPM™ for culture establishment of other rare orchids in our conservation program, we used in vitro explants of *Dendrobium officinale, Nervilia fordii, Nervilia plicata* of MS media supplemented with 0.1% PPM™. The explants were sterilized and transferred to MS media supplemented with 0.1% PPM™. General explant growth and development were observed.

Statistical analysis and data analysis

In this research, a one-factor simple experiment design was used. Replicates' positions in the growth chamber

were randomized using Microsoft Excel®. Chi-squared analysis was used for qualitative data such as seed germination rates. Statistical analysis was performed using R software (version 4.2.2).

Result and Discussion

Most tropical countries with the highest biodiversity also have low-income populations. Plant conservation and reintroduction research are often underestimated and poorly funded (Heywood 2017). To establish a sustainable yet high-impact plant conservation program in this context, the working procedures must be simple, low-cost, and effective (Ribaudo 2017). This study highlighted the use of commercial products for rare explants in general. Simple and effective in vitro explant establishment results are the foundation for more successful in vitro conservation and restoration projects in low-income countries.

The active ingredients in PPM^{TM} are mainly isothiazolones, including methylchloroisothiazolinone and methylisothiazolinone (US patent US5750402A). These biofilm inhibitor compounds could be harmless to plant tissue at low levels and therefore were used as preservatives or germicidal (Guri et al. 1998). Our data showed no inhibitory effect on *Dendrobium thyrsiflorum* embryo explants at low levels, such as 0.1-0.2% (Table 1). After 10 weeks of in vitro culture, the germination rate of *Dendrobium thyrsiflorum* seeds was not affected at the 0.1-0.2% PPM^{TM} concentrations. No statistical differences were detected between the control and low-level treatments. No unusual organogenesis was observed, and protocorms grew normally on this media for months after experimenting (Table 1 & Fig. 1). Arditti and Yam also documented this low dosage for orchid seed (Arditti and Yam 2017) or melon explants (Compton and Koch 2001).

At the high level of PPM^{TM} supplementation (above 0.2%), *Dendrobium thyrsiflorum* protocorm explants were either damaged or starting to show inhibitory effects (Table 1). Stages of protocorm development were delayed as a sign of growth inhibition (Fig. 1). Browning and necrotic tissue were detected on protocorms. Growth inhibition by PPM^{TM} supplementation was also documented by Paul et al. in *Arabidopsis thaliana* (Paul et al. 2001) and Niedz and Bausher in *Citrus sinensis* (Niedz and Bausher 2002). Besides growth inhibition, PPM^{TM} can also negatively affect organogenesis in sensitive crops, such as petunia (Compton and Koch 2001) and chrysanthemum (George and Tripepi 2001).

Seeds of Orchidaceae members have morphological dormancy (Baskin and Baskin 2001). The underdeveloped embryos with dozens of cells required either endomycorrhizal fungus or exogenous sugar supplementation for their further germination stages (Rasmussen 1992). Seed maturation is merged into embryo growth and protocorm development (Fang et al. 2021). After a successful germination initiation, marked by water imbibition, the protocorms develop further into seedlings with true leaflets (Hoang et al. 2016). Normal embryo survival and protocorm development of *D. thyrsiflorum* Rchb.f. on medium supplemented with low PPM^{TM} concentration (Fig. 1) indicate that the recipe could also be used safely for seedlings explants, which commonly have higher tolerance.

Three-month old *Dendrobium thyrsiflorum* Rchb.f. seedlings developed normally on MS media supplemented with 0.1% PPM^{TM} during the experiment and months later. However, signs of damage were observed in the seedlings on media supplemented with higher PPM^{TM} concentration (0.2% to 1.6%), particularly at root zones (Fig. 2). In seedlings with damage, root tissue demonstrated higher sensitivity to PPM^{TM} than leaf tissues; there are no significant - differences among the percent of leaves and roots damaged soon after

Table 1 Seed germination rates and developmental stages of *D. thyrsiflorum* after 6 and 10 weeks of culture on MS (Murashige & Skoog) medium supplemented with different PPM^{TM} concentrations

Treatment	No. of seeds	Germination rate (%)		Developmental stage with damage (10 weeks)
		6 weeks	10 weeks	
0.0% PPM^{TM}	317	88.64	88.33	-
0.1% PPM^{TM}	260	90.38	88.85	-
0.2% PPM^{TM}	383	84.33	82.33	-
0.4% PPM^{TM}	341	78.01*	66.86**	IV
0.8% PPM^{TM}	274	59.85**	45.26**	III
1.6% PPM^{TM}	367	28.88**	6.54**	II

The asterisks * and ** indicate significant chi-square differences between treatments with PPM^{TM} and control (0.0% PPM^{TM}), with significance levels set at 0.05 and 0.001, respectively.

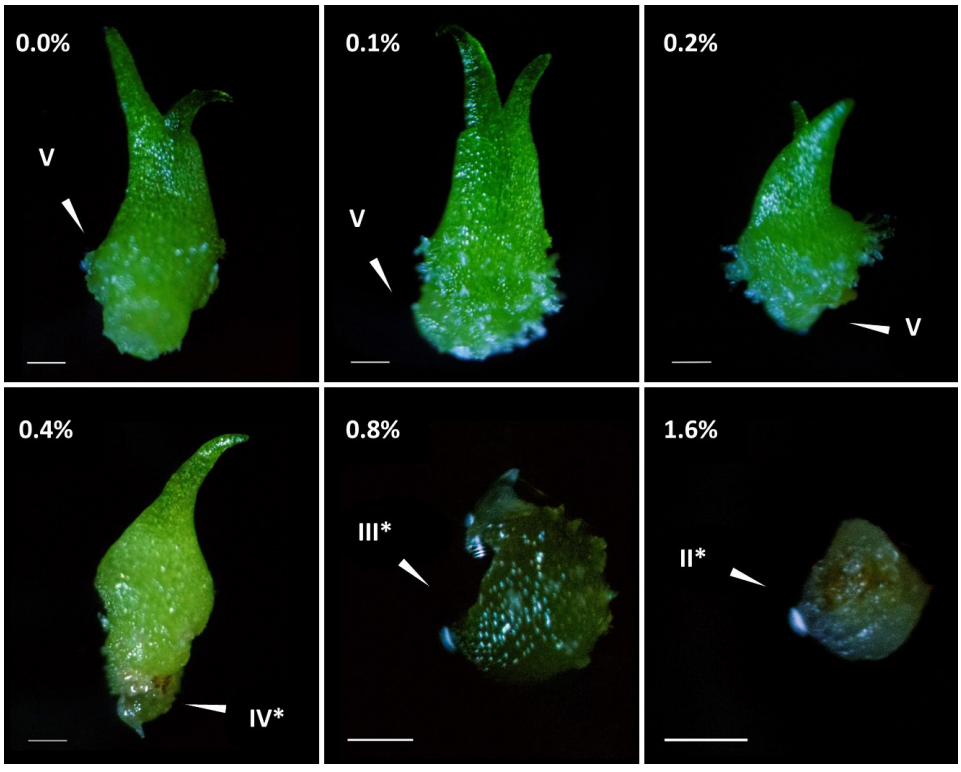

Fig. 1 Seed germination of *D. thyrsiflorum* after 10 weeks of culture at different PPM^{TM} concentrations. "V" refers to developmental stage V without damage at PPM^{TM} concentrations of 0.0%, 0.1%, and 0.2%. "IV*" (at 0.4% PPM^{TM}), "III*" (at 0.8% PPM^{TM}), and "II*" (at 1.6% PPM^{TM}) refer to the damage that appeared in developmental stages IV, III, and II, respectively. Scale bar = 200 μm

Fig. 2 Adaptation of *D. thyrsiflorum* seedlings after 20 days of culture at different PPM^{TM} concentrations. The 3-month-old seedlings showed normal development after culture at 0.0% and 0.1% PPM^{TM} (N). However, at 0.2% and 0.4% PPM^{TM}, signs of damage were observed in roots (RD). At higher PPM^{TM} concentrations (0.8% and 1.6%), signs of damage were also observed in leaves (LD). Scale bar = 5 mm

10 days on media supplemented with 1.6% PPM™ or 20 days on media supplemented with 0.8% PPM™ (Table 2).

As mentioned above, seedlings showed damaging signs at different levels. PPM™ damage symptoms were commonly observed on roots but rarely on leaves except for very high concentrations, e.g., 0.8 and 1.6% (Fig. 2). The supplementation of PPM™ at 0.1% and 0.2% could not affect the growth, but from 0.4% to 1.6% inhibited growth and decreased the seedlings' height and biomass after 20 days (Table 3). Growth inhibition when PPM™ is supplemented at levels above 0.2% is also observed on a few other species, such as Walnut (*Juglans regia* L.) (Kushnarenko et al. 2022), American birch, chrysanthemum, and rhododendron (George and Tripepi 2001).

In fact, by testing sensitive materials such as orchid embryos and seedlings, media supplemented with low dosage (0.1% PPM™) is considerably safer to use for rare explant establishment. During conservation programs, quality and amount of field-collected plant material are usually low (Sarasan 2010). The result could be poor in vitro establishment efficiency and a high-chance of losing valuable explants. This data is significant for in vitro conservation of rare plant species. We successfully used different versions of basal media supplemented with 0.1% PPM™ for in vitro culture establishment of rare species in our conservation program, including *Dendrobium officinales* (Critically Endangered-IUCN Red List), *Dendrobium anosmum, Nervilia plicata, or Pyrenaria jonquieriana* (unpublished data).

In summary, we used *Dendrobium thyrsiflorum* Rchb. f. as a model explant to illustrate the effectiveness of PPM™ supplementation to culture media at low concentrations (e.g., 0.1%). Similar to many other Ag-formulation, PPM™ usage is commonly seen in the commercial sector. We discussed PPM™'s potential in Stage I - explant establishment for rare explants. In the extinction era, we firmly believe that enhancement solutions, such as PPM™ and many other innovative agriculture solutions, should be discussed and explicitly implemented for plant conservation.

Table 2 Response of 3-month-old *D. thyrsiflorum* seedlings after 10 and 20 days of culture on MS (Murashige & Skoog) medium supplemented with different PPM™ concentrations (30 replicates for each treatment)

Treatment	Percentage of seedlings with damage symptoms (%)					
	Total		Leaf		Root	
	10 days	20 days	10 days	20 days	10 days	20 days
0.0% PPM™	0.00	0.00	NA	NA	NA	NA
0.1% PPM™	0.00	0.00	NA	NA	NA	NA
0.2% PPM™	6.66	20.00*	0.00	0.00*	100.00	100.00
0.4% PPM™	50.00**	56.67**	0.00**	11.76**	100.00	100.00
0.8% PPM™	93.33**	96.67**	78.57*	96.55	100.00	100.00
1.6% PPM™	100.00**	100.00**	86.67	100.00	100.00	100.00

The asterisks * and ** indicate significant chi-square differences between treatments with PPM™ and control (0.0% PPM™), with significance levels set at 0.05 and 0.001, respectively. NA: not available.

Table 3 Growth of *D. thyrsiflorum* after 0, 10, and 20 days of culture at different PPM™ concentrations

Treatment	Height (mm)			Fresh weight (mg)			Dried weight (mg)		
	0 DAT	10 DAT	20 DAT	0 DAT	10 DAT	20 DAT	0 DAT	10 DAT	20 DAT
0.0% PPM™	8.75	9.04^{a}	9.63^{ab}	6.52	6.79	8.03	0.80	0.90	1.10
0.1% PPM™	10.26	10.93^{a}	11.57^{b}	11.34	12.31	14.50	1.20	1.70	1.90
0.2% PPM™	10.73	10.99^{a}	11.51^{ab}	14.59	16.04	17.21	1.40	1.50	1.70
0.4% PPM™	9.39	9.53^{a}	9.90^{ab}	9.98	9.73	9.62	0.90	0.80	0.90
0.8% PPM™	9.97	10.10^{a}	10.05^{a}	8.52	8.32	8.19	1.00	0.90	0.80
1.6% PPM™	9.31	9.48^{a}	9.29^{a}	9.33	7.44	6.60	0.90	0.70	0.60

Each treatment had 10 replicates, with 30 seedlings in total (3 subreplicates). The means of the seedling height, fresh weight, and dried weight were calculated using data from 10 replicates with 3 seedlings in each tube. Mean height values with different letters are significantly different at $\alpha = 0.05$ according to one-way ANOVA and Tukey post-hoc test. Fresh weight and dried weight data were pooled. DAT: day after transplant.

Conflict of Interest

The authors declare that there is no conflict of interest.

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References

Agrawal A, Sanayaima R, Tandon R, Tyagi RK (2010) Cost-effective in vitro conservation of banana using alternatives of gelling agent (isabgol) and carbon source (market sugar). Acta Physiol Plant 32(4):703-711. doi.org/10.1007/s11738-009-0450-9

Arab MM, Yadollahi A, Hosseini-Mazinani M, Bagheri S (2014) Effects of antimicrobial activity of silver nanoparticles on in vitro establishment of G × N15 (hybrid of almond × peach) rootstock. J Genet Eng Biotechnol 12(2):103-110. doi.org/10.1016/j.jgeb.2014.10.002

Arditti J, Yam TW (2017) Appendix Eight: Plant Preservative Mixture. Micropropag Orchid 9:2124-2137. doi.org/10.1002/9781119187080.app8

Ban NT, Ly TD, Tap N, Dung VV, Thin NN, Tien NV, Khoi NK (2007) Vietnam Red Data Book (Part II. Plants). Publishing house Sci and Technol 399-477

Baskin C, Baskin JM (2001) Seed Ecology, Biogeography, and Evolution of Dormancy and Germination. Academy Press. pp 360

Compton ME, Koch JM (2001) Influence of plant preservative mixture (PPM)™ on adventitious organogenesis in melon, petunia, and tobacco. Vitr Cell Dev Biol - Plant 37(2):259-261. doi.org/10.1007/s11627-001-0046-6

Faizy HS, AL-Zubaydi SR, Nair M (2017) Effect of Plant Preservative Mixture PPM™ on the Shoot Regeneration of Watercress (*Nasturtium officinale*). Sci J Univ Zakho 5(2):187. doi.org/10.25271/2017.5.2.366

Fang L, Kong X, Wen Y, Li J, Yin Y, Li L, Zeng S, Kong X, Wen Y, Li J, Yin Y, Li L, Wu K, Zeng S (2021) Characterization of embryo and protocorm development of *Paphiopedilum spicerianum*. Plant Physiol Biochem 167:1024-1034. doi.org/10.1016/j.plaphy.2021.09.001

George MW, Tripepi RR (2001) Plant Preservative Mixture™ can affect shoot regeneration from leaf explants of chrysanthemum, European birch, and rhododendron. HortScience 36(4):768-769. doi.org/10.21273/hortsci.36.4.768

Givnish TJ, Spalink D, Ames M, Lyon SP, Hunter SJ, Zuluaga A, Doucette A, Caro GG, McDaniel J, Clements MA, Arroyo MTK, Endara L, Kriebel R, Williams NH, Cameron KM (2016) Orchid historical biogeography, diversification, Antarctica and the paradox of orchid dispersal. J Biogeogr 43(10):1905-1916. doi.org/10.1111/jbi.12854

Guri AZ, Cherry Hill NJ, Patel KN, Dobbs Ferry NY (1998) Compositions and methods to prevent microbial contamination of plant tissue culture media. United States Pat No USOO5750402A (May 12, 1998)

Heywood VH (2017) The future of plant conservation and the role of botanic gardens. Plant Divers 39(6):309-313. doi.org/10.1016/j.pld.2017.12.002

Hoang NH, Kane ME, Radcliffe EN, Zettler LW, Larry W (2016) Comparative seed germination and seedling development of the ghost orchid, *Dendrophylax lindenii* (Orchidaceae), and molecular identification of its mycorrhizal fungus from South South Florida. Ann Bot 119(3):379-393. doi.org/10.1093/aob/mcw220

Izarra ML, Panta AL, Maza CR, Zea BC, Cruzado J, Gutarra LR, Rivera CR, Ellis D, Kreuze JF (2020) Identification and Control of Latent Bacteria in in vitro Cultures of Sweetpotato (*Ipomoea batatas* (L.) Lam). Front Plant Sci, 11(July). doi.org/10.3389/fpls.2020.00903

Kushnarenko S, Aralbayeva M, Rymkhanova N, Reed BM (2022) Initiation pretreatment with Plant Preservative Mixture™ increases the Initiation pretreatment with Plant Preservative Mixture™ increases the percentage of aseptic walnut shoots. Vitr Cell Dev Biol - Plant July. doi.org/10.1007/s11627-022-10279-4

Leão JRA, Raposo A, da Silva ACL, Sampaio PDTB (2020) Control of contaminants in the in vitro establishment of *Guadua latifolia*. Pesqui Agropecu Trop 50:1-7. doi.org/10.1590/1983-40632020V5063541

Murashige T, Skoog F (1962) A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Physiologia Plantarum 15(3), 473-497. doi.org/10.1111/j.1399-3054.1962.tb08052.x

Niedz RP, Bausher MG (2002) Control of in vitro contamination of explants from greenhouse- and field-grown trees. Vitr Cell Dev Biol - Plant 38(5):468-471. doi.org/10.1079/IVP2002316

Paul AL, Semmer C, Kucharek T, Ferl RJ (2001) The fungicidal and phytotoxic properties of benomyl and PPM in supplemented agar media supporting transgenic arabidopsis plants for a Space Shuttle flight experiment. Appl Microbiol Biotechnol 55(4):480-485. https://doi.org/10.1007/s002530000521

Pujasatria GC, Miura C, Kaminaka H (2020) In vitro symbiotic germination: A revitalized heuristic approach for orchid species conservation. Plants 9(12):1-15. doi.org/10.3390/plants9121742

Rasmussen HN (1992) Seed dormancy patterns in Epipactis pahistris (Orchidaceae): Requirements for germination and establishment of mycorrhiza. Physiol Plant 86(1):161-167. doi.org/10.1111/j.1399-3054.1992.tb01325.x

Ribaudo MO (2017). Conservation Programs Can Accomplish More with Less by Improving Cost-Effectiveness. Choices 32(4):1-6. doi.org/10.22004/ag.econ.265838

Rihan HZ, Al-Issawi M, Al-swedi F, Fuller MP (2012) The effect of using PPM (plant preservative mixture) on the development

of cauliflower microshoots and the quality of artificial seed produced. Sci Hortic 141:47-52. doi.org/10.1016/j.scienta.2012.03.018

Romadanova NV, Tolegen AB, Kushnarenko SV, Zholdybayeva EV, Bettoni JC (2022) Effect of Plant Preservative Mixture™ on Endophytic Bacteria Eradication from In Vitro-Grown Apple Shoots. Plants 11:1-12. doi.org/10.3390/plants11192624

Santos-Díaz M del S, Santos-Díaz M denormally developed L, Alvarado-Rodríguez J (2022) In vitro regeneration of the endangered cactus *Turbincarpus mombergeri* (Riha), a hybrid of *T. laui* × *T. pseudopectinatus*. Plant Cell Tissue Organ Cult 148(2):271-279. doi.org/10.1007/s11240-021-02181-5

Sarasan V (2010) Importance of in vitro technology to future conservation programmes worldwide. Kew Bull 65(4):549-554. doi.org/10.1007/s12225-011-9250-7

Seaton P, Kendon JP, Pritchard HW, Puspitaningtyas DM, Marks TR (2013) Orchid conservation: The next ten years. Lankesteriana 13(1-2):93-101. doi.org/10.15517/lank.v0i0.11545

Shao SC, Wang QX, Beng KC, Zhao DK, Jacquemyn H (2020) Fungi isolated from host protocorms accelerate symbiotic seed germination in an endangered orchid species (*Dendrobium chrysotoxum*) from southern China. Mycorrhiza 30(4):529-539. doi.org/10.1007/s00572-020-00964-w

Wraith J, Pickering C (2018) Quantifying anthropogenic threats to orchids using the IUCN Red List. Ambio 47(3):307-317. https://doi.org/10.1007/s13280-017-0964-0

Zhu B, Luo X, Gao Z, Feng Q, Hu L, Weng Q (2019) The complete chloroplast genome sequence of *Dendrobium thyrsiflorum* (Orchidaceae) and its phylogenetic analysis. Mitochondrial DNA B Resour 4(2):4085-4086. doi.org/10.1080/23802359.2019.1691947

Zi XM, Sheng CL, Goodale UM, Shao SC, Gao JY (2014) In situ seed baiting to isolate germination-enhancing fungi for an epiphytic orchid, *Dendrobium aphyllum* (Orchidaceae). Mycorrhiza 24(7). doi.org/10.1007/s00572-014-0565-8