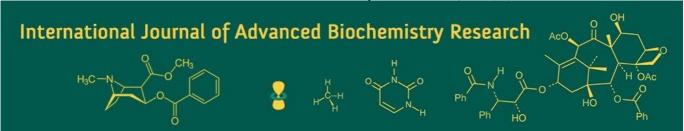
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Synergistic effects of biological agents on the biohardening of in vitro-raised Spathiphyllum plantlets

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Abstract

Spathiphyllum is a popular and widely cultivated indoor ornamental plant, valued across the world for its aesthetic appeal and adaptability to indoor environments. The efficiency of in vitro multiplication in Spathiphyllum is constrained by low survival rates and slow plantlet growth. Hence, the current study was under taken to study the influence of microbial inoculants on survival, growth, physiological traits and disease dynamics of micro-propagated Spathiphyllum plantlets during acclimatization. The present investigation was carried out at Regional Horticultural Research Station, Kovvur, East Godavari District of Andhra Pradesh during the year 2024-25. The experiment was conducted with different bioagent combinations viz., B₁ (Trichoderma reesei + AMF), B₂ (Trichoderma reesei + Azotobacter), B₃ (Trichoderma reesei + PSB), B₄ (Pseudomonas fluorescens + AMF), B₅ (Pseudomonas fluorescens + Azotobacter), B₆ (Pseudomonas fluorescens + PSB), B₇ (Trichoderma reesei + Pseudomonas fluorescens), and B₈ (control). Among the treatments, significant increase in plant survival (92%), maximum plant height (8.17 cm), number of leaves (12.3), leaf area (32.5 cm²), chlorophyll content (SPAD 45.8), root length (5.92 cm) was recorded in potting media enriched with Pseudomonas fluorescens and Phosphate solubilizing bacteria. Higher root system development and significant increase in plant survival was also recorded in AMF in combination with Pseudomonas fluorescens and Trichoderma reesei.

Keywords: Bioagents, Arbuscular mycorrhizal fungi (AMF), Phosphate solubilizing bacteria (PSB)

Introduction

The Peace Lily (Spathiphyllum), an evergreen ornamental of about 47 species in the family Araceae, is valued for its attractive spathes, glossy foliage, shade tolerance and minimal care needs (Ramírez-Malagón et al., 2001) [2]. It is also listed among NASA's top air-purifying plants, known for removing harmful indoor pollutants such as benzene, formaldehyde and xylene (Wolverton et al., 1989) [1], which has enhanced its demand in urban gardening and indoor greening programs. However, nursery production is constrained by its high susceptibility to fungal, bacterial and viral pathogens, especially root wilt in humid climates, which reduces its aesthetic quality and marketability (Zhang et al., 2020) [19]. In vitro culture offers an efficient method for large-scale propagation of genetically uniform, pathogen-free planting material, but acclimatization to ex vitro conditions remains difficult due to poor survival and weak physiological development. Bio-hardening with plant growth-promoting microorganisms such as arbuscular mycorrhizal fungi (AMF), Trichoderma spp., Pseudomonas fluorescens, phosphate solubilizing bacteria (PSB) and Azotobacter has proven effective in improving root establishment, nutrient uptake, stress tolerance and disease suppression. For instance, Trichoderma harzianum enhanced survival and root biomass of gerbera plantlets (Anand and Chandrasekaran, 2008) [5], while AMF inoculation significantly increased shoot growth and chlorophyll content in chrysanthemum (Srivastava et al., 2014) [10]. Considering these aspects, the present study aims to evaluate the influence of various combinations of bio-stimulants on the survival and growth of in vitro raised Spathiphyllum plantlets.

Materials and Methods

The experiment was conducted at Dr. Y.S.R.H.U-RHRS, Kovvur and geographically situated at 17.000 N latitude, 81.430 E longitude and altitude of 34 m above mean sea level. The location falls under Agroclimatic zone-10 with Southern plateau and hills region (Andhra Pradesh) with an average annual rainfall of 1017.67 mm.

Experimental details

Trichoderma reesei, Pseudomonas fluorescens, Arbuscular Mycorrhizal Fungi (AMF), Azotobacter and Phosphorus Solubilizing Bacteria (PSB) were obtained from Biocontrol laboratory, Amaravathi, in powder as well as liquid forms. IIHR Consortium was obtained from ICAR-IIHR, Hessaraghatta, Bangalore in powder form. Previously standardized potting media was prepared by mixing red earth, vermicompost, coco peat and neem cake in 4:3:2:1 ratio respectively. All the different bio-agents in combinations were added @ 20 grams or 20 ml per kilo of potting media and mixed thoroughly before filling up of pro trays. The experiment was laid out in a Completely Randomized Design (CRD) with eight treatments and three replications, each replication consisting of 25 plants, making a total of 75 plantlets per treatment. Observations were recorded 45 days after primary hardening from 20 randomly selected plantlets per replication, while 10 plantlets from each replication were sampled at the end of hardening for shoot and leaf parameters and the mean values were used for analysis. Data collected included survival and mortality percentages, plant height (cm), number of leaves per plant, fully open young leaf area (cm²), leaf chlorophyll content (SPAD value), number of roots per plant, microbial colony forming unit (CFU) and AMF colonization. All the growth parameters and survival of the biohardened plantlets were recorded 45 days after hardening. The SPAD values were recorded by using SPAD-502 Plus chlorophyll meter. Leaf area of fully expanded young leaves was measured using a Systronics Leaf Area Meter (Model 211). Microbial colony forming units (CFU) were enumerated by spread plate method on PDA after serial dilution, incubated at 27 °C and calculated using standard formula. Root colonization was assessed using trypan blue staining (Phillips & Hayman, 1970) and calculated as percentage colonized root length. All the standard cultural operations were followed during the period of primary hardening of Spathiphyllum.

Treatment details

| Treatments | Treatment details | | | |
|-----------------------|--|--|--|--|
| \mathbf{B}_1 | Trichoderma reesei + Arbuscular mycorrhizal fungi (AMF) | | | |
| \mathbf{B}_2 | Trichoderma reesei+ Azotobacter | | | |
| B ₃ | Trichoderma reesei + Phosphate solubilizing Bacteria (PSB) | | | |
| B4 | Pseudomonas fluorescens + Arbuscular mycorrhizal fungi (AMF) | | | |
| B 5 | Pseudomonas fluorescens + Azotobacter | | | |
| B ₆ | Pseudomonas fluorescens + Phosphate solubilizing Bacteria (PSB) | | | |
| B ₇ | IIHR Consortium | | | |
| \mathbf{B}_{8} | Control medium (Without any bio-stimulant) | | | |

Results and Discussion

As per the data envisaged in Table 1, highest plant survival (99%) was recorded in B₆ (*Pseudomonas fluorescens* +

PSB) which was on par with B4 (Pseudomonas fluorescens + AMF) (97%) and significantly superior with all other treatments. Minimum survival was recorded in control (B₈) (74%). Enhanced survival in B₆ and B₄ was attributed to improved root biomass, nutrient uptake and AMF colonization (up to 85%) leading to better acclimatization. The synergistic action of microbial consortia induced stresstolerant metabolism, improved water-use efficiency and suppressed soil-borne pathogens like Fusarium and Pythium (Umer & Ahammed, 2025; Duc et al., 2017; Yao et al., 2023) [21, 16, 19]. These results agree with earlier reports that Trichoderma, Pseudomonas and AMF enhance growth and survival in ornamentals such as Gerbera and Ornithogalum (Anand & Chandrasekaran, 2008; Begum *et al.*, 2019) [5, 13]. Conversely, poor survival in the control was due to weak roots and stress susceptibility (Karthikeyan et al., 2005) [4]. Overall, inoculation with P. fluorescens + PSB (B₆) and P. fluorescens + AMF (B4) significantly improved survival and reduced mortality demonstrating their crucial role in sustainable acclimatization of tissue-cultured ornamentals. The poor survival rates of tissue culture plantlets might be attributed to lack of proper functional root system, lack of epicuticular wax on leaf surface and non functioning of stomata In these circumstances, the sudden exposure of in vitro grown sensitive plants to harsh ex vitro conditions leads higher mortality. The maximum mortality of micropropagated plantlets also occurs during the acclimatization process as plantlets undergo several morphological, physiological, and biochemical changes (Prathyusha et al., 2024) [20]

In the present investigation, significantly highest ex vitro survival was recorded in potting media enriched with *Pseudomonas fluorescens* and PSB over control i.e devoid of biological agents. This may be due to the effect of bioagents which promotes root initiation through more Phosphorous and other micronutrient uptake, root cell elongation and initiation. *Pseudomonas fluorescens* considered a potent biocontrol agent, that can survive under stressed conditions, antagonistic against phyto-pathogenic microorganisms, induces a defence mechanism in plantlets, and helps in plant growth promotion by secreting several secondary metabolites in pomegranate plantlets and Prathyusha *et al.*, 2024 [20] in banana.

Growth characters

The growth of Spathiphyllum plantlets during primary hardening was significantly affected by microbial inoculation (Table 1). The highest plant height (8.17 cm) was recorded in B₆ (Pseudomonas fluorescens + PSB) which was statistically on par with B₄ (P. fluorescens + AMF) (7.45 cm), while the control (B₈) showed the lowest height (5.57 cm) (Fig. 1). Enhanced height in B₆ and B₄ resulted from early development of functional root system and there by improvement of nutrient uptake and microbial symbiosis that boosted nitrogen and phosphorus assimilation essential for cell elongation and shoot growth (Karthikeyan et al., 2005; Choudhary & Johri, 2009) [4, 6]. AMF colonization in B₄ (up to 85%) further improved phosphorus absorption, enhancing energy metabolism and shoot vigor (Begum et al., 2019) [13]. Microbial inoculation also increased chlorophyll content and photosynthetic efficiency, consistent with findings in Heliconia and Tuberose, where Trichoderma and Pseudomonas treatments enhanced plant height and biomass (Gowda & Yadav, 2014; Chatterjee &

Bandyopadhyay, 2010) [11, 9]. Poor performance in the control was due to limited nutrient uptake and weak root systems (Padmadevi, 2004; Srivastava *et al.*, 2010) [3, 10]. Overall, *P. fluorescens* + PSB (B₆) and P. fluorescens + AMF (B₄) significantly improved plant height through enhanced nutrient acquisition, chlorophyll synthesis and photosynthetic capacity, highlighting microbial biofertilizers as sustainable tools for vigorous growth and quality improvement in ornamental plantlets (Weng *et al.*, 2022; Yao *et al.*, 2023) [18, 19].

Similarly, the highest number of leaves (5.80), largest leaf area (1.46 cm²) and highest SPAD value (16.1) were recorded in B₆ (Pseudomonas fluorescens + PSB) which were on par with B₄ (P. fluorescens + AMF), while the control (B₈) showed the lowest values (4.03 leaves, 0.84 cm² and 12.8 SPAD). Enhanced leaf development in B₆ and B₄ was attributed to improved nitrogen and phosphorus uptake, better root growth and microbial production of IAA promoting leaf initiation and expansion (Choudhary & Johri, 2009; Jalali & Ghanbari, 2014) [6,8]. AMF colonization in B4 improved phosphorus use and water absorption, enhancing turgor and photosynthetic efficiency (Weng et al., 2022; Umer & Ahammed, 2025) [18, 21]. Similar effects were reported in China aster and Heliconia where microbial inoculation increased foliage growth and chlorophyll content (Anand & Chandrasekaran, 2008; Krishna et al., 2020; Gowda & Yadav, 2014) [5, 17]. The control showed poor performance due to limited nutrient uptake and weak root systems (Padmadevi, 2004) [3]. Overall, inoculation Pseudomonas fluorescens + PSB (B6) and Pseudomonas fluorescens + AMF (B4) effectively enhanced leaf production, area and chlorophyll content, demonstrating their importance in promoting vigorous vegetative growth and sustainable acclimatization of Spathiphyllum plantlets. The number of roots per Spathiphyllum plantlet during primary hardening was significantly influenced by microbial inoculation (Table 1). The highest number of roots (6.30) was recorded in B₁ (Trichoderma reesei + Arbuscular Mycorrhizal Fungi), which was on par with all bio-hardened treatments except the control (B₈) that recorded the lowest (4.66). The enhanced root proliferation in bioagent-treated plantlets was due to auxin production by Pseudomonas fluorescens, stimulating root initiation and lateral branching, while phosphate-solubilizing bacteria improved phosphorus availability supporting root meristem activity (Choudhary & Johri, 2009; Jalali & Ghanbari, 2014) [6, 8]. AMF colonization in B₁ and B₄ further enhanced root formation by improving nutrient and water absorption, leading to increased root branching and biomass (Weng *et al.*, 2022; Muthukumar, 2009) [18, 7]. Fewer roots in control plantlets were resulted from poor nutrient uptake and weak root systems, limiting physiological development during acclimatization (Padmadevi, 2004) [3].

Microbial Colony Forming Units (CFU)

The microbial population (CFU $\times 10^{5}$) associated with Spathiphyllum plantlets during primary hardening was also calculated to verify the presence and increase of microbial load in the potting media during the primary hardening stage (Table 2). The highest CFU (10.5×10^{5}) was recorded in B₆ (*Pseudomonas fluorescens* + Phosphate Solubilizing Bacteria), which was on par with B₂ (9.5×10^{5}), B₃ (9.3×10^{5}) and B₅ (9.4×10^{5}), while the lowest (0.51×10^{5}) was observed in the control (B₈). The superior microbial

population under B₆ was due to the synergistic action of *Pseudomonas fluorescens* and PSB, which efficiently multiplied in the rhizosphere using root exudates, enhance enzymatic activities and improve nutrient cycling through phosphorus solubilization and nitrogen mineralization (Muthukumar, 2009; Karthikeyan *et al.*, 2005; Choudhary & Johri, 2009) [7, 4, 6]. Similarly, B₂ (*Trichoderma reesei* + Azotobacter) also showed a high CFU count due to Trichoderma-induced root exudation and nitrogen fixation by Azotobacter, creating a nutrient-rich rhizospheric environment (Jalali & Ghanbari, 2014; Weng *et al.*, 2022) ^[8, 18]. These findings confirm that B₆ was the most effective treatment in enhancing rhizospheric microbial populations, while the control recorded the least due to the absence of bioagents.

Arbuscular mycorrhizal fungi (AMF) colonization (%)

AMF was inoculated in B1 and B4 treatments along with the potting media. Hence, the highest root colonization (85.50%) was recorded in B₄ (Pseudomonas fluorescens + AMF) which was statistically on par with B₁ (*Trichoderma* reesei + AMF, 82.20%) (Table 2 & Fig.2)., Very insignificant AMF root colonization was observed in other biohardening treatments and no colonization was recorded in control. Enhanced colonization in B4 resulted from the synergistic effect of *Pseudomonas fluorescens* promoting root growth, exudation and secretion of bioactive compounds that stimulate AMF spore germination and hyphal penetration (Choudhary & Johri, 2009) [6]. Similarly, Trichoderma in B₁ improved root receptivity for AMF colonization. AMF established arbuscules and vesicles within root cortical cells, facilitating phosphorus uptake, water relations and chlorophyll content, thereby enhancing plant vigor (Kurimella et al., 2014) [12].

Table 1: Effect of different combinations of biological agents on survival and growth of *in vitro* raised plantlets of Spathiphyllum after primary hardening

| Treatment | Survival rate (%) | Plant height (cm) | Number of leaves | Leaf area (cm²) | SPAD Value | Number of roots |
|----------------|-------------------|-------------------------|---------------------|-----------------------|---------------|-----------------|
| \mathbf{B}_1 | 95.0 | 6.63 | 5.13 | 1.12 | 15.3 | 6.30 |
| \mathbf{B}_2 | 91.0 | 6.36 | 5.07 | 1.08 | 14.5 | 6.07 |
| \mathbf{B}_3 | 96.0 | 7.09 | 5.20 | 1.21 | 15.0 | 6.23 |
| B4 | 97.0 | 7.45 | 5.43 | 1.35 | 16.0 | 6.25 |
| B5 | 93.0 | 6.66 | 4.67 | 1.10 | 14.9 | 6.08 |
| B ₆ | 99.0 | 8.17 | 5.80 | 1.46 | 16.2 | 6.29 |
| B 7 | 92.0 | 6.88 | 5.07 | 1.11 | 15.1 | 5.97 |
| B ₈ | 74.0 | 5.57 | 4.03 | 0.84 | 12.8 | 4.66 |
| SE(m) ± | 1.76 | 0.14 | 0.20 | 0.08 | 0.41 | 0.15 |
| CD at 5% | 5.29 | 0.44 | 0.62 | 0.24 | 1.23 | 0.47 |

 B_1 : $Trichoderma\ reesei\ +\ Arbuscular\ Mycorrhizal\ Fungi\ (AMF)$

B₂: *Trichoderma reesei*+ Azotobacter

B₃: *Trichoderma reesei* + Phosphate Solubilizing Bacteria (PSB)

B₄: Pseudomonas fluorescens + Arbuscular Mycorrhizal Fungi (AMF)

 $B_5: \textit{Pseudomonas fluorescens} + Azotobacter$

B₆: *Pseudomonas fluorescens* + Phosphate Solubilizing Bacteria (PSB)

B₇: IIHR Consortium

B₈: Control medium (Without any bio-stimulant)

Table 2: Estimation of Microbial Colony Forming Units (CFU) and AMF root colonization in potting media of *in vitro* raised plantlets of Spathiphyllum during primary hardening stage

| Treatment | CFU (x 10 ⁵) | AMF Colonization (%) |
|-----------------------|--------------------------|----------------------|
| \mathbf{B}_1 | 5.2 | 82.20 |
| \mathbf{B}_2 | 9.3 | 4.79 |
| \mathbf{B}_3 | 9.5 | 5.63 |
| B_4 | 5.6 | 85.50 |
| B 5 | 9.4 | 5.98 |
| B ₆ | 10.5 | 5.22 |
| B ₇ | 9.1 | 4.69 |
| \mathbf{B}_8 | 0.51 | 0.00 |
| SE(m) ± | 0.42 | 0.60 |
| CD at 5% | 1.26 | 0.81 |



Fig 1: Effect of biological agents on plant growth of *in vitro* raised plantlets of Spathiphyllum during the primary hardening stage a)

Potting media inoculated with *Pseudomonas fluorescens* +

Phosphate Solubilizing Bacteria (B₆) and b) Control (Without any biological agents) (B₈)



Fig 2: Arbuscular mycorrhizal fungi (AMF) root colonization in micro-propagated Spathiphyllum plant lets during primary hardening stage a) *Pseudomonas fluorescens* + Arbuscular mycorrhizal fungi (B₄), b) *Trichoderma reesei* + Arbuscular mycorrhizal fungi (B₁) and c) Control (B₈)

Conclusion

Through the results of this experimentation, it was revealed that microbial inoculation significantly improved plant survival, growth and physiological performance of in vitro raised Spathiphyllum plantlets during primary hardening. Pseudomonas fluorescens + PSB (B6) showed the highest survival (99%), plant height (8.17 cm), leaf number (5.80), leaf area (1.46 cm²) and SPAD value (16.1), along with enhanced rhizospheric microbial populations (10.5 ×10⁵ CFU). Pseudomonas fluorescens + AMF (B₄) and Trichoderma reesei + AMF (B1) also promoted root development and AMF colonization (up to 85%), improving nutrient uptake, photosynthesis and stress resilience. Overall, B6 and B4 were most effective, demonstrating the of microbial biofertilizers for sustainable acclimatization and vigorous growth of tissue-cultured Spathiphyllum plantlets.

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