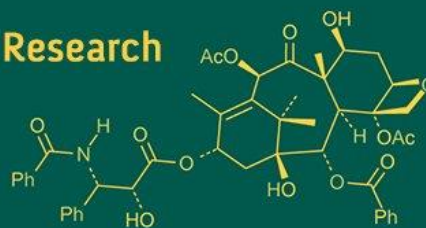
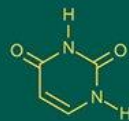
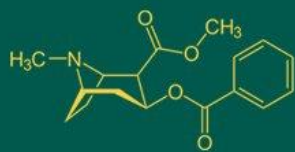


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Effect of native microbial bioinoculants on the early growth, biomass accumulation and quality of big-leaf mahogany (*Swietenia macrophylla* King) seedlings

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Abstract

Big-leaf mahogany (*Swietenia macrophylla* King) is a high-value tropical timber whose nursery establishment is often limited by slow early growth and a reliance on mineral fertilisers. Microbial bioinoculants offer a low-input route to improving seedling vigour, but comparative evidence across functionally distinct inoculant groups is scarce for this species. A six-month nursery experiment was conducted at Ponnampet, in the central Western Ghats of India, in a randomised complete block design with nine treatments (T₁, uninoculated control; T₂, vesicular-arbuscular mycorrhiza; T₃, microbial consortium; T₄, *Azotobacter chroococcum*; T₅, *Azospirillum brasilense*; T₆, phosphate-solubilising bacteria, powder; T₇, phosphate-solubilising bacteria, liquid; T₈, potassium-solubilising bacteria, liquid; and T₉, *Rhizobium*), each replicated three times. Analysis of variance showed significant treatment effects on seedling height, collar diameter, total leaf area, total biomass and the Dickson Quality Index (DQI; $p \leq 0.05$), whereas leaf number, shoot and root dry weight, root length and the root-to-shoot ratio did not differ significantly. *Azospirillum brasilense* (T₅) produced the tallest seedlings (9.24 cm), the largest leaf area (104.7 cm²) and the greatest total biomass (0.413 g), exceeding the control by 14%, 133% and 109%, respectively, while the microbial consortium (T₃) recorded the largest collar diameter (1.87 mm) and the highest DQI (0.048). Total biomass was very strongly correlated with shoot dry weight ($r = 0.99$) and leaf area ($r = 0.89$), indicating that the inoculation response was driven mainly by aboveground development. Principal component analysis and Ward clustering placed *Azospirillum brasilense* and the consortium together in a high-performance group, opposite the control and the mycorrhizal, PSB-powder and *Rhizobium* treatments. *Azospirillum brasilense* and the microbial consortium are therefore identified as the most effective bioinoculants for raising vigorous *S. macrophylla* planting stock under low-input nursery conditions.

Keywords: *Swietenia macrophylla*, *Azospirillum brasilense*, biofertilizer, Dickson Quality Index, nursery seedlings, plant growth-promoting microorganisms, sustainable forestry

Introduction

Big-leaf mahogany (*Swietenia macrophylla* King; Meliaceae) is one of the most commercially important tropical hardwoods in the world, valued for its dimensionally stable, richly coloured timber and for a range of bioactive metabolites recovered from its leaves, bark and seeds (Gullison *et al.*, 1996). Although the species is native to the Neotropics, from Mexico through Central America into the Amazon basin, sustained over-exploitation and habitat loss prompted its listing on Appendix II of the Convention on International Trade in Endangered Species (CITES) in 2002 and a Vulnerable assessment on the IUCN Red List (Grogan & Barreto, 2005; Grogan *et al.*, 2010). As a consequence, most mahogany now entering international trade originates from plantations established across South and Southeast Asia, Oceania and the Pacific, where the absence of the mahogany shoot borer (*Hypsipyla grandella*) permits rapid, straight-stemmed growth (Newton *et al.*, 1993; Mayhew & Newton, 1998), although in parts of its introduced range the species has itself become invasive (Coracero, 2023). India is among the countries in which plantation cultivation has expanded substantially, increasing the demand for vigorous, uniform planting stock from forest nurseries [11, 8, 10, 20, 16, 4].

The quality of nursery seedlings is a decisive determinant of subsequent field survival and growth, yet conventional nursery production relies heavily on synthetic fertilisers that raise costs, acidify soils and disrupt beneficial soil biota.

Bioinoculants-formulations of beneficial bacteria and arbuscular mycorrhizal fungi-provide an ecologically benign alternative (Vessey, 2003; Glick, 2012). VAM fungi in particular have been shown to stimulate growth of tropical tree seedlings, including neem (Sumana & Bagyaraj, 2002, 2003) and leguminous timber trees (Sumana & Bagyaraj, 1996, 1998), as well as cashew rootstock (Lakshmipathy *et al.*, 2004). Through nitrogen fixation, phosphate and potassium solubilisation, siderophore release and the production of phytohormones such as indole-3-acetic acid, these microorganisms can enhance nutrient acquisition, root architecture and stress tolerance, thereby improving seedling vigour while reducing chemical inputs (Spaepen *et al.*, 2007; Backer *et al.*, 2018) [31, 7, 29, 30, 27, 28, 15, 25, 1].

A considerable body of evidence documents positive responses of agricultural and forest species to microbial inoculation. Co-inoculation with nitrogen-fixing and phosphate-solubilising organisms has improved shoot length, chlorophyll content and biomass in pulses (Rodríguez & Fraga, 1999), while plant growth-promoting rhizobacteria and mycorrhizae have enhanced root surface area, nutrient uptake and drought tolerance in several tropical tree seedlings (Muthukumar *et al.*, 2001; Muthukumar & Udaiyan, 2010, 2018; Sumana & Bagyaraj, 1996, 1998). Despite this, most forestry studies have examined a single inoculant or a narrow functional group, and direct comparisons among mycorrhizal, free-living diazotrophic, phosphate-solubilising and potassium-solubilising inoculants on a single host remain uncommon. For an exacting, slow-establishing species such as big-leaf mahogany, identifying which functional group delivers the most reliable early growth benefit has clear practical value [29, 30, 27, 28].

The present study therefore evaluated nine inoculation treatments, spanning the principal functional categories of commercial bioinoculants, on the early growth and biomass partitioning of *S. macrophylla* seedlings under uniform nursery conditions. The specific objectives were (i) to quantify the effect of contrasting bioinoculants on seedling growth parameters (height, collar diameter, leaf area), and (ii) to assess their effect on biomass parameters (shoot and root dry weight, total biomass) and on integrated measures of seedling quality. We hypothesised that inoculants capable of biological nitrogen fixation and phytohormone production would most strongly stimulate aboveground growth.

2. Materials and methods

2.1. Study site and plant material

The experiment was carried out over six months in the open nursery of the College of Forestry, Ponnampet (12.1445° N, 75.9382° E; 860 m above mean sea level), in the Kodagu district of the central Western Ghats, Karnataka, India. The locality experiences a humid tropical monsoon climate. Uniform, vigorous polybag-raised seedlings of comparable age were selected as experimental material, and seedlings were measured before treatment to verify uniformity of the starting population.

2.2. Experimental design and treatments

The trial was laid out in a randomised complete block design (RCBD) comprising nine treatments and three replications (blocks), with 15 seedlings constituting each treatment plot within a block, giving 45 seedlings per treatment and 405 seedlings in total. The nine treatments were:

- T₁ - Uninoculated control;
- T₂ - Vesicular-arbuscular mycorrhiza (VAM);
- T₃ - Microbial consortium;
- T₄ - *Azotobacter chroococcum*;
- T₅ - *Azospirillum brasilense*;
- T₆ - Phosphate solubilising bacteria (PSB-P);
- T₇ - Phosphate solubilising bacteria (PSB-L);
- T₈ - Potassium solubilising bacteria (KSB-L);
- T₉ - *Rhizobium Spp.*

Note: P- Powder formulation; L-Liquid Formulation

2.3. Inoculation procedure

A shallow circular trench was opened around the collar of each seedling to form a uniform application ring. Powder-based inoculants were applied at 10 g per seedling, distributed evenly within the ring and covered with soil to ensure intimate root contact; liquid inoculants were applied as a 10 mL suspension around the collar and backfilled in the same manner. Treated seedlings were then left undisturbed for 20 days to allow microbial establishment and root colonisation before the first measurement.

2.4. Growth and biomass measurements

The first observation was recorded at the end of the 20-day colonisation phase, after which readings were taken at 15-day intervals throughout the six-month study; a final, comprehensive harvest was conducted at six months. Growth variables comprised seedling height (cm), collar diameter (mm), number of leaves and total leaf area (cm²), the latter determined by the graphical (graph-paper) method. For biomass determination, seedlings were carefully uprooted, separated into shoots and roots, oven-dried to constant weight and weighed to obtain shoot dry weight, root dry weight and total biomass (g); root length (cm) and the root-to-shoot ratio were also recorded. Overall seedling quality was expressed using the Dickson Quality Index (DQI; Dickson *et al.*, 1960): $DQI = \text{total dry mass} \div [(\text{height} / \text{collar diameter}) + (\text{shoot dry mass} / \text{root dry mass})]$.

2.5. Statistical analysis

The replicate (plot-level) data were subjected to analysis of variance appropriate to the RCBD, partitioning variation into treatment (8 degrees of freedom), block (2) and error (16) sources. Treatment effects were tested by the F-test, and where significant the means were separated by the critical difference (CD; least significant difference) and a compact-letter display at $p \leq 0.05$. The standard error of the mean (S.E.m ±) and coefficient of variation (CV) were obtained from the error mean square, and two effect-size statistics-eta-squared (η^2) and the less biased omega-squared (ω^2)-were derived to gauge the magnitude of the treatment effects. The multivariate structure of the response was examined by (i) Pearson correlation analysis among treatment means (7 degrees of freedom); (ii) principal component analysis (PCA) of the nine treatments described by eight standardized traits, summarised as a biplot; and (iii) agglomerative hierarchical clustering of the standardized treatment profiles by Ward's minimum-variance method. Analyses were performed in Python (NumPy, SciPy). Throughout, effects were considered significant at $p \leq 0.05$; "ns" denotes non-significant.

3. Results

3.1. Overall treatment effects and effect sizes

The analysis of variance (Table 1) revealed significant treatment effects on seedling height ($p = 0.009$), collar diameter ($p < 0.001$), total leaf area ($p = 0.038$), total biomass ($p = 0.014$) and the Dickson Quality Index ($p = 0.024$). Leaf number, shoot and root dry weight, root length and the root-to-shoot ratio were not significantly affected ($p > 0.05$); shoot

dry weight was only marginal ($p = 0.087$). The effect sizes for the significant traits were moderate to large, being greatest for collar diameter ($\eta^2 = 0.78$; $\omega^2 = 0.68$), followed by seedling height, total biomass, the Dickson Quality Index and total leaf area ($\eta^2 = 0.58$ - 0.65). Coefficients of variation were lowest for collar diameter (4.6%) and height (12.1%) and highest for leaf area (41.6%), reflecting the greater variability of canopy measurements.

Table 1: Analysis of variance and effect sizes for the measured traits of *S. macrophylla* seedlings (treatment df = 8; error df = 16).

Trait	F	p	η^2	ω^2	CV (%)	Sig.
Seedling height	4.01	0.009	0.654	0.481	12.1	**
Collar diameter	8.28	<0.001	0.781	0.679	4.6	***
Number of leaves	0.70	0.684	0.255	0.000	16.3	ns
Total leaf area	2.79	0.038	0.581	0.363	41.6	*
Shoot dry weight	2.19	0.087	0.518	0.273	33.4	ns
Root dry weight	1.70	0.175	0.455	0.181	36.6	ns
Total biomass	3.59	0.014	0.638	0.450	24.8	*
Root length	1.77	0.158	0.464	0.195	24.2	ns
Root: shoot ratio	0.27	0.966	0.110	0.000	64.1	ns
Dickson Quality Index	3.17	0.024	0.593	0.397	25.4	*

η^2 , eta-squared; ω^2 , omega-squared; CV, coefficient of variation. Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, non-significant

3.2. Seedling height and collar diameter

Treatments differed significantly in both height and collar diameter (Table 2; Figs 1 and 2). *Azospirillum brasilense* (T₅) produced the tallest seedlings (9.24 cm), statistically on par with *Azotobacter chroococcum* (T₄, 8.81 cm) and the control (T₁, 8.13 cm) but significantly taller than the VAM treatment

(T₂, 6.01 cm), the shortest (CD = 1.58). Collar diameter was greatest under the microbial consortium (T₃, 1.87 mm), which significantly exceeded most other treatments and was thickest of all, while *Rhizobium* (T₉, 1.46 mm) and the PSB-powder treatment (T₆, 1.49 mm) produced the slenderest stems (CD = 0.13). Leaf number did not differ among treatments.

Table 2: Influence of bioinoculants on the growth performance of *S. macrophylla* seedlings at final harvest.

Treatment	Seedling height (cm)	Collar diameter (mm)	Number of leaves
T ₁	8.127 ^{abc}	1.610 ^{bcd}	2.140
T ₂	6.013 ^e	1.651 ^{bc}	1.830
T ₃	6.530 ^{de}	1.870 ^a	1.873
T ₄	8.813 ^{ab}	1.673 ^{bc}	1.790
T ₅	9.243 ^a	1.733 ^b	1.907
T ₆	6.907 ^{cde}	1.492 ^{de}	2.003
T ₇	7.720 ^{abcd}	1.559 ^{cde}	1.777
T ₈	7.373 ^{bcde}	1.621 ^{bcd}	1.643
T ₉	7.060 ^{cde}	1.460 ^e	1.757
S.Em ($\pm$)	0.527	0.043	0.174
CD (0.05)	1.580	0.130	NS

Within a column, means with a different letter differ significantly at $p \leq 0.05$. NS, non-significant.

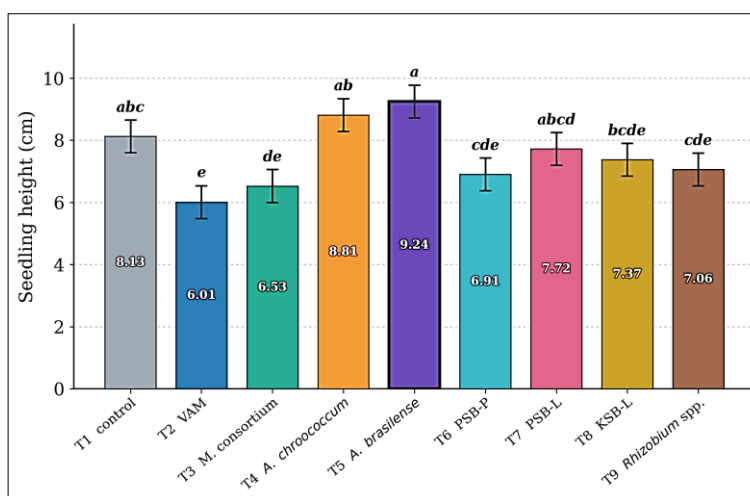


Fig 1: Effect of bioinoculant treatments on seedling height of *S. macrophylla*. Error bars denote the standard error of the mean; bars with a different letter differ significantly at $p \leq 0.05$.

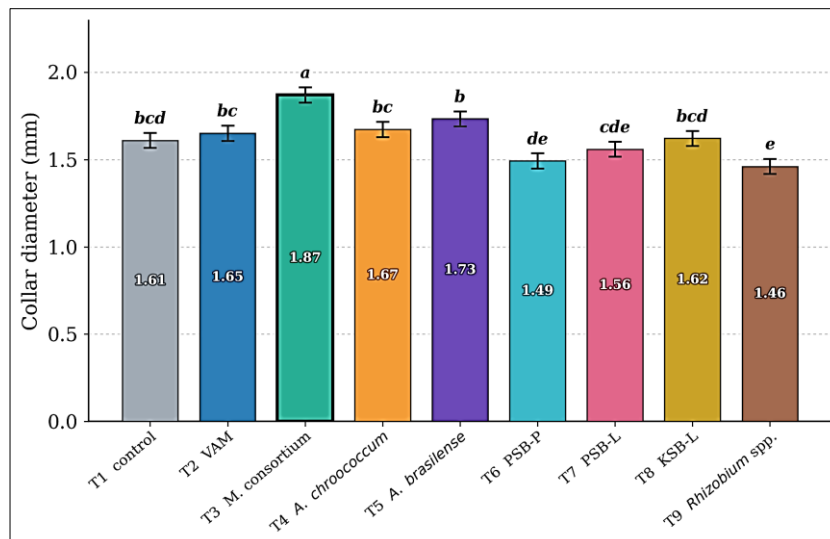


Fig 2: Effect of bioinoculant treatments on collar diameter of *S. macrophylla*. Error bars denote S.Em $\pm$; different letters indicate significant differences at $p \leq 0.05$.

3.3. Leaf area and seedling quality

Total leaf area varied markedly among treatments (CD = 41.9; CV = 41.6%; Table 3, Fig. 3). *Azospirillum brasilense* (T₅) recorded the largest canopy (104.7 cm²), followed by the microbial consortium (T₃, 82.8 cm²), and was significantly greater than the VAM treatment (T₂, 26.9 cm²), the smallest.

In contrast to the original analysis, the Dickson Quality Index also differed significantly among treatments ($p = 0.024$): the consortium (T₃, 0.048) and *Azospirillum brasilense* (T₅, 0.047) achieved the highest values, roughly double that of the control (T₁, 0.021), indicating genuinely sturdier, better-balanced planting stock rather than merely larger seedlings.

Table 3: Influence of bioinoculants on total leaf area and the Dickson Quality Index of *S. macrophylla* seedlings at final harvest.

Treatment	Total leaf area (cm ²)	Dickson Quality Index
T ₁	45.000 ^{bc}	0.021 ^d
T ₂	26.900 ^c	0.031 ^{bcd}
T ₃	82.833 ^{ab}	0.048 ^a
T ₄	63.233 ^{abc}	0.040 ^{abc}
T ₅	104.733 ^a	0.047 ^a
T ₆	54.667 ^{bc}	0.026 ^{cd}
T ₇	57.767 ^{bc}	0.043 ^{ab}
T ₈	45.433 ^{bc}	0.041 ^{abc}
T ₉	42.700 ^{bc}	0.031 ^{bcd}
S.Em ($\pm$)	13.980	0.005
CD (0.05)	41.913	0.016

Within a column, means with a different letter differ significantly at $p \leq 0.05$.

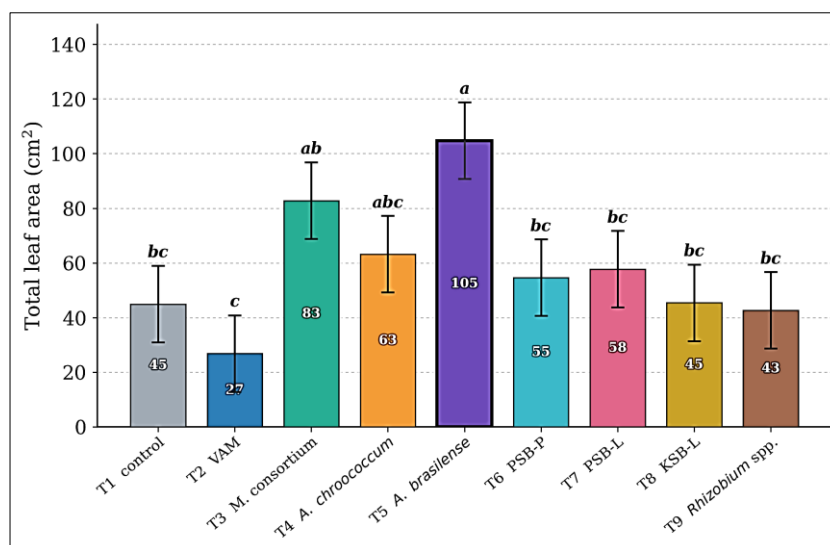


Fig 3: Effect of bioinoculant treatments on total leaf area of *S. macrophylla*. Error bars denote S.Em $\pm$; different letters indicate significant differences at $p \leq 0.05$.

3.4. Biomass and root parameters

Total biomass differed significantly among treatments (Table 4; Figs 4 and 5), whereas shoot dry weight (marginal, $p = 0.087$), root dry weight, root length and the root-to-shoot ratio did not. *Azospirillum brasilense* (T₅) accumulated the greatest total biomass (0.413 g) and the microbial consortium (T₃) ranked a close second (0.392 g), both significantly exceeding

the control (T₁, 0.198 g; CD = 0.126)-an increase of about 109% for T₅. Root dry weight ranged narrowly from 0.039 g to 0.095 g and root length from 4.89 cm (T₁) to 9.21 cm (T₇), but neither, nor the root-to-shoot ratio, differed significantly. The stacked partitioning of dry matter (Fig. 5) shows that the biomass advantage of the responsive treatments was expressed mainly in the shoot fraction.

Table 4: Influence of bioinoculants on biomass and root parameters of *S. macrophylla* seedlings at final harvest.

Treatment	Shoot dry wt (g)	Root dry wt (g)	Total biomass (g)	Root length (cm)	Root: shoot
T ₁	0.159	0.039	0.198 ^d	4.887	0.254
T ₂	0.159	0.051	0.210 ^{cd}	7.100	0.334
T ₃	0.319	0.073	0.392 ^a	6.470	0.290
T ₄	0.255	0.082	0.337 ^{ab}	6.687	0.314
T ₅	0.318	0.095	0.413 ^a	7.513	0.300
T ₆	0.186	0.052	0.238 ^{bcd}	6.143	0.475
T ₇	0.248	0.088	0.335 ^{abc}	9.210	0.354
T ₈	0.213	0.082	0.294 ^{abcd}	7.400	0.401
T ₉	0.167	0.063	0.230 ^{bcd}	5.507	0.369
S.Em (±)	0.043	0.015	0.042	0.945	0.127
CD (0.05)	NS	NS	0.126	NS	NS

Within a column, means with a different letter differ significantly at $p \leq 0.05$. NS, non-significant.

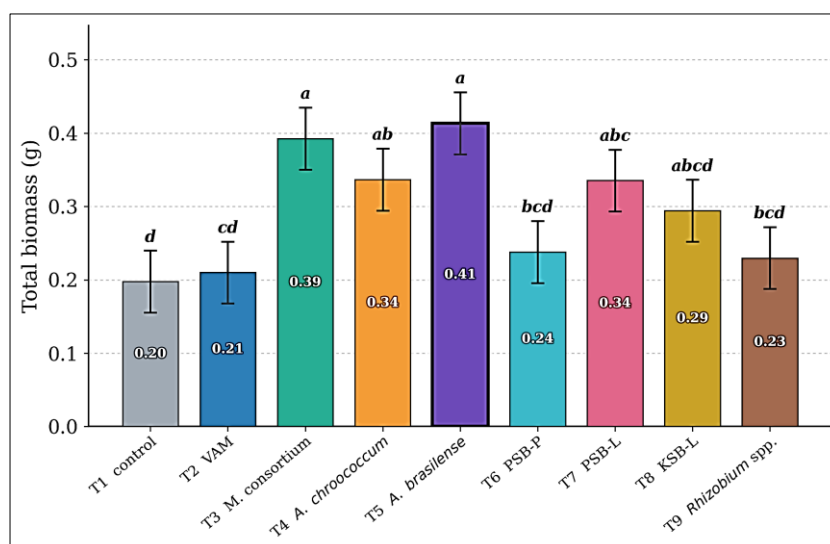


Fig 4: Effect of bioinoculant treatments on total biomass of *S. macrophylla*. Error bars denote S.Em ±; different letters indicate significant differences at $p \leq 0.05$.

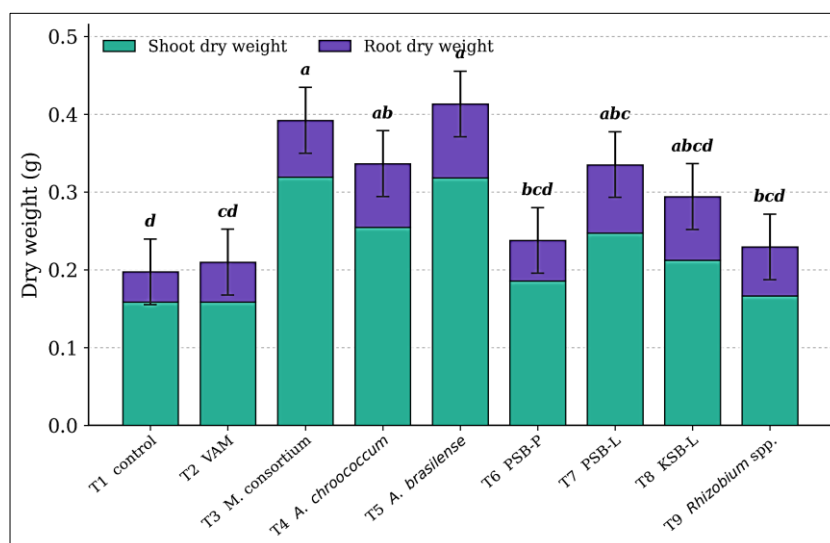


Fig 5: Partitioning of seedling dry matter into shoot and root fractions across treatments; the biomass advantage of the responsive treatments was expressed mainly through the shoot fraction.

3.5. Associations among traits

Pearson correlations among treatment means revealed a tightly integrated aboveground growth syndrome (Fig. 6). Total biomass was almost perfectly correlated with shoot dry weight ($r = 0.99$) and closely correlated with leaf area ($r = 0.89$), collar diameter ($r = 0.67$) and the Dickson Quality Index ($r = 0.94$), but only weakly with seedling height ($r =$

0.45) and root length ($r = 0.53$). The Dickson Quality Index was itself strongly tied to biomass, root dry weight ($r = 0.91$) and shoot dry weight ($r = 0.90$), confirming that it captures genuine dry-matter accumulation. These patterns indicate that the inoculation response was governed chiefly by leaf-area-driven shoot growth, with root length contributing comparatively little to overall mass.

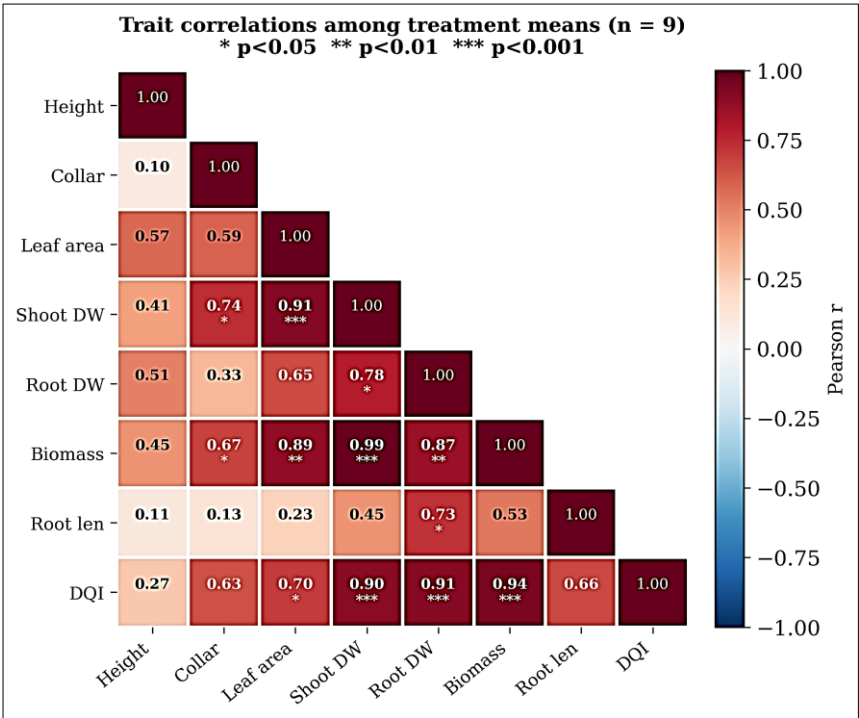


Fig 6: Pearson correlation matrix among the measured traits, computed across the nine treatment means ($n = 9$). Only the lower triangle is shown; asterisks denote significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.6. Multivariate structure of the treatment response

Principal component analysis condensed the eight standardized traits into a few interpretable axes (Table 5, Fig. 7). The first component (PC1) captured 67.6% of the variation and loaded negatively and fairly evenly on all growth and biomass traits, representing a general seedling-vigour gradient; the second (PC2, 14.3%) contrasted root

length and root dry weight against collar diameter and leaf area, describing a root-versus-shoot axis. Together the first two axes explained 81.8% of the variation. In the biplot, *Azospirillum brasilense* (T_5) and the consortium (T_3) occupied the high-vigour extreme, diametrically opposite the control and the VAM and PSB-powder treatments.

Table 5: Trait loadings on the first three principal components and the variance explained by each.

Trait	PC1	PC2	PC3
Seedling height	-0.212	-0.214	-0.797
Collar diameter	-0.286	-0.406	0.508
Total leaf area	-0.373	-0.346	-0.141
Shoot dry weight	-0.418	-0.155	0.106
Root dry weight	-0.385	0.325	-0.190
Total biomass	-0.427	-0.047	0.040
Root length	-0.252	0.711	0.048
Dickson Quality Index	-0.407	0.176	0.188
Variance explained (%)	67.6	14.3	12.7
Cumulative (%)	67.6	81.8	94.5

Loadings with the largest absolute values on each component define its biological meaning.

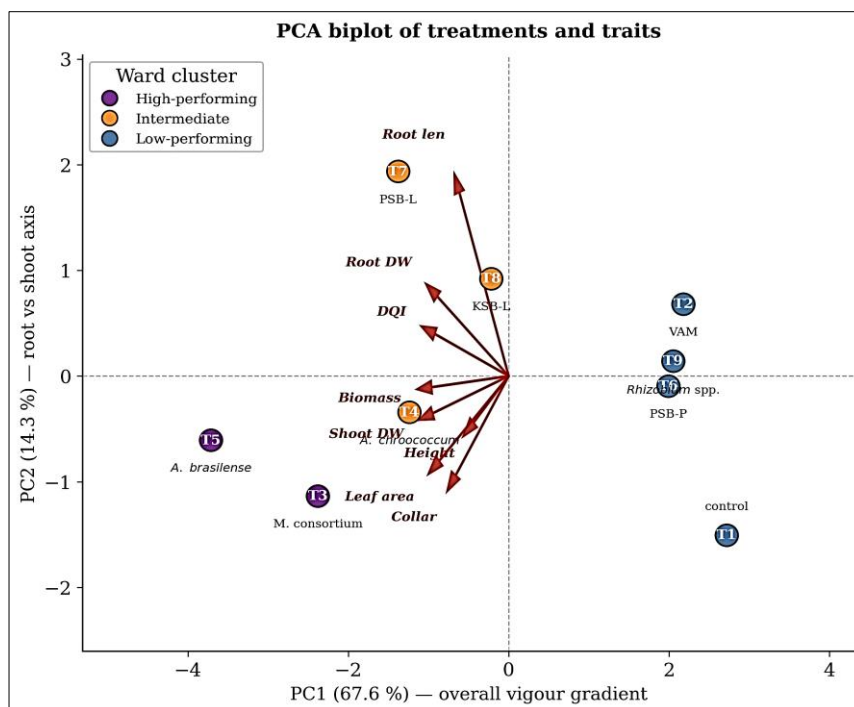


Fig 7: Principal component analysis biplot of the nine treatments (points, coloured by Ward cluster) and the eight traits (red vectors). PC1 is an overall vigour gradient and PC2 a root-versus-shoot axis.

Ward's hierarchical clustering of the standardized treatment profiles recovered three well-separated groups (Fig. 8) that mirror the ordination: a high-performance cluster comprising the microbial consortium (T₃) and *Azospirillum brasilense* (T₅); an intermediate, comparatively root-oriented cluster of *Azotobacter chroococcum* (T₄) and the liquid PSB and KSB treatments (T₇, T₈); and a low-performance cluster grouping

the control (T₁) with the VAM (T₂), PSB-powder (T₆) and *Rhizobium* (T₉) treatments. The standardized trait heat map (Fig. 9), with treatments ordered from most to least vigorous, makes the same pattern explicit, the upper rows being dominated by positive (green) z-scores across the shoot and biomass traits and the lower rows by negative (red) values.

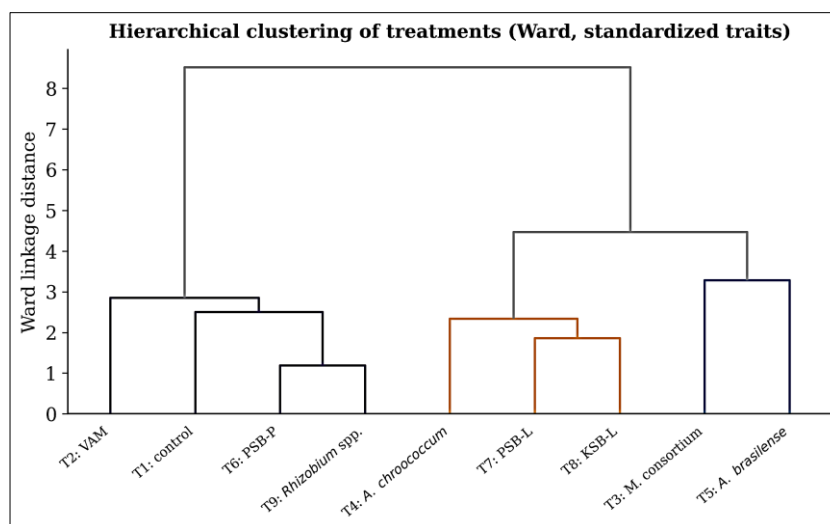


Fig 8: Dendrogram from Ward's clustering of the standardized treatment profiles, resolving high-performance (T₃, T₅), intermediate (T₄, T₇, T₈) and low-performance (T₁, T₂, T₆, T₉) clusters.

4. Discussion

Across the nine treatments, the clearest and most consistent benefit was associated with *Azospirillum brasilense* (T₅) and the microbial consortium (T₃), which together maximised seedling height, leaf area, total biomass and the Dickson Quality Index. The convergence of these advantages across functionally related aboveground traits is the central result of the study.

The strong performance of *Azospirillum brasilense* is mechanistically plausible. Free-living diazotrophs of this genus (Steenhoudt & Vanderleyden, 2000; Bashan *et al.*, 2004) are known to stimulate plant growth less through the quantity of nitrogen they fix than through the secretion of phytohormones-particularly indole-3-acetic acid-that promote cell elongation, lateral branching and the proliferation of fine absorptive surfaces (Spaepen *et al.*, 2007; Bashan & de-Bashan, 2010) [26, 3, 2].

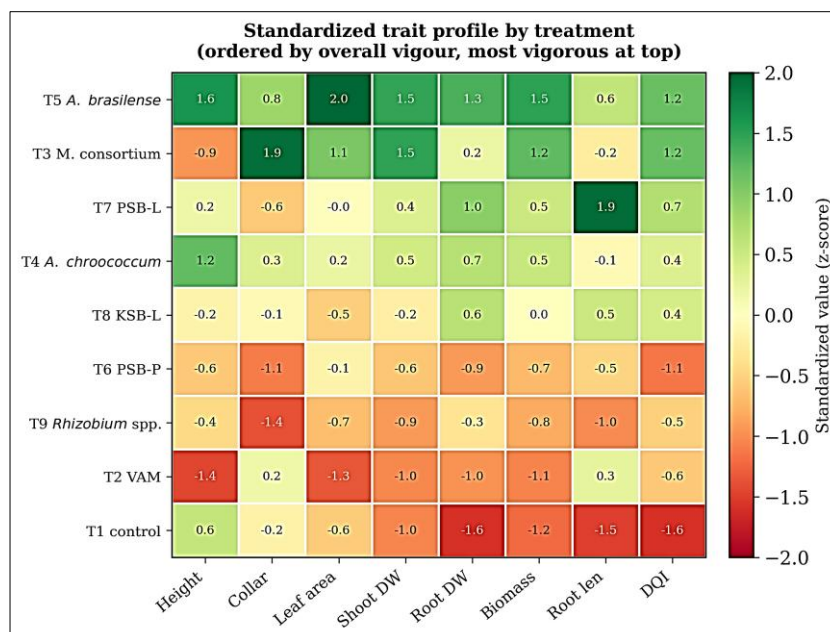


Fig 9: Heat map of standardized (z-scored) trait values by treatment, ordered from most vigorous (top) to least vigorous (bottom) along PC1. Green denotes above-average and red below-average values.

The resulting improvement in resource capture is typically expressed as enhanced shoot elongation and leaf expansion, exactly the pattern recorded here, where the leaf area of T₅ more than doubled that of the control. Comparable hormone-mediated growth promotion has been reported in cereals (Kapulnik *et al.*, 1985; Hungria *et al.*, 2010) and in several tree seedlings (Muthukumar *et al.*, 2001), lending external support to the present findings [3, 2, 14].

The very strong correlation between total biomass and shoot dry weight ($r = 0.99$), together with the weak correlations involving root length, indicates that the inoculation response was an aboveground phenomenon. This is reinforced by the non-significant treatment effects on root dry weight, root length and the root-to-shoot ratio: the inoculants enhanced the production of photosynthetic tissue rather than reorganising biomass allocation between shoot and root. From a nursery standpoint this is advantageous, because larger leaf area and stem diameter underpin greater carbon capture and mechanical robustness in outplanting stock. Importantly, in this dataset the Dickson Quality Index—an integrative measure of sturdiness and balance—was significantly higher under the consortium and *Azospirillum brasilense*, so the gains reflect genuinely better-quality seedlings and not merely larger ones. The multivariate analyses lend coherence to these trait-by-trait results. Because the first principal component—an undifferentiated vigour axis—absorbed about two-thirds of the variation, the treatments differed mainly in how strongly they stimulated growth overall rather than in the particular combination of traits they favoured. Ward clustering distilled this into three interpretable groups, and the convergence of the consortium and *Azospirillum brasilense* in the high-performance cluster, set against a low-performance cluster that included the untreated control, provides a robust, assumption-light corroboration of the univariate F-tests.

It is notable that the largest collar diameter was obtained with the microbial consortium (T₃) rather than *Azospirillum brasilense*. Consortium products combine complementary functional groups, and their advantage for radial stem thickening may reflect a broader spectrum of nutrient mobilisation, especially of phosphorus, which is closely tied

to secondary growth (Rodríguez & Fraga, 1999; Etesami *et al.*, 2017) [6]. The *Azotobacter chroococcum*, PSB and KSB treatments produced intermediate, trait-specific gains but none matched the all-round performance of the two leaders. Two results ran counter to expectation. The VAM treatment (T₂) consistently ranked among the poorest, producing the shortest seedlings and the smallest canopy. Mycorrhizal symbioses often establish slowly and may impose an initial carbon cost on the host before net benefits accrue (Smith & Read, 2008; Sumana & Bagyaraj, 1996); six months in a fertile, well-watered nursery medium—conditions under which the host is least dependent on fungal phosphorus delivery—may have been too short and too benign for the symbiosis to express its potential. Such delayed or context-dependent VAM benefits have been noted in nursery studies on tropical tree species, including neem (Sumana & Bagyaraj, 2003) and cashew rootstock (Lakshmipathy *et al.*, 2004), where VAM response varied with fungal species, soil fertility and establishment period. *Rhizobium* (T₉) likewise underperformed, which is unsurprising on a non-leguminous host that does not form the nodular symbiosis through which *Rhizobium* exerts its principal effect.

Several limitations temper these conclusions. The experiment was conducted at a single nursery over a single growing season, and the high variability of the leaf-area and biomass measurements (CV 25–42%) indicates that larger sample sizes or repeated trials would strengthen inference. Direct measures of microbial establishment and seedling nutrient status were not available, so the proposed mechanisms remain inferential. Finally, nursery performance does not always translate into field success; the value of *Azospirillum brasilense*- and consortium-treated stock should ultimately be confirmed through field outplanting trials (Grogan *et al.*, 2003) under the water- and nutrient-limited conditions in which microbial benefits are typically most pronounced (Jing *et al.*, 2023) [13].

5. Conclusion

Among nine functionally distinct bioinoculants screened on big-leaf mahogany seedlings, *Azospirillum brasilense* and the

microbial consortium were the most effective, together raising seedling height, leaf area, total biomass and the Dickson Quality Index; *Azospirillum brasilense* increased total biomass by about 109% over the uninoculated control, while the consortium produced the thickest collar and the highest quality index. The mycorrhizal, PSB-powder and *Rhizobium* treatments were the least effective on this non-leguminous host over the nursery period studied, and the response was driven primarily by leaf-area-mediated shoot growth rather than by changes in root allocation. *Azospirillum brasilense* and a well-formulated microbial consortium are therefore recommended as practical, low-input amendments for raising vigorous, good-quality *S. macrophylla* planting stock, pending validation in field establishment trials.

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