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Effects of polyamine type and concentration on growth and physiological traits of in vitro *Dendrobium parishii* plantlets

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Key Message

Clump growth and PSII efficiency identified Spm 0.6, Spd 0.8 and Spm 0.3 mg L^{-1} as candidate media for improving in vitro *Dendrobium parishii* plantlet quality.

Abstract

Dendrobium usually require more diversified nutritional support during long-term tissue culture; polyamines (putrescine, spermidine, and spermine), as a class of small-molecule aliphatic amines extensively involved in plant growth and development, morphogenesis, and stress responses, have demonstrated promising application prospects in tissue culture. We compared putrescine (Put), spermidine (Spd) and spermine (Spm), each at 0.3–0.8 mg L^{-1}, with paclobutrazol and an unsupplemented control (CK) in *Dendrobium parishii* tissue culture. Clump-level leaf number differed among treatments in a vessel-cluster-preserving permutation test (Holm-adjusted $P = 0.024$). Spd 0.8 had the greatest mean shoot number, whereas Put 0.8 had the maximum mean fresh weight and leaf number. The highest descriptive Fv/Fm means at 30 d occurred in Put 0.4 (0.768), Spd 0.4 (0.768) and Put 0.6 (0.766); at 120 d, they occurred in Spm 0.3 (0.755), Spd 0.8 (0.749) and Spm 0.6 (0.737). An exploratory index integrating six morphological traits with endpoint Fv/Fm ranked Spm 0.6, Spd 0.8 and Spm 0.3 closely ($I = 0.696$, 0.681 and 0.678). Biochemical responses were not uniformly aligned with this ranking: Spm 0.6 combined the lowest endpoint MDA with the highest H_2O_2, while CK had the highest CAT activity. These complementary developmental and physiological profiles indicate that, according to the culture objective, either a specific type of polyamine may be selected or combinations of different polyamines may be considered.

Abbreviations

CAT, catalase; CK, unsupplemented culture control; Fv/Fm, maximum quantum efficiency of photosystem II photochemistry; FW, fresh weight; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; MS, Murashige and Skoog; POD, peroxidase; PP, paclobutrazol; PSII, photosystem II; Put, putrescine; ROS, reactive oxygen species; SD, standard deviation; Spd, spermidine; Spm, spermine

Introduction

Dendrobium is one of the largest orchid genera and includes many species valued as ornamentals, medicinal plants. *Dendrobium parishii* H.Low is an epiphytic orchid noted for its fragrant flowers and can be propagated through asymbiotic seed culture and nursery establishment (Vu and Nguyen 2023). Reliable production of vigorous plantlets is important both to germplasm conservation and to the development of cultivated sources that reduce dependence on wild material. In *Dendrobium*, regeneration and subsequent plantlet development depend on genotype, explant, basal medium, growth regulators and culture conditions (Teixeira da Silva et al. 2015). Conditions that support early protocorm development may not sustain shoot proliferation, biomass accumulation, rooting and leaf function during prolonged culture of *Dendrobium*. Preliminary observations suggested that polyamine supplementation could improve shoot and root development in our *D. parishii* cultures, motivating a comparison across these components of plantlet quality.

Putrescine, spermidine and spermine participate in plant development and stress responses through regulated metabolism and interactions with cellular macromolecules (Chen et al. 2019; Blázquez 2024). Their tissue-culture applications include embryogenesis, shoot development and rooting, with responses depending on compound, concentration, genotype and developmental stage (Rakesh et al. 2021; Redha and Suleman 2011). In *Dendrobium 'Sonia'*, putrescine produced a non-linear response in protocorm-like-body production, while spermidine and spermine had distinct response profiles (Saiprasad et al. 2004). In *D. huoshanense*, exogenous polyamines affected protocorm-like-body conversion and the cytokinin-to-auxin balance (Wang et al. 2009). These findings motivate direct comparison of polyamine identity and concentration in *D. parishii* rather than extrapolation from other species or developmental stages.

Physiological measurements can help distinguish sustained vigor from a transient or stress-associated growth response. A single trait may not adequately describe plantlet quality. Clumps with more new shoots can have smaller average stems despite greater propagation output. Antioxidant enzyme activities can reflect both oxidative demand and protective capacity, so higher activity does not necessarily indicate better plant performance (Xu et al. 2025). Dark-adapted Fv/Fm provides a complementary measure of maximum photosystem II (PSII) photochemical efficiency (Murchie and Lawson 2013). In *D. nobile*, morphology, antioxidant metabolism and fluorescence captured different responses to culture conditions (Guo et al. 2023). We therefore evaluated clump proliferation, biomass, leaf and root traits as developmental outcomes, used Fv/Fm as a functional descriptor in an exploratory integrated ranking, and interpreted biochemical measurements as physiological context.Materials and methods

Plant material and culture conditions

Cultivated *Dendrobium parishii* plants were obtained from Chishui, Guizhou, China, identified by Prof. Depo Yang, and maintained in the greenhouse of Guangzhou Caizhilin Pharmaceutical Co., Ltd. Capsules were produced by self-pollination. Culture conditions were a 12 h photoperiod, approximately 1,200 lx, 28 °C and 60% relative humidity. No voucher specimen was deposited for this study.

Capsule surfaces were wiped with 75% ethanol, disinfected with 0.1% mercuric chloride for 4 min 30 s and rinsed three times with sterile water. Seeds were aseptically excised and inoculated onto hormone-free half-strength Murashige and Skoog (MS) medium containing 30 g L^{-1} sucrose and 6.8 g L^{-1} agar at pH 6.0.

Polyamine treatments

Plantlet clumps of comparable initial size were transferred to half-strength MS-based media containing Put, Spd or Spm at 0.3, 0.4, 0.6 or 0.8 mg L^{-1}, paclobutrazol (PP333; PP) at 0.5 mg L^{-1}, or neither supplement (CK; Table 1). Initial shoots per clump and clumps inoculated per vessel were comparable across treatments. Fifteen independent culture vessels were established for each of the 14 treatments in one experimental batch. Contaminated vessels were excluded during culture. At 30 d, five uncontaminated vessels per treatment were selected at random for photography and physiological sampling. At 120 d, all remaining uncontaminated vessels represented in the endpoint record were measured; records were not screened by phenotype.

Measurement of dark-adapted PSII photochemical efficiency

Dark-adapted chlorophyll fluorescence was measured with a PAM-2500 fluorometer (Heinz Walz GmbH, Germany) after 30 min of dark adaptation, using non-apical leaves and avoiding major veins. Fv/Fm was used to describe maximum PSII photochemical efficiency (Murchie and Lawson 2013). One leaf was measured from each of six distinct clumps per treatment at 30 d and eight distinct clumps per treatment at 120 d. Each retained reading therefore represented one sampled clump. Means and sample SDs were calculated across these clump-representative readings. The two sampling times were not treated as paired observations; no leaf-level reading was counted as an additional replicate within a clump.

Determination of antioxidant enzyme activities and oxidative indicators

Leaves were assayed for peroxidase (POD) and catalase (CAT) activities and malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) concentrations using Suzhou Comin Biotechnology kits POD-1-Y, CAT-1-W, MDA-1-Y and H_2O_2-1-Y, respectively. Three independent vessel-derived biological samples were analysed per treatment at each time. Each sample contained 0.1 g fresh tissue extracted with 1 mL extraction solution. Measurements used 96-well plates without additional dilution and were calculated according to the kit instructions. CAT reactions lasted 10 min, and POD readings were separated by 1 min. Technical readings were averaged within each biological sample. All assays used three technical readings per sample.

Morphology and hierarchical data processing

Morphology records were organized as treatment–vessel–clump–individual measurements. At 120 d, 111 clumps from 55 vessels across 14 treatments were included. Individual stem measurements were averaged within clumps. Shoot number was the number of recorded plantlets in a clump. Repeated leaf counts and leaf dimensions were averaged within clumps; fresh mass and root number were clump-level records. Mean root length was calculated from roots recorded within each clump. Treatment descriptions are unweighted means and sample SDs across observed clumps, with sample counts reported by treatment.

A maximum-leaf area proxy was calculated for each leaf record as $0.75 \times L \times B / 100$, where L and B were length and width in mm. Repeated leaf-area records were averaged within a clump. This variable is an average proxy for repeatedly measured maximum leaves rather than total clump leaf area. The factor 0.75 followed the recording convention and was not validated as a species-specific shape coefficient. Stem height, stem diameter and longest-root length were reported descriptively but were not included in the composite index.

Exploratory composite index

The exploratory 120 d index combined six morphological treatment means with a clump-representative Fv/Fm treatment mean: Fv/Fm (F), shoot number (N), fresh mass (W), leaf number (B), maximum-leaf area proxy (A), root number (C) and mean root length (D). Morphology and fluorescence were summarized separately and combined only at treatment level; individual records were not matched across datasets. Each indicator was standardized across the 14 groups' mean using their unweighted mean μ_j and sample SD s_j (denominator 13), not within treatments (OECD et al. 2008):

$$Z_{ij} = (\bar{x}_{ij} - \mu_j) / s_j$$

The growth, leaf and root domain scores were:

$$G_i = (Z_iN + Z_iW) / 2; \quad L_i = (Z_iB + Z_iA) / 2; \quad R_i = (Z_iC + Z_iD) / 2$$

The composite score and its display scale were:

$$I_i = 0.5\, Z_iF + (G_i + L_i + R_i) / 6$$

$$S_i = 100 \times (I_i - min\, I) / (max\, I - min\, I)$$

Fv/Fm therefore had nominal weight 1/2 and each morphological variable weight 1/12. The index was calculated once per treatment. All indicator directions were defined as positive for this exploratory screen. Rankings used unrounded I values; S is a relative display scale rather than a percentage of efficacy. POD, CAT, MDA and H_2O_2 were analyzed separately as physiological indicators. Antioxidant enzyme activity alone is not a reliable proxy for stress tolerance (Xu et al. 2025). Stem dimensions were excluded because new shoots can reduce average stem size without reducing clump propagation output.

Statistical analysis

Treatment-adjusted intraclass correlations (ICCs) described within-vessel clustering of clump traits and the 120 d clump-representative Fv/Fm readings. For each 120 d morphological endpoint, a one-way F statistic was calculated from clump values. Treatment labels were permuted among whole vessels 49,999 times while preserving the observed numbers of vessels per treatment. Monte Carlo P values included the observed arrangement and were Holm-adjusted across nine morphology endpoints (Holm 1979; Phipson and Smyth 2010). Fluorescence was summarized descriptively: distinct clumps within a vessel were not assumed to be independent treatment replicates (Lazic et al. 2018). For each biochemical endpoint and time, rank-based Kruskal–Wallis H was evaluated by 49,999 label permutations among independent vessel-derived biological samples and Holm-adjusted across eight assays. Concentration rankings and cross-time comparisons were descriptive; no pairwise or paired-time significance was inferred from omnibus tests.

AI-assisted preparation

OpenAI tools assisted with data-processing and language editing. The authors are responsible for verifying the analyses, interpretation, references and final manuscript.

Table 1 Culture-medium treatments in the 14-group screen

Treatment	PP mg L^{-1}	Put mg L^{-1}	Spd mg L^{-1}	Spm mg L^{-1}
CK	0	0	0	0
PP 0.5	0.5	0	0	0
Put 0.3	0	0.3	0	0
Put 0.4	0	0.4	0	0
Put 0.6	0	0.6	0	0
Put 0.8	0	0.8	0	0
Spd 0.3	0	0	0.3	0
Spd 0.4	0	0	0.4	0
Spd 0.6	0	0	0.6	0
Spd 0.8	0	0	0.8	0
Spm 0.3	0	0	0	0.3
Spm 0.4	0	0	0	0.4
Spm 0.6	0	0	0	0.6
Spm 0.8	0	0	0	0.8

All treatments used half-strength MS-based medium. PP denotes paclobutrazol, and CK was unsupplemented. Fifteen independent vessels were initially established per treatment. Endpoint morphology and fluorescence sample counts are reported in Table S1.

Results

Seed-derived cultures produced green protocorms

The photographic record showed visible development after 15 d and green protocorm material after 45 d on hormone-free half-strength MS medium (Fig. 1). These images document the induction sequence qualitatively without supporting a germination percentage or a treatment comparison.

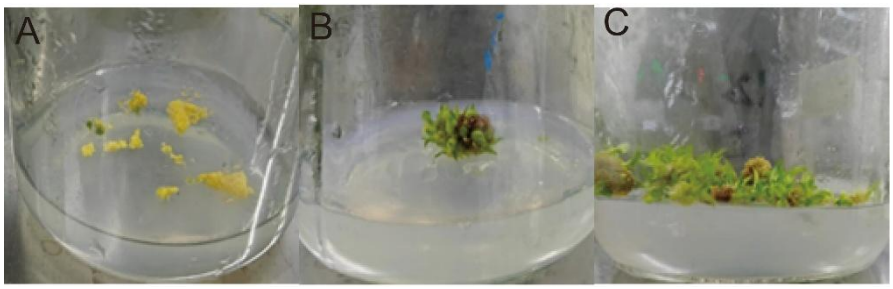


Fig. 1 Photographic sequence of *D. parishii* protocorm induction. (A) Freshly inoculated seeds; (B) 15 d after inoculation; (C) 45 d after inoculation. Different fields and magnifications should not be compared as quantitative growth measurements.

Polyamine identity and concentration were associated with distinct clump growth profiles

Growth responses to polyamines were trait and concentration dependent at the clump level (Table 2). Spd 0.8 had the greatest mean shoot number (4.50 ± 2.74 shoots clump^{-1}), while Put 0.8 had the greatest mean leaf number (12.38 ± 4.17) and fresh mass (6.87 ± 3.50 g clump^{-1}). Put 0.4 had the greatest maximum-leaf area proxy (4.57 ± 1.64 cm²). Spm 0.6 had the highest mean root number (28.88 ± 5.36), whereas CK had the greatest mean root length (25.27 ± 5.40 mm) but variable root number (28.00 ± 17.09). In the vessel-cluster-preserving omnibus tests, leaf number differed among treatments after Holm correction (adjusted $P = 0.024$); stem diameter approached but did not reach the threshold (adjusted $P = 0.052$). No other morphology endpoint had an adjusted $P < 0.05$, and the omnibus result does not identify a particular treatment–CK contrast. The complete clump descriptions are in Table S2.

Table 2 Clump-level morphology at 120 d

Treatment	Shoots clump^{-1}	Fresh mass g clump^{-1}	Leaves clump^{-1}	Leaf area cm²	Roots clump^{-1}	Mean root length mm
CK	3.25 ± 1.49	4.65 ± 2.64	1.86 ± 0.98	1.06 ± 0.59	28.00 ± 17.09	25.27 ± 5.40
PP 0.5	3.62 ± 1.41	6.20 ± 2.74	9.12 ± 3.91	3.36 ± 1.21	25.12 ± 4.85	22.27 ± 10.19
Put 0.3	3.89 ± 1.05	4.13 ± 1.51	9.33 ± 4.47	3.49 ± 2.10	18.44 ± 7.95	15.76 ± 2.29
Put 0.4	2.88 ± 1.13	4.20 ± 1.62	9.38 ± 3.11	4.57 ± 1.64	21.25 ± 3.81	17.59 ± 5.97
Put 0.6	3.50 ± 0.93	3.20 ± 2.37	9.12 ± 1.64	2.57 ± 1.74	18.88 ± 5.25	17.97 ± 5.02
Put 0.8	3.75 ± 1.28	6.87 ± 3.50	12.38 ± 4.17	3.87 ± 1.98	27.62 ± 8.60	17.75 ± 8.61
Spd 0.3	2.88 ± 1.13	4.65 ± 1.72	7.00 ± 2.83	3.94 ± 1.27	23.25 ± 7.21	18.59 ± 6.34
Spd 0.4	3.50 ± 1.51	3.33 ± 0.74	7.75 ± 3.20	3.49 ± 0.84	20.00 ± 4.04	15.80 ± 4.85
Spd 0.6	3.25 ± 1.04	4.40 ± 2.12	8.75 ± 3.99	3.87 ± 2.27	23.38 ± 6.91	18.84 ± 6.75

Treatment	Shoots clump^{-1}	Fresh mass g clump^{-1}	Leaves clump^{-1}	Leaf area cm^2	Roots clump^{-1}	Mean root length mm
Spd 0.8	4.50 ± 2.74	4.12 ± 1.82	9.33 ± 9.24	2.99 ± 1.19	25.33 ± 5.65	20.94 ± 12.59
Spm 0.3	3.75 ± 1.16	6.32 ± 4.11	10.50 ± 5.10	3.16 ± 0.99	23.50 ± 8.94	18.86 ± 6.21
Spm 0.4	3.62 ± 1.19	5.20 ± 3.13	11.38 ± 6.12	3.65 ± 1.25	25.50 ± 9.86	17.18 ± 8.39
Spm 0.6	3.62 ± 0.92	6.55 ± 3.04	11.38 ± 2.83	3.73 ± 1.43	28.88 ± 5.36	17.80 ± 8.10
Spm 0.8	3.50 ± 1.20	2.40 ± 0.66	10.88 ± 3.72	2.09 ± 0.78	19.50 ± 5.29	10.66 ± 2.41

Values are mean ± sample SD across observed clumps (n = 6–9 clumps per treatment). Leaf area is the corrected maximum-leaf proxy in cm²; mean root length is expressed in mm. Clumps within the same vessel were kept together during permutation testing. Stem height, stem diameter and longest-root length are reported in Table S2. No treatment-specific significance letters are assigned from the omnibus test.

Stage- and Treatment-Dependent Variation in Fv/Fm

At 30 d, CK had an Fv/Fm mean of 0.572 ± 0.047, below the descriptive means of all supplemented treatments (Fig. 2a). Put 0.4 (0.768 ± 0.013), Spd 0.4 (0.768 ± 0.020) and Put 0.6 (0.766 ± 0.008) had the highest unrounded means. Within the Put series, means ranked 0.4 > 0.6 > 0.8 > 0.3 mg L^{-1}; within the Spd series, 0.4 > 0.6 > 0.8 > 0.3; and within the Spm series, 0.8 > 0.4 > 0.6 > 0.3. At 120 d, Spm 0.3 (0.755 ± 0.022), Spd 0.8 (0.749 ± 0.017) and Spm 0.6 (0.737 ± 0.037) had the highest descriptive means, while CK (0.560 ± 0.090) and Spm 0.8 (0.605 ± 0.199) had the lowest (Fig. 2b). The highest within-family means shifted to Put 0.3, Spd 0.8 and Spm 0.3. The treatment-adjusted within-vessel ICC at 120 d was 0.455.

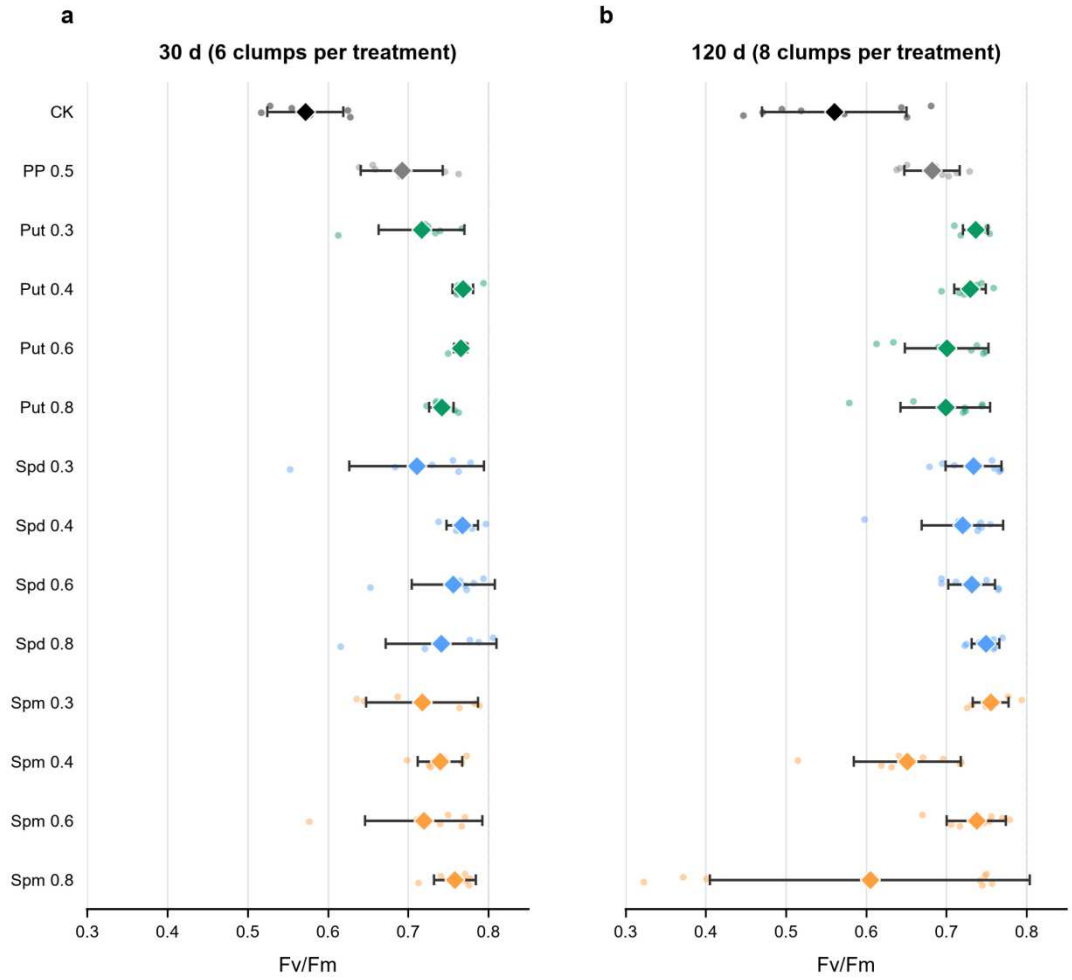


Fig. 2 Dark-adapted maximum quantum efficiency of photosystem II (Fv/Fm) at 30 d (a) and 120 d (b). Filled circles represent one leaf from each of six distinct clumps per treatment at 30 d and eight distinct clumps at 120 d. Diamonds and horizontal bars show the mean ± sample SD across the sampled clumps. Offsets along the treatment axis separate points without changing Fv/Fm values. The dashed line at Fv/Fm = 0.8 is a visual reference, not a significance threshold. Treatment concentrations are in mg L^{-1}.

Exploratory composite scoring identified suitable polyamine types and concentrations for tissue culture of *D. parishii*

Using treatment means for six clump-level developmental traits and 120 d Fv/Fm, the exploratory index ranked Spm 0.6 first (I = 0.696), Spd 0.8 second (I = 0.681), and Spm 0.3 third (I = 0.678; Fig. 3; Table 3). Their score range was only 0.018, so the numerical order should not be interpreted as three distinct optima. Within each polyamine family, the scores ranked Put 0.8 > Put 0.3 > Put 0.4 > Put 0.6, Spd 0.8 > Spd 0.6 > Spd 0.3 > Spd 0.4, and Spm 0.6 > Spm 0.3 > Spm 0.4 > Spm 0.8. Spm 0.6 combined positive growth, leaf, and root domains; Spd 0.8 combined the highest mean shoot number with high Fv/Fm; and Spm 0.3 had the highest endpoint Fv/Fm. Put 0.8 ranked fourth (I = 0.419), reflecting its high leaf number and fresh mass. CK ranked last because its high root-domain score was offset by low leaf and Fv/Fm scores. The index is an exploratory summary of this experiment and should be interpreted together with the original traits.

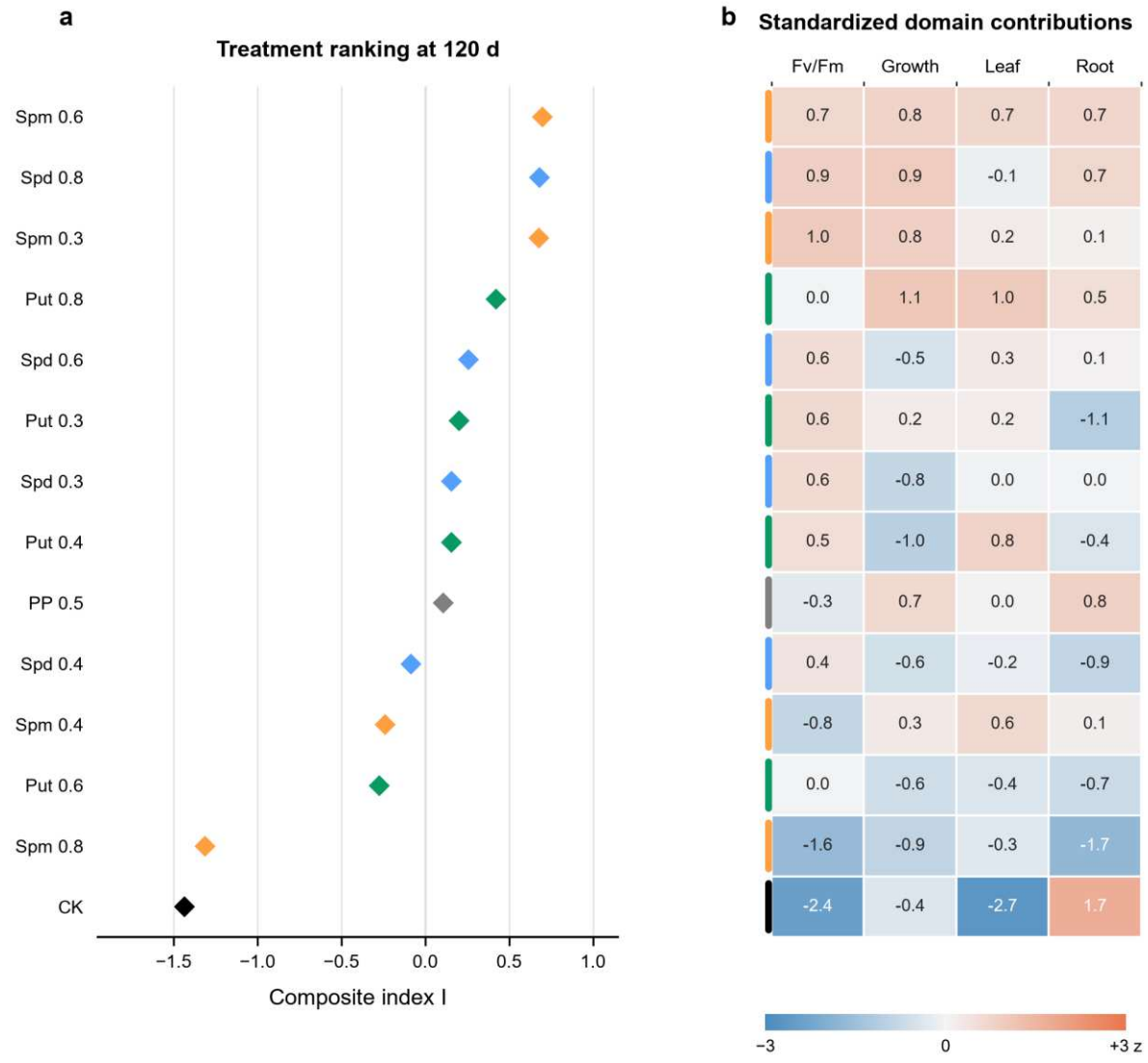


Fig. 3 Exploratory integrated ranking of the 14 treatments at 120 d. (a) Each diamond represents one treatment-level composite index (I), ordered by its unrounded value. Error bars and replicate points are omitted because the index combines treatment-level summaries. (b) The heat map shows standardized Fv/Fm and the growth (G), leaf (L) and root (R) domains. G averages standardized shoot number and fresh mass; L averages leaf number and maximum-leaf area proxy; R averages root number and mean root length. I = 0.5 × Z(Fv/Fm) + (G + L + R)/6.

Table 3 Exploratory 120 d composite scores and domain contributions

Rank	Treatment	n	Z Fv/Fm	Growth G	Leaf L	Root R	Index I	Display 0–100
1	Spm 0.6	8	0.663	0.781	0.691	0.713	0.696	100.0
2	Spd 0.8	6	0.865	0.943	-0.126	0.672	0.681	99.3
3	Spm 0.3	8	0.974	0.850	0.197	0.098	0.678	99.2
4	Put 0.8	8	-0.009	1.051	0.969	0.525	0.419	87.0

Rank	Treatment	n	Z Fv/Fm	Growth G	Leaf L	Root R	Index I	Display 0–100
5	Spd 0.6	8	0.561	-0.470	0.260	0.076	0.258	79.5
6	Put 0.3	9	0.648	0.204	0.157	-1.094	0.202	76.8
7	Spd 0.3	8	0.600	-0.834	-0.046	0.020	0.157	74.7
8	Put 0.4	8	0.522	-0.998	0.775	-0.417	0.154	74.6
9	PP 0.5	8	-0.297	0.653	0.040	0.841	0.107	72.4
10	Spd 0.4	8	0.356	-0.563	-0.150	-0.865	-0.085	63.4
11	Spm 0.4	8	-0.837	0.280	0.645	0.135	-0.242	56.0
12	Put 0.6	8	0.023	-0.611	-0.403	-0.703	-0.274	54.5
13	Spm 0.8	8	-1.646	-0.909	-0.333	-1.705	-1.314	5.7
14	CK	8	-2.423	-0.377	-2.676	1.704	-1.436	0.0

Z scores were calculated across the 14 treatment means. Morphology inputs are means across observed clumps; Fv/Fm inputs are descriptive means across eight sampled clumps, one leaf per clump. I = 0.5 Z(Fv/Fm) + (G + L + R)/6. Display scores rescale the minimum and maximum treatment-level I to 0 and 100 and are not percentages of efficacy. n is the morphology clump count.

Biochemical responses varied with polyamine identity, concentration and sampling time

At 30 d, CK POD activity was 928.89 ± 214.30 U g^{-1} FW (Fig. 4). Most supplemented treatments had higher descriptive means; Spd 0.6 had the highest (3,253.33 U g^{-1} FW), whereas Spm 0.6 was lower than CK (840.00 U g^{-1} FW). Within-family means ranked Put 0.6 > 0.3 > 0.4 > 0.8, Spd 0.6 > 0.8 > 0.4 > 0.3, and Spm 0.4 > 0.8 > 0.3 > 0.6. At 120 d, CK had the highest mean (1,391.11 U g^{-1} FW). The within-family orders were Put 0.6 > 0.8 > 0.3 > 0.4, Spd 0.8 > 0.6 > 0.4 > 0.3, and Spm 0.6 > 0.8 > 0.4 > 0.3. All supplemented treatments except Spm 0.6 had lower means at 120 d than at 30 d.

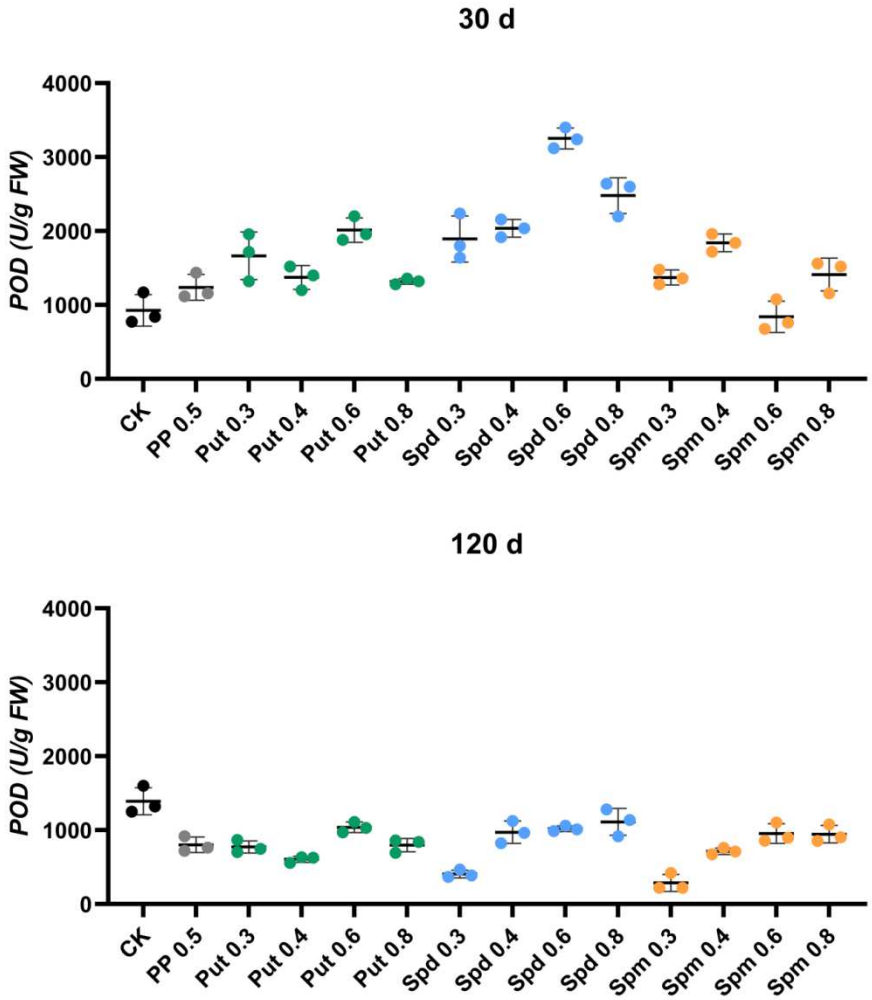


Fig. 4 Peroxidase activity at 30 d (a) and 120 d (b). Points represent three independent biological samples per treatment, with mean ± SD after averaging technical triplicates. One unit is defined by $\Delta A_{470} = 0.005$ min^{-1} under kit conditions. Omnibus tests are reported in Table S8; no pairwise or cross-time significance is assigned. Treatment concentrations are in mg L^{-1}.

At 30 d, CK CAT activity was 99.71 ± 11.21 U g^{-1} FW (Fig. 5). Supplemented-treatment means ranged from 8.43 in Spm 0.6 to 127.18 U g^{-1} FW in Spm 0.3. Within-family means ranked Put 0.4 > 0.3 > 0.6 > 0.8, Spd 0.3 > 0.6 > 0.8 > 0.4, and Spm 0.3 > 0.8 > 0.4 > 0.6. At 120 d, CK had the highest mean (101.42 ± 4.66 U g^{-1} FW), while Spd 0.3 had the lowest (0.91 U g^{-1} FW). Concentration orders were Put 0.6 > 0.4 > 0.8 > 0.3, Spd 0.4 > 0.8 > 0.6 > 0.3, and Spm 0.8 > 0.6 > 0.4 > 0.3. Spm 0.6 and Spd 0.8 had endpoint CAT means near 8.4 U g^{-1} FW despite their leading composite scores, showing that the numerical CAT ranking differed from the integrated growth ranking.

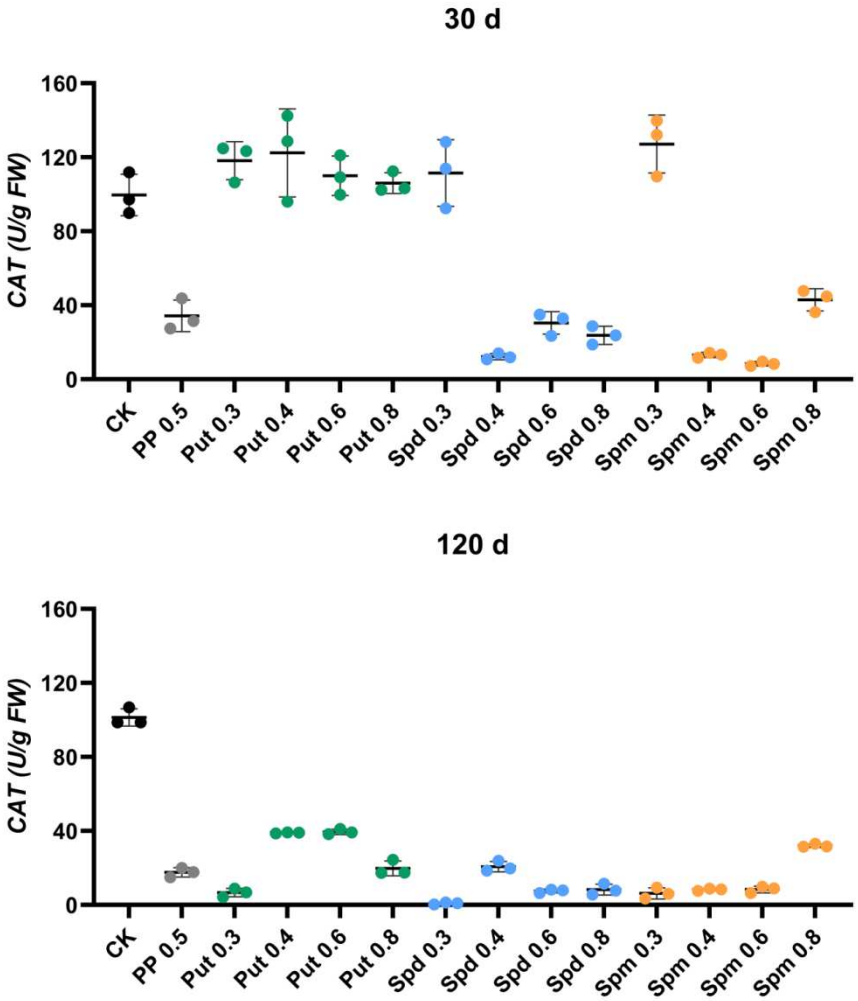


Fig. 5 Catalase activity at 30 d (a) and 120 d (b). Points represent three biological samples per treatment, with mean ± SD after averaging technical triplicates. One unit corresponds to 1 μmol H_2O_2 decomposed min^{-1} under kit conditions. Omnibus tests cover the 14 treatments (Table S8). No pairwise or cross-time significance is assigned. Treatment concentrations are in mg L^{-1}.

At 30 d, CK MDA was 18.40 ± 16.87 nmol g^{-1} FW (Fig. 6). Spd 0.6 had the lowest treatment mean (8.43 nmol g^{-1} FW), and Spm 0.8 had the highest (88.58 nmol g^{-1} FW). Within-family means ranked Put 0.8 > 0.3 > 0.6 > 0.4, Spd 0.4 > 0.3 > 0.8 > 0.6, and Spm 0.8 > 0.3 > 0.6 > 0.4. At 120 d, Spm 0.6 had the lowest mean (12.03 nmol g^{-1} FW), Spm 0.4 the highest (44.49 nmol g^{-1} FW), and CK was 20.98 ± 4.17 nmol g^{-1} FW. The within-family orders were Put 0.6 > 0.4 > 0.8 > 0.3, Spd 0.4 > 0.3 > 0.6 > 0.8, and Spm 0.4 > 0.3 > 0.8 > 0.6. The contrasting concentration orders at the two times do not support a uniform concentration response.

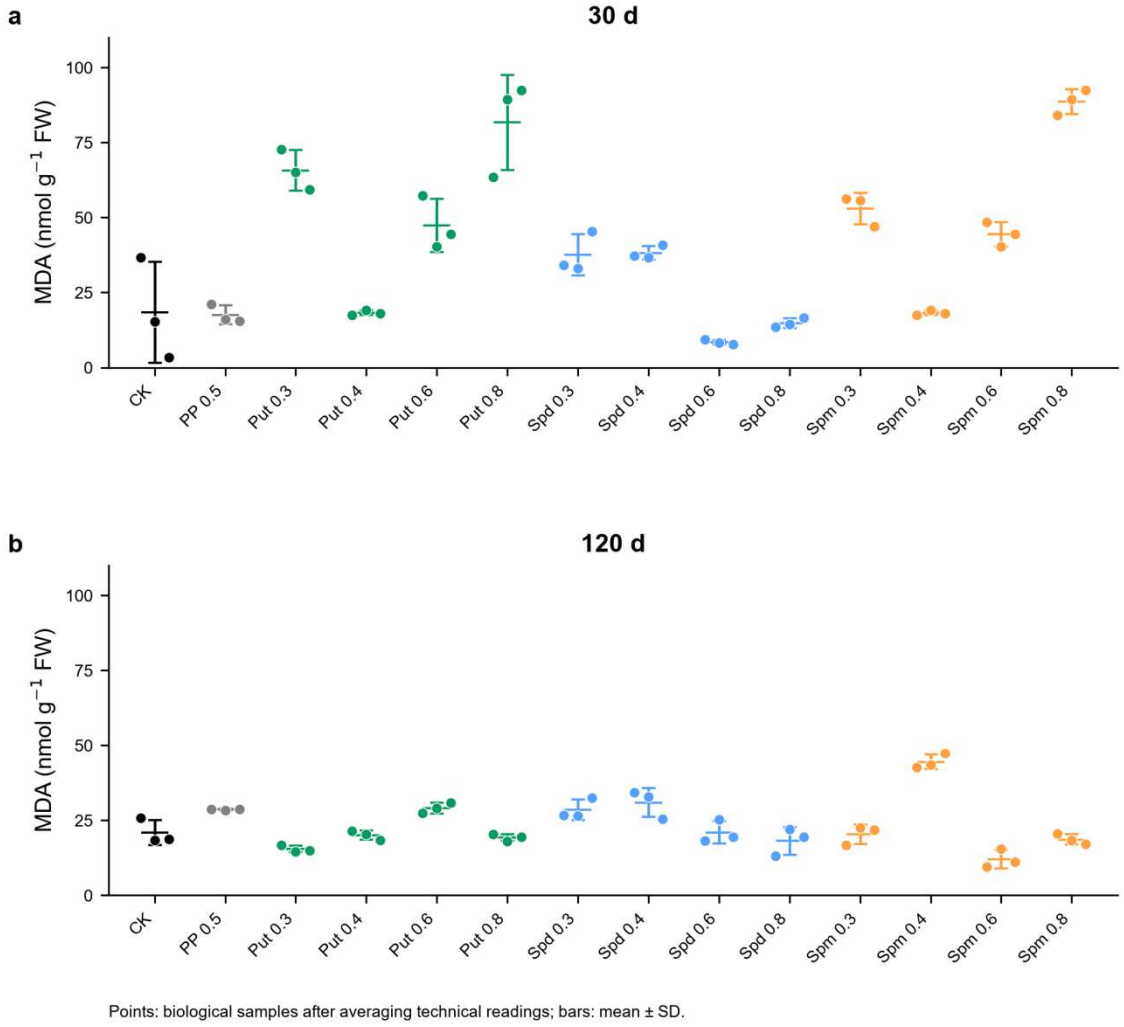


Fig. 6 Malondialdehyde concentration at 30 d (a) and 120 d (b). Points represent three biological samples per treatment, with mean ± SD after averaging technical readings. Horizontal offsets separate sample points without changing concentrations. Omnibus tests are reported in Table S8. Treatment concentrations are in mg L^{-1}.

At 30 d, CK had a mean H_2O_2 concentration of 11.81 ± 3.58 μmol g^{-1} FW, while the supplemented treatments ranged from 5.38 μmol g^{-1} FW in Spd 0.6 to 10.90 μmol g^{-1} FW in PP 0.5 (Fig. 7a). Within-family means ranked Put 0.4 > Put 0.8 > Put 0.6 > Put 0.3, Spd 0.8 > Spd 0.4 > Spd 0.3 > Spd 0.6, and Spm 0.6 > Spm 0.8 > Spm 0.3 > Spm 0.4. At 120 d, CK had the lowest mean H_2O_2 concentration (2.74 ± 0.48 μmol g^{-1} FW), while all supplemented treatments had higher descriptive means. Spm 0.6 had the highest endpoint mean (11.75 μmol g^{-1} FW). The endpoint mean was lower than the 30 d mean in CK, Put 0.4, Put 0.6, and Spm 0.8, whereas ten supplemented treatments showed the opposite descriptive pattern. All eight biochemical omnibus tests remained significant after Holm correction (adjusted $P < 0.001$; Table S8). These tests establish overall treatment heterogeneity but do not identify specific pairwise differences from CK.

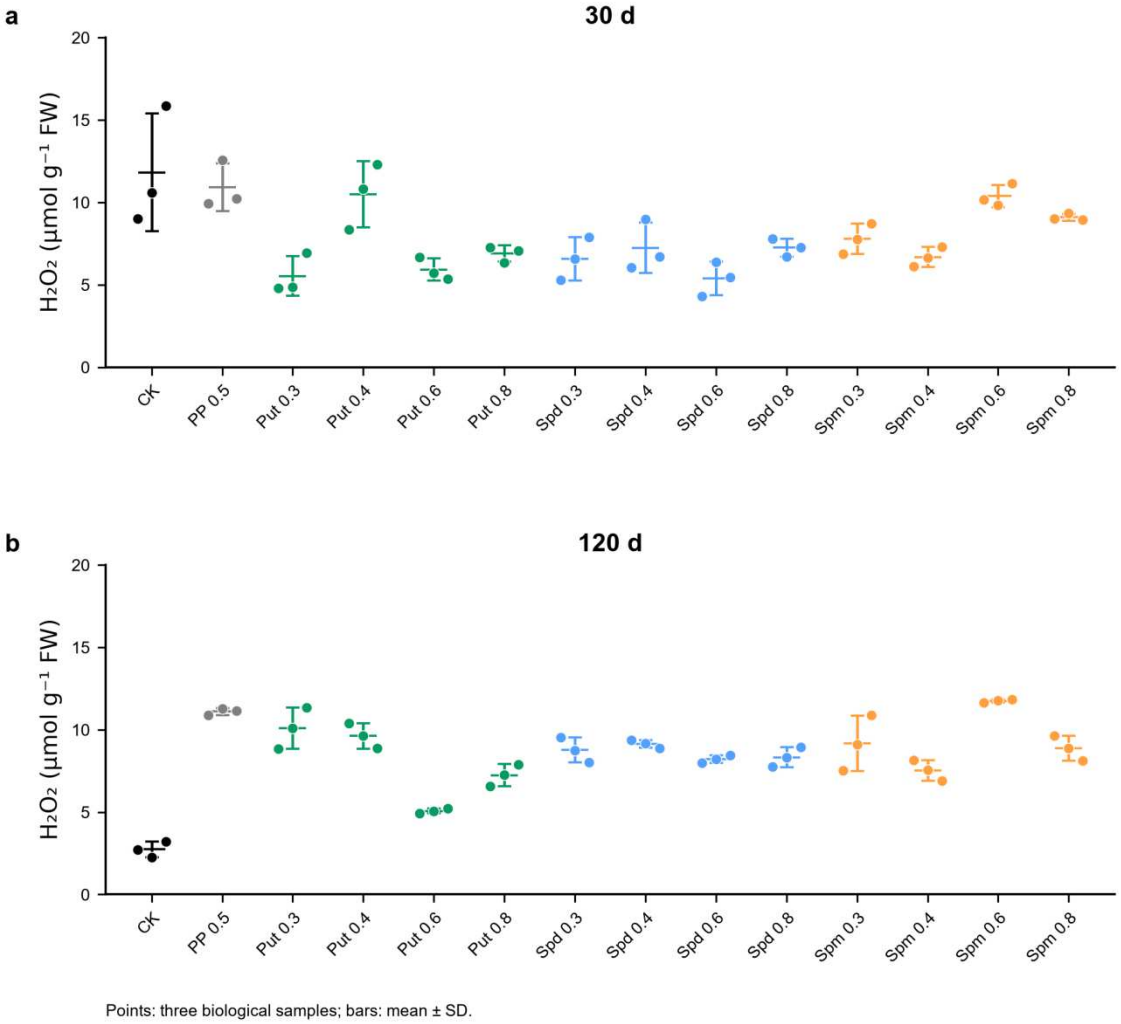


Fig. 7 Hydrogen peroxide concentration at 30 and 120 d. (a) H_2O_2 concentration at 30 d. (b) H_2O_2 concentration at 120 d. Points represent three independent biological samples, and bars show the mean ± SD. Technical readings were averaged within each biological sample before plotting. No pairwise or cross-time tests were performed. Treatment concentrations are in mg L^{-1}.

Discussion

Different polyamines promoted different aspects of plantlet development

Each polyamine improved a different component of plantlet quality. Spd 0.8 mainly promoted shoot proliferation, produced the highest mean shoot number, and ranked second in the composite index. Spm supported both rooting and PSII performance: Spm 0.6 had the highest mean root number and ranked first overall, while Spm 0.3 had the highest endpoint Fv/Fm and ranked third. Put 0.8 supported biomass and leaf development, with the highest fresh mass and leaf number, while Put 0.4 had the largest maximum-leaf area proxy. These complementary responses show that medium selection should reflect the propagation goal. Treatment-dependent responses have also been reported in *Dendrobium 'Sonia'* (Saiprasad et al. 2004), wheat anther culture (Redha and Suleman 2011), tulip culture (Podwyszyńska et al. 2015), and organ development under polyamine treatments (Kıymaz and Acemi 2025). However, the present data do not show whether these responses arose from changes in polyamine metabolism, nutrient allocation, or hormone signalling. Mechanistic interpretation will require measurements of endogenous polyamines, nitrogen status, and hormone-related pathways.

Clump development and PSII efficiency captured different aspects of plantlet quality promotion

The clump hierarchy addresses the proliferation-versus-size problem that motivated the index. A clump containing more new shoots can have smaller mean stems even when propagation output increases. Since new and original shoots were not distinguished, mean stem dimensions cannot separate this compositional effect from growth restriction. Shoot number, fresh mass, leaves and roots were therefore retained as distinct domains, with stem dimensions reported separately. Rooting and shoot conversion are central quality endpoints in *Dendrobium* micropropagation (Luo et al. 2008).

Fv/Fm added a functional measure that was not interchangeable with morphology. For each polyamine, the concentration with the highest mean Fv/Fm differed between 30 and 120 d. At 120 d, Put 0.8 had the highest mean fresh mass per clump, while Spm 0.3 had the highest mean Fv/Fm. Greater biomass therefore did not always coincide with higher PSII efficiency. The 120 d ICC of 0.455 indicates within-vessel similarity among sampled clumps under the fitted variance decomposition. *Dendrobium* cultures can also show different responses in shoot formation, pigments, biomass and fluorescence under altered light conditions (Lin et al. 2011; Guo et al. 2023). In senescing barley leaf discs, polyamines preserved chlorophyll even as photosystem I and II activities declined (Cohen et al. 1979). Because Fv/Fm measures dark-adapted maximum PSII efficiency rather than pigment concentration, carbon assimilation or whole-plant vigor (Murchie and Lawson 2013), it was used here for descriptive comparison and exploratory integration.

Biochemical indicators showed different treatment responses

The biochemical indicators did not follow a single pattern across treatments. Their rankings changed between 30 and 120 d and also differed among POD, CAT, MDA, and H_2O_2. At 30 d, CK had lower Fv/Fm and lower POD activity than most supplemented treatments, but this does not necessarily indicate weaker antioxidant defence. Enzyme activity can reflect oxidative demand, scavenging capacity, or both. Similarly, a high CAT or POD value cannot be interpreted as beneficial without considering oxidative damage and plant performance. The omnibus tests confirmed overall differences among treatments, but they did not show which treatments differed from CK.

At 120 d, CK had the highest CAT activity but the lowest Fv/Fm and a low composite score. Spm 0.6 showed a different pattern, with the lowest MDA concentration and the highest H_2O_2 concentration. These contrasting

results show that the biochemical indicators and plantlet quality did not change in parallel. H_2O_2 is both a reactive oxygen species and a signalling molecule, and its measured level depends on production, removal, and cellular compartmentation (Chen et al. 2019; Blázquez 2024). The present assays therefore cannot identify the underlying pathway or show that higher H_2O_2 caused better growth. Previous in vitro studies have also reported treatment-specific changes in antioxidant enzymes and lipid peroxidation under polyamine or elicitor treatments (Tokarz et al. 2018; Samsurizal et al. 2025). In this study, the biochemical measurements helped describe physiological state but were not used as direct measures of plantlet quality.

The integrated ranking supports medium selection for different propagation goals

The composite index gave balanced weight to proliferation, biomass, organ development, and PSII performance. Spm 0.6, Spd 0.8, and Spm 0.3 formed a close leading group, but they reached these scores through different trait profiles. Instead of defining one universal optimum, the results identify candidate media for different propagation goals. Spd 0.8 is a candidate when shoot proliferation is the main priority, Spm 0.6 when rooting and balanced growth are important, and Spm 0.3 when endpoint PSII efficiency is emphasized. Put 0.8 remains a candidate for biomass and leaf production. These media provide a basis for refining the *D. parishii* propagation system, but their performance should be confirmed in independent cultures and during acclimatization. The ranking is study specific because it depends on the selected indicators, stated weights, and correlations among traits (Becker et al. 2017).

Conclusion

Polyamine type and concentration shaped different aspects of *D. parishii* plantlet development. Spd 0.8 promoted shoot proliferation, Spm 0.6 supported rooting and balanced growth, Spm 0.3 had the highest endpoint Fv/Fm, and Put 0.8 supported biomass and leaf development. The exploratory composite index ranked Spm 0.6, Spd 0.8, and Spm 0.3 as the leading candidates, while the biochemical indicators provided complementary information on physiological state. Together, these results identify media for different propagation goals and provide a basis for improving the *D. parishii* micropropagation system. Independent culture and acclimatization experiments are needed to confirm whether these advantages remain stable in later propagation stages.

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Statements and Declarations

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Author contributions

Chong Feng: Investigation, Formal analysis, Writing – original draft. Jun Liu: Investigation (assisted with experiments). Enyao Ma: Funding acquisition, Resources. Yu'e Chen: Funding acquisition, Resources. Mengru Zhang: Data curation (assisted with experimental data processing). Zhimin Zhao: Conceptualization, Writing – review & editing. Depo Yang: Conceptualization, Methodology, Funding acquisition, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Data availability

The data supporting the findings of this study are available from the corresponding author on reasonable request.

Supplementary information

Table S1 provides the sample counts; Table S2, clump-level morphology; Table S3, treatment-level index inputs; Table S4, within-vessel correlations; Table S5, cluster-preserving morphology tests; Table S6, composite scores; Table S7, 30 d Fv/Fm summaries; Table S8, biochemical omnibus tests; Table S9, CK biochemical results at 120 d; and Table S10, within-polyamine concentration orders. Figure S1, Appearance of in vitro *Dendrobium parishii* cultures at 0, 30 and 120 d under different medium treatments.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables10.18.xlsx
- SupplementFigure.pdf