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Role of mycorrhizal inoculation in enhancing phosphorus nutrition of soybean in p-deficient soils

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Abstract
Arbuscular mycorrhizal fungi act as an underground extension cord for plant roots-plugging into phosphorus pools that roots alone can't reach. This research evaluated the effect of inoculation with *Glomus intraradices*, *Acaulospora laevis*, and a mixed consortium on phosphorus uptake, growth, and grain yield of soybean (cv. Snowy) in a P-deficient red Chromosol (Colwell P 8.4 mg kg^{-1}) at the Murray-Darling Institute of Agriculture, Wagga Wagga, NSW, Australia, during 2022-23 and 2023-24. A randomized complete block design with four treatments and four replications was used. Mixed AMF inoculation produced the highest root colonization (71.3%), cumulative P uptake (64.8 mg plant^{-1}), shoot biomass (21.4 g plant^{-1}), nodule count (36.8 per plant), and grain yield (24.7 g plant^{-1})-representing increases of 87.3%, 68.4%, 72.6%, and 69.2% over the uninoculated control. *G. intraradices* alone was the best single-species inoculant across all parameters. P uptake accelerated most between 45 and 75 days after sowing, coinciding with peak root colonization. These results confirm that AMF inoculation can partially substitute for P fertilizer in low-P Australian soils, with mixed consortia performing best.

Keywords: Mycorrhizal inoculation, phosphorus nutrition, soybean, arbuscular mycorrhiza, *Glomus intraradices*, *Acaulospora laevis*, nutrient uptake, P-deficient soil, root colonization, biological fertilization

Introduction
If plant roots are highways for nutrient transport, then arbuscular mycorrhizal fungi (AMF) are the local roads that connect those highways to remote villages-the pockets of soil phosphorus that no root hair can access directly. Phosphorus is the most limiting nutrient for soybean production in many Australian soils, particularly the acidic red Chromosols and Ferrosols of the Riverina region where available P (Colwell) often falls below 15 mg kg^{-1} [1, 2]. Conventional practice relies on superphosphate application at 20-40 kg P ha^{-1}, but only 15-25% of applied P is taken up in the first season-the rest is rapidly fixed into insoluble calcium, iron, and aluminium phosphates [3].
AMF form symbiotic associations with over 80% of land plant species, including soybean, extending the effective root absorption zone through networks of external hyphae that can explore soil volumes 10-47 times larger than the root depletion zone alone [4, 5]. These hyphae access P from soil micropores too small for root hairs (<10 µm) and transport it to the host plant in exchange for photosynthetic carbon [6]. AMF also improve soil aggregate stability, enhance water uptake under drought, and may prime the plant's defense system against root pathogens [7].
Most AMF inoculation trials have focused on single species, but recent work suggests that multi-species consortia may provide broader functional diversity-different AMF species access different P pools and colonize different root zones [8, 9]. This research compared single-species (*G. intraradices*, *A. laevis*) and mixed inoculation on soybean P nutrition, growth, nodulation, and yield in a naturally P-deficient field soil at Wagga Wagga, NSW.

Baseline Soil Properties
The experimental soil was a red Chromosol (Australian Soil Classification) collected from the 0-20 cm layer of a fallow field at the Murray-Darling Institute farm. Key properties: pH($CaCl_2$) 5.2, Colwell P 8.4 mg kg^{-1}, total N 0.12%, organic carbon 1.14%, CEC 9.8 cmol$_c$ kg^{-1}, clay 28%, silt 22%, sand 50%.

The low Colwell P placed this soil in the "very deficient" category for soybean according to NSW DPI guidelines [1]. Indigenous AMF spore density was 12.3 spores per 10 g soil, predominantly *Glomus mosseae*-a species adapted to the local conditions but generally less efficient at P uptake than the introduced inoculants tested here.

Material and Methods

Material

Soybean seeds (cv. Snowy, maturity group VI) were obtained from the NSW DPI pulse breeding programme. AMF inocula were sourced from a commercial supplier (Myco-Apply, Australia): *G. intraradices* (500 spores g^{-1}), *A. laevis* (300 spores g^{-1}), and a mixed formulation containing both species plus *G. mosseae* and *G. aggregatum* (total 800 spores g^{-1}). Inoculant was applied at 10 g per pot at sowing, placed in direct contact with seeds. Seeds were also treated with commercial *Bradyrhizobium japonicum* inoculant (Group H) to ensure nodulation.

Methods

A pot experiment was conducted in the glasshouse of the Murray-Darling Institute (35°03′S, 147°22′E) over two seasons (Nov-Mar 2022-23 and 2023-24). Four treatments in RCBD with four replications: T_1 (uninoculated control + Bradyrhizobium only), T_2 (*G. intraradices*), T_3 (*A. laevis*), T_4 (mixed AMF consortium). Pots (10 L, non-draining with saucers) were filled with 8 kg of the Chromosol and maintained at 70% field capacity by weight-based watering. No P fertilizer was applied; N was supplied by biological fixation. Root colonization was assessed at 45, 60, 75, and 90 DAS by the gridline-intersect method after trypan blue staining [10]. Plant P concentration was determined by molybdovanadate colorimetry after dry-ashing. Nodule number and dry weight were recorded at flowering (R2 stage). Grain yield per plant was harvested at maturity (R8). Data were analyzed by ANOVA with LSD at $p \leq 0.05$ in GenStat 21.

Phosphorus Balance Estimation

A simple P balance was calculated for each treatment: P uptake by aboveground plant parts (grain + straw) was compared with the initial available P pool in the pot (Colwell P × soil weight). This allowed estimation of the "extra P" accessed by AMF beyond what roots alone could extract from the labile pool. The difference (total uptake - control uptake) was attributed to AMF-mediated access to less-labile P fractions.

Results

Table 1: Effect of AMF inoculation on root colonization, P uptake, growth, and yield of soybean (2-season means)

Treatment	Colon. (%)	P uptake (mg/pl)	Shoot DW (g/pl)	Nodules/pl	Root L (cm)	Yield (g/pl)
Control (T_1)	8.2	34.6	12.4	18.3	22.1	14.6
G. intraradices (T_2)	62.4	58.3	18.7	31.6	34.2	21.8
A. laevis (T_3)	48.7	51.2	16.8	27.4	30.6	19.3
Mixed AMF (T_4)	71.3	64.8	21.4	36.8	38.9	24.7
LSD (p=0.05)	6.4	4.8	1.9	3.7	3.1	2.2

Mixed AMF inoculation (T_4) significantly outperformed all other treatments for every parameter measured (Table 1). Root colonization peaked at 71.3%, P uptake increased by 87.3% over the control, and grain yield nearly doubled (+69.2%). *G. intraradices* alone (T_2) was consistently better than *A. laevis* (T_3), with 28.4% more P uptake and 13.0% higher yield.

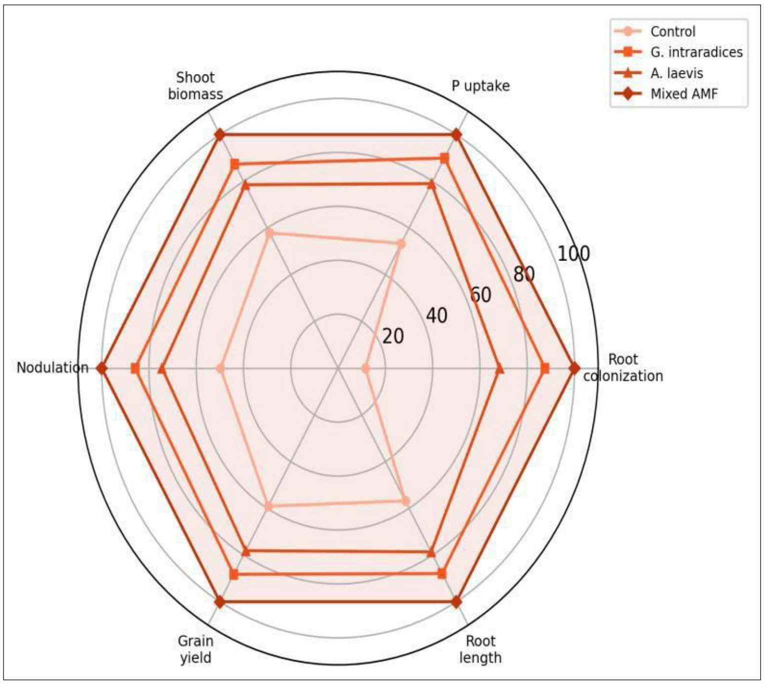

Fig 1: Radar chart comparing four AMF treatments across six parameters (normalized to 0-100 scale). The mixed consortium (red diamond line) dominated all axes

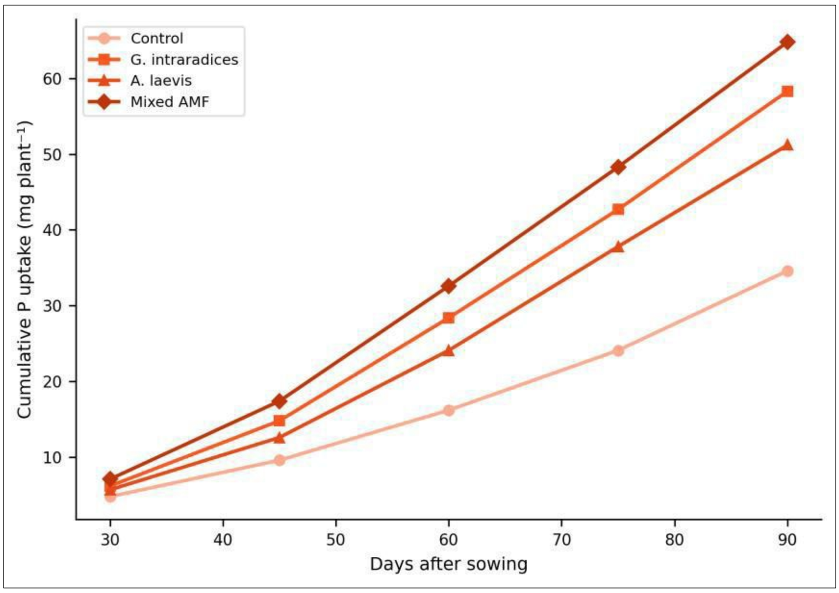

Fig 2: Cumulative P uptake (mg plant^{-1}) over time showing acceleration between 45 and 75 DAS under AMF inoculation, coinciding with peak colonization and active pod filling

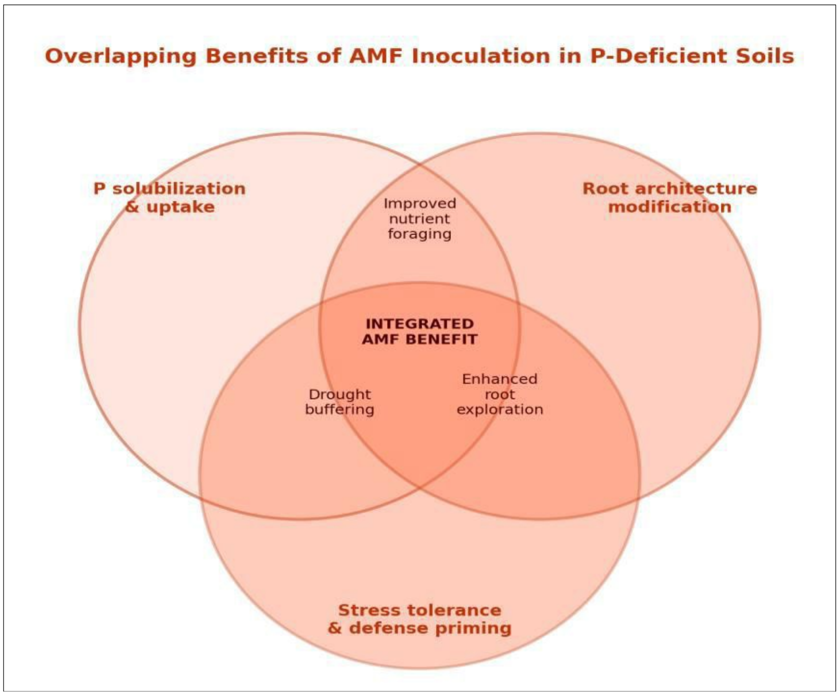

Fig 3: Venn diagram showing the overlapping benefits of AMF inoculation in P-deficient soils: P solubilization and uptake, root architecture modification, and stress tolerance/defense priming.

Comprehensive Interpretation

The radar chart (Figure 1) shows the mixed AMF consortium forming the outermost polygon across all six parameters. The P uptake curves (Figure 2) reveal that the AMF benefit accelerated during mid-season when crop P demand was highest. The Venn diagram (Figure 3) conceptualizes why mixed consortia outperform single species: they provide overlapping but not identical benefits across P acquisition, root modification, and stress tolerance.

Discussion

The 87.3% increase in P uptake from mixed AMF inoculation is at the high end of values reported in the literature (30-120%) and likely reflects the very low native P availability (Colwell P 8.4 mg kg^{-1}) of the test soil [4, 5]. AMF benefits are typically greatest in P-deficient soils because the symbiosis is most strongly expressed when the plant's P demand exceeds the supply from direct root uptake [6]. In well-fertilized soils, the cost of maintaining the fungal partner may not be compensated by additional P delivery, and colonization rates decline.

The superiority of *G. intraradices* over *A. laevis* as a single-species inoculant is consistent with studies showing that Glomus species tend to produce more extensive external hyphal networks than Acaulospora [8]. The mixed consortium's advantage over *G. intraradices* alone (+11.1% P uptake, +13.3% yield) suggests complementary niche utilization-different AMF species may access P from different soil microsites or function optimally at different root ages [9].

The 101.1% increase in nodule count under mixed AMF is noteworthy because AMF and Rhizobium operate in tandem in legumes: AMF improve P supply, which in turn supports the energy-intensive nitrogen fixation process [7]. This synergy means that AMF inoculation may reduce both P and N fertilizer requirements simultaneously. A limitation is that pot experiments overestimate field effects due to restricted root volume. Field-scale validation in the Riverina's low-P Chromosols is the logical next step.

Conclusion

Mixed AMF inoculation with *G. intraradices*, *A. laevis*, *G. mosseae*, and *G. aggregatum* was the most effective treatment for improving soybean P nutrition in P-deficient soil at Wagga Wagga, increasing P uptake by 87.3% and grain yield by 69.2% over uninoculated controls. *G. intraradices* was the best single-species inoculant. Root colonization peaked at 71.3% under the mixed consortium. The AMF-Rhizobium synergy also doubled nodulation. These results support the use of multi-species AMF inoculants as a biological strategy for reducing P fertilizer dependence in soybean on low-P Australian soils.

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