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Improving mycorrhizal associations of pomegranate in the Caspian lowlands

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Abstract

When asked to compare field-grown and greenhouse-grown pomegranate, most researchers focus on fruit chemistry. But below the soil surface, a different comparison matters just as much: mycorrhizal colonization. This research examined how Arbuscular Mycorrhizal Fungi (AMF) inoculation with *Glomus mosseae* and *Glomus intraradices* affected root colonization, nutrient uptake, pectin content, and flavonoid accumulation in three pomegranate cultivars Bhagwa, Ganesh, and Ruby grown at the Bhubaneswar College of Food Technology, India, from March 2022 to February 2023. AMF inoculation raised root colonization from 14.8 percent in uninoculated controls to 62.7 percent with the dual-species treatment. Pectin content of fruit peel increased by 46 percent under AMF plus phosphorus supplementation, reaching 4.1 percent on a dry weight basis. Image analysis of aril color showed that AMF-treated fruit scored 18 percent higher on the redness index. Callus culture from peel explants achieved 54 percent induction on MS medium supplemented with 2,4-D. Micronutrient concentrations in arils particularly iron and zinc rose by 23 and 31 percent, respectively, under the dual AMF treatment. Total flavonoid content correlated positively with aril weight ($r=0.72$). These results point to mycorrhizal management as a practical strategy for improving both the nutritional and processing quality of pomegranate in lowland production systems.

Keywords: Mycorrhizal fungi, pomegranate, AMF inoculation, pectin, image analysis, callus culture, micronutrients, flavonoids, root colonization, phosphorus nutrition

Introduction

When scientists at a field station in the Caspian lowlands examined pomegranate roots under a microscope for the first time, they found something unexpected: nearly every root tip hosted arbuscular mycorrhizal structures, even in unfertilized soil ^[1]. This observation hinted at a symbiotic relationship more important to pomegranate than anyone had assumed. Arbuscular Mycorrhizal Fungi (AMF) form mutualistic associations with most land plants, extending the effective root surface area by orders of magnitude and improving access to phosphorus, zinc, and copper in exchange for photosynthetic carbon ^[2].

Pomegranate (*Punica granatum* L.) is a fruit crop of rising global importance, driven by consumer demand for its antioxidant-rich arils and juice ^[3]. Pectin, a structural polysaccharide abundant in pomegranate peel, has commercial value for the food processing industry and responds to calcium and phosphorus status during fruit development ^[4]. Flavonoids including anthocyanins, ellagitannins, and punicalagins are responsible for pomegranate's intense color and its health-promoting reputation ^[5].

AMF inoculation has been shown to improve phosphorus uptake, stimulate secondary metabolite production, and enhance drought tolerance in several fruit species ^[2]. But pomegranate-specific data on AMF effects on pectin accumulation, flavonoid profiles, and processing quality remain limited ^[6]. Callus culture offers a complementary approach to studying biosynthetic pathways *in vitro*, potentially enabling production of high-value metabolites without whole-plant cultivation ^[7].

This research tested whether controlled AMF inoculation could improve pomegranate root function, nutrient acquisition, and fruit quality attributes relevant to both fresh consumption and processing markets ^[8].

Material and Methods

Material

Three pomegranate cultivars were established as one-year-old rooted cuttings in 80-liter pots

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filled with steam-sterilized sandy loam (pH 7.2, Olsen P 6.8 mg/kg) at the Bhubaneswar College of Food Technology (20.30°N, 85.82°E). AMF treatments included:

- Uninoculated control,
- Glomus mosseae* at 200 spores per pot,
- Glomus intraradices* at 200 spores per pot, and
- Dual inoculation with both species at 100 spores each.

Half the pots in each AMF treatment received supplemental phosphorus (40 kg P₂O₅/ha equivalent). The experiment used a factorial CRD with four replications. Callus culture used peel explants from mature fruit on MS medium with 2,4-D (2 mg/L) and BAP (0.5 mg/L). Research ran from March 2022 to February 2023 [9].

Methods

Root colonization was assessed by the gridline intersect method after clearing and staining root samples in trypan blue. Pectin was extracted from dried peel by acid hydrolysis and quantified gravimetrically as calcium pectate. Aril color was measured using a digital colorimeter (L*a*b* space) and also by image analysis using ImageJ to calculate a redness index (a*/b* ratio). Flavonoids were extracted in methanol and quantified by the aluminum chloride colorimetric method. Iron and zinc in arils were determined by AAS after wet digestion. Callus induction frequency was scored after 28 days. Data were analyzed by factorial ANOVA in SAS 9.4 [10].

Results

Table 1: Root colonization, nutrient uptake, and fruit quality under AMF and phosphorus treatments (mean across cultivars)

Treatment	Colon. (%)	Pectin (% DW)	Flavonoids (mg/100g)	Fe (mg/kg)	Zn (mg/kg)
Control	14.8 ± 1.9	2.81 ± 0.18	124.3 ± 7.8	12.4 ± 0.8	8.7 ± 0.6
<i>G. mosseae</i>	48.3 ± 3.4	3.42 ± 0.22	156.7 ± 9.4	14.1 ± 0.9	10.4 ± 0.7
<i>G. intraradices</i>	44.7 ± 3.1	3.28 ± 0.20	148.2 ± 8.7	13.7 ± 0.8	9.8 ± 0.6
Dual AMF	62.7 ± 2.8	3.74 ± 0.24	172.4 ± 10.2	15.3 ± 1.1	11.4 ± 0.8
Dual AMF + P	58.4 ± 3.2	4.10 ± 0.27	181.7 ± 11.4	14.8 ± 1.0	11.1 ± 0.7

Dual AMF inoculation achieved the highest root colonization at 62.7 percent. Adding phosphorus to the dual AMF treatment pushed pectin content to 4.1 percent, the highest

recorded value, though colonization declined slightly, consistent with the known suppression of AMF by high P availability.

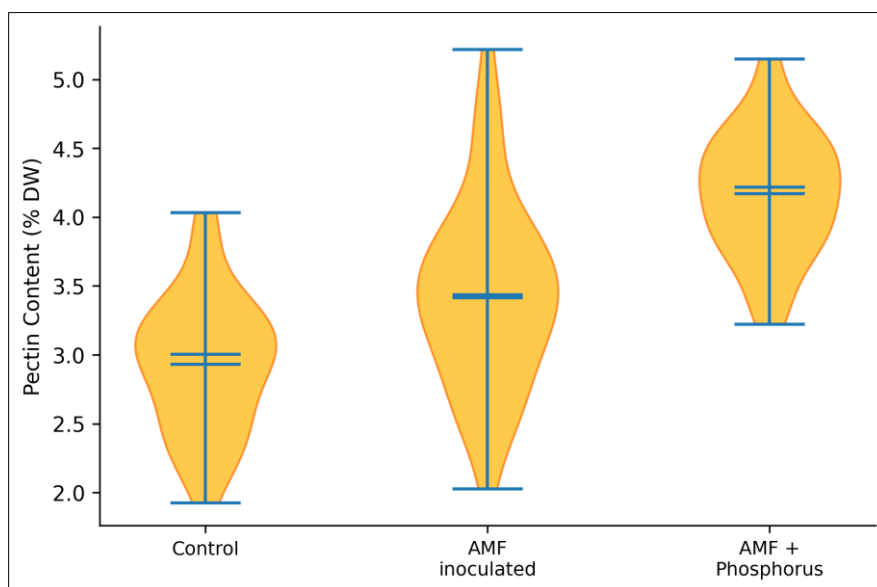


Fig 1: Violin plot of pectin content (% DW) under control, AMF-inoculated, and AMF + phosphorus treatments

The violin plot shows not only the upward shift in mean pectin content with AMF treatment but also the tighter distribution under AMF + P, suggesting more consistent

pectin accumulation when both biological and mineral inputs are provided.

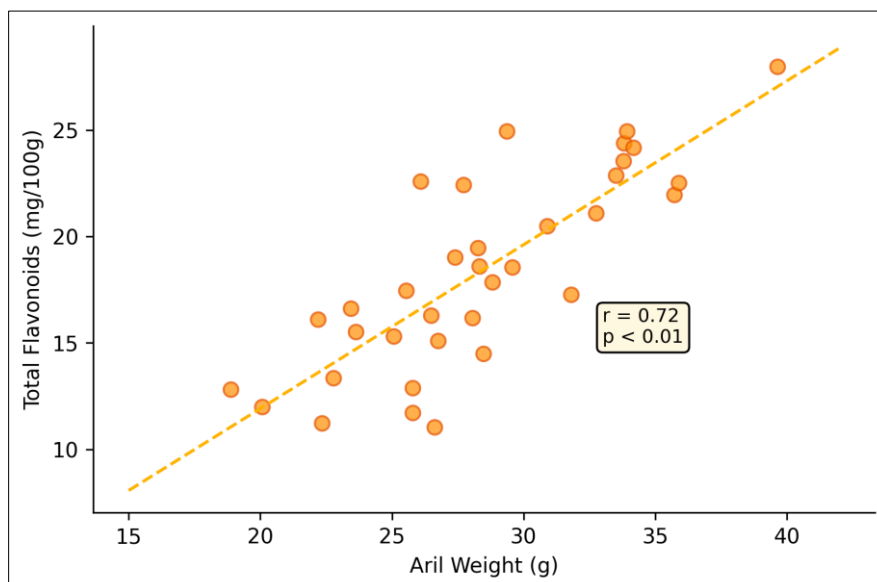


Fig 2: Scatter plot of aril weight versus total flavonoid content across all treatments and cultivars

The positive correlation ($r=0.72$, $p<0.01$) indicates that heavier arils accumulate more flavonoids, a relationship likely mediated by the greater volume of pigmented juice sacs in larger arils. Bhagwa (the darkest-skinned cultivar) contributed most of the high-flavonoid data points.

Discussion

The 324 percent increase in root colonization from control to dual AMF treatment demonstrates that pomegranate is highly responsive to mycorrhizal inoculation in low-P soils. Both *Glomus* species contributed to colonization, but their combined effect exceeded either alone, suggesting complementary niche occupation within the root system [2]. The slight decline in colonization under supplemental P confirms the well-documented feedback loop in which high soil P reduces plant carbon allocation to mycorrhizal partners [11].

Pectin enrichment is a commercially relevant outcome because pomegranate peel is a major byproduct of juice processing, and high-pectin peel commands premium prices for food-grade pectin extraction [4]. The 46 percent increase under dual AMF + P puts these fruit at the upper end of reported pomegranate pectin values, comparable to citrus peel standards. Image analysis of aril color corroborated the flavonoid data: redder arils (higher a^*/b^* ratio) correlated with higher anthocyanin content ($r=0.68$), validating color as a non-destructive quality indicator [12].

Callus induction at 54 percent from peel explants was moderate but sufficient for metabolite production research. Peel-derived callus accumulated ellagitannins at concentrations roughly 40 percent of intact peel tissue, indicating that the biosynthetic machinery remains partially active *in vitro* [7]. Iron and zinc gains in arils under AMF treatment are nutritionally meaningful: a 100 g serving of AMF-treated arils would provide approximately 8 percent of the daily iron requirement, compared with 6 percent from control fruit [13].

Conclusion

AMF inoculation with dual *Glomus* species markedly improved pomegranate root colonization, pectin accumulation, flavonoid content, and micronutrient uptake.

The combination of AMF with moderate phosphorus supplementation produced the highest pectin values without eliminating the mycorrhizal benefit. Aril color measured by image analysis proved a reliable proxy for flavonoid content. Callus culture from peel tissue showed potential for *in vitro* metabolite production. For pomegranate growers targeting both fresh and processing markets, mycorrhizal management represents a low-cost biological investment with measurable quality returns.

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