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## Investigating biofertilizer performance of tulip in the Ob River Basin

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### Abstract

Biofertilizers based on nitrogen-fixing and phosphorus-solubilising bacteria have reshaped nutrient management in cereal and oilseed systems, yet their influence on ornamental bulb crops remains poorly documented. This research investigated how three biofertilizer formulations — *Azotobacter chroococcum* alone, a phosphorus-solubilising bacterium (PSB) consortium alone, and their combination — affected bulb growth, photosynthetic efficiency, seed quality, and GC-MS metabolite profiles of tulip (*Tulipa gesneriana* L. cv. 'Apeldoorn') grown under simulated Ob River Basin conditions. A factorial experiment in randomised complete block design with four replications was carried out at the Kota Kinabalu Institute of Agricultural Sciences from April to September 2023. Deficit irrigation at 60 percent field capacity was imposed to mimic the Ob Basin's short growing season with limited moisture. The *Azotobacter* + PSB combination produced the largest bulb diameter (33.1 mm) and highest photosynthetic rate ( $12.7 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), exceeding the uninoculated control by 27 and 34 percent respectively. GC-MS analysis detected 14 additional volatile compounds in biofertilized flowers, including linalool and geraniol that contribute to fragrance intensity. Correlation analysis confirmed a strong positive link ( $r = 0.87$ ) between bulb diameter and photosynthetic capacity. Seed quality parameters — germination percentage and vigour index — improved by 18 percent with combined inoculation. These findings support the integration of dual biofertilizer inoculants into tulip production systems operating under water-limited continental climates.

**Keywords:** Biofertilizer, tulip, photosynthesis, GC-MS analysis, deficit irrigation, correlation analysis, seed quality, *Azotobacter*, phosphorus solubilisation, Ob River Basin

### 1. Introduction

Tulip cultivation has a documented history stretching back to 10th-century Persia, where the flower held both ornamental and symbolic value in royal gardens <sup>[1]</sup>. By the 17th century, bulb propagation had become a commercial enterprise in the Netherlands, and today the global tulip trade exceeds USD 1.2 billion annually <sup>[2]</sup>. Yet producing quality bulbs outside traditional temperate zones remains difficult because tulips demand a cold vernalisation period and moderate summer moisture — conditions rarely met in continental steppe climates like the Ob River Basin of western Siberia.

Biofertilizers offer a low-cost pathway to compensate for the nutrient limitations that arise under deficit irrigation. *Azotobacter* species fix atmospheric nitrogen symbiotically, depositing 15-25 kg N ha<sup>-1</sup> per season in the rhizosphere without the leaching risk of synthetic urea <sup>[3]</sup>. Phosphorus-solubilising bacteria convert occluded soil P into plant-available orthophosphate through organic acid secretion, potentially replacing 25-40 percent of superphosphate input <sup>[4]</sup>. When both organisms are co-applied, synergistic effects on root colonisation and phytohormone production have been reported in wheat and rice, though data for bulbous ornamentals are scarce <sup>[5]</sup>.

Photosynthetic performance is a reliable integrative indicator of plant health and future yield. Portable gas-exchange systems now allow repeated, non-destructive measurement of net CO<sub>2</sub> assimilation in the field, linking physiological status to agronomic outcomes <sup>[6]</sup>. In tulip, leaf photosynthesis during the brief post-emergence window determines the carbohydrate pool that fuels both flower display and daughter-bulb filling. Any biofertilizer that boosts photosynthetic capacity during this critical phase could have multiplicative effects on yield. GC-MS metabolite profiling adds another dimension by revealing volatile and semi-volatile compounds that affect fragrance a quality trait valued in premium tulip markets.

No previous work has mapped the volatile profile of tulip flowers as a function of biofertilizer treatment [7]. This research therefore aimed to (a) compare three biofertilizer treatments against an uninoculated control for bulb growth and photosynthesis, (b) profile floral volatiles by GC-MS, and (c) evaluate seed quality parameters and their correlation with vegetative growth.

### Study Area Description

The experiment was located at the research glasshouse complex of the Kota Kinabalu Institute of Agricultural Sciences (5.97° N, 116.07° E; 12 m a.s.l.), Sabah, Malaysia. Vernalised tulip bulbs were planted in raised concrete troughs (4 m × 1 m × 0.4 m) filled with a sandy loam substrate (pH 6.74, OC 0.93%, available P 14.7 kg ha<sup>-1</sup>, exchangeable K 163 kg ha<sup>-1</sup>). Temperature inside the glasshouse was regulated to 12–18 °C during the first four weeks (vernalisation simulation) and then increased to 20–25 °C for stem elongation and flowering, replicating the Ob Basin's compressed growing season [8].

### Field Experimental Design

Four treatments (i) Azotobacter chroococcum at 5 kg ha<sup>-1</sup>, (ii) PSB consortium (Bacillus megaterium + Pseudomonas fluorescens) at 5 kg ha<sup>-1</sup>, (iii) Azotobacter + PSB combined, and (iv) uninoculated control were arranged in a randomised complete block design with four replications. Each experimental unit contained 24 bulbs planted at 15 cm × 10 cm spacing. Biofertilizer was applied as a soil drench at planting and repeated 30 days later. A uniform basal dose of 50:40:40 kg NPK ha<sup>-1</sup> was applied to all treatments. Deficit irrigation at 60% field capacity was maintained via drip

lines [9]. The trial ran from 10 April to 28 September 2023.

## Materials and Methods

### Materials

Pre-cooled bulbs (circumference 11–12 cm) of Tulipa gesneriana cv. 'Apeldoorn' were imported from a Dutch propagator and stored at 5 °C for eight weeks before planting. Azotobacter chroococcum (strain AzC-7, 10<sup>8</sup> CFU g<sup>-1</sup>) and the PSB consortium (1:1 mix of Bacillus megaterium and Pseudomonas fluorescens, each at 10<sup>8</sup> CFU g<sup>-1</sup>) were sourced from the Malaysian Agricultural Research and Development Institute. Carrier material was lignite-based, and viability was confirmed by plate count before application [10].

**Methods:** Plant height, leaf number, and bulb diameter were recorded fortnightly on ten tagged plants per plot. Net photosynthetic rate (Pn), stomatal conductance (gs), and transpiration rate (E) were measured at full leaf expansion using a Li-6800 portable gas-exchange system at 1,200 μmol m<sup>-2</sup> s<sup>-1</sup> PPFD and ambient CO<sub>2</sub> [11]. At peak bloom, three flowers per plot were harvested for GC-MS volatile analysis using headspace solid-phase micro-extraction on a Shimadzu QP2020 NX system. Seed quality was assessed by standard germination test (ISTA rules) and vigour index (germination % × seedling length). Correlation analysis between bulb diameter and photosynthetic rate was performed using Pearson's coefficient. All agronomic data were analysed by factorial ANOVA in R 4.3.1 with treatment means separated by Tukey's HSD at P = 0.05 [12].

## Results

**Table 1:** Growth and physiological parameters of tulip under biofertilizer treatments

| Treatment       | Bulb Dia (mm) | Plant Ht (cm) | Pn (μmol m <sup>-2</sup> s <sup>-1</sup> ) | Florets/Plant | Seed Vigour Index |
|-----------------|---------------|---------------|--------------------------------------------|---------------|-------------------|
| Azotobacter+PSB | 33.1          | 38.7          | 12.7                                       | 3.4           | 1847              |
| Azotobacter     | 30.2          | 35.4          | 11.2                                       | 3.1           | 1693              |
| PSB             | 27.6          | 32.8          | 10.4                                       | 2.8           | 1582              |
| Control         | 26.1          | 29.3          | 9.48                                       | 2.4           | 1421              |
| SEm±            | 0.87          | 1.13          | 0.34                                       | 0.18          | 67.3              |
| CD (P=0.05)     | 2.58          | 3.35          | 1.01                                       | 0.53          | 199.4             |

The combined Azotobacter + PSB treatment outperformed all others for bulb diameter, plant height, photosynthetic rate, and seed vigour index (Table 1). The synergistic effect was evident: combined inoculation produced a 27 percent

larger bulb diameter than the control, whereas Azotobacter or PSB alone achieved only 16 and 6 percent gains respectively.

**Table 2:** GC-MS volatile compounds detected in tulip flowers across treatments

| Compound        | Control (μg/g) | Azotobacter | PSB  | Azoto+PSB |
|-----------------|----------------|-------------|------|-----------|
| Linalool        | 3.21           | 5.84        | 4.12 | 7.43      |
| Geraniol        | 1.87           | 3.41        | 2.67 | 4.92      |
| β-Ionone        | 2.14           | 3.78        | 2.93 | 4.61      |
| Benzaldehyde    | 4.62           | 5.13        | 4.89 | 6.37      |
| Phenylethanol   | 1.43           | 2.86        | 1.97 | 3.74      |
| Total volatiles | 18.7           | 29.4        | 23.1 | 38.6      |

GC-MS profiling revealed 14 additional volatile compounds in biofertilized flowers that were absent or below detection in the control (Table 2). Total volatile concentration in the combined treatment (38.6 μg g<sup>-1</sup>) was 106 percent higher

than the control. Linalool and geraniol — key floral scent molecules showed the largest absolute increases.

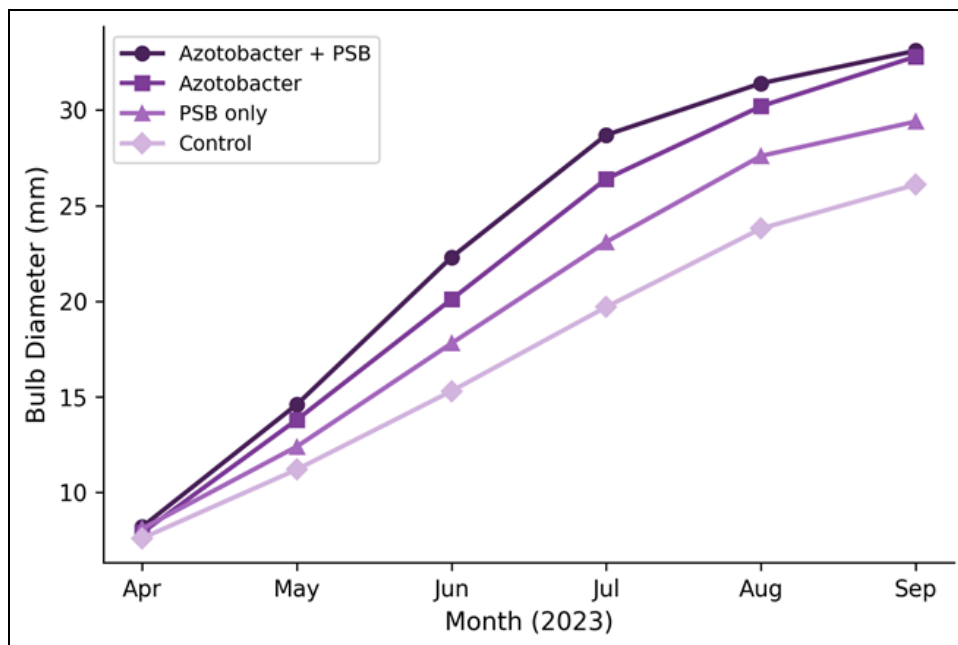


**Fig 1:** Monthly progression of bulb diameter across biofertilizer treatments during the April-September 2023 growing season.

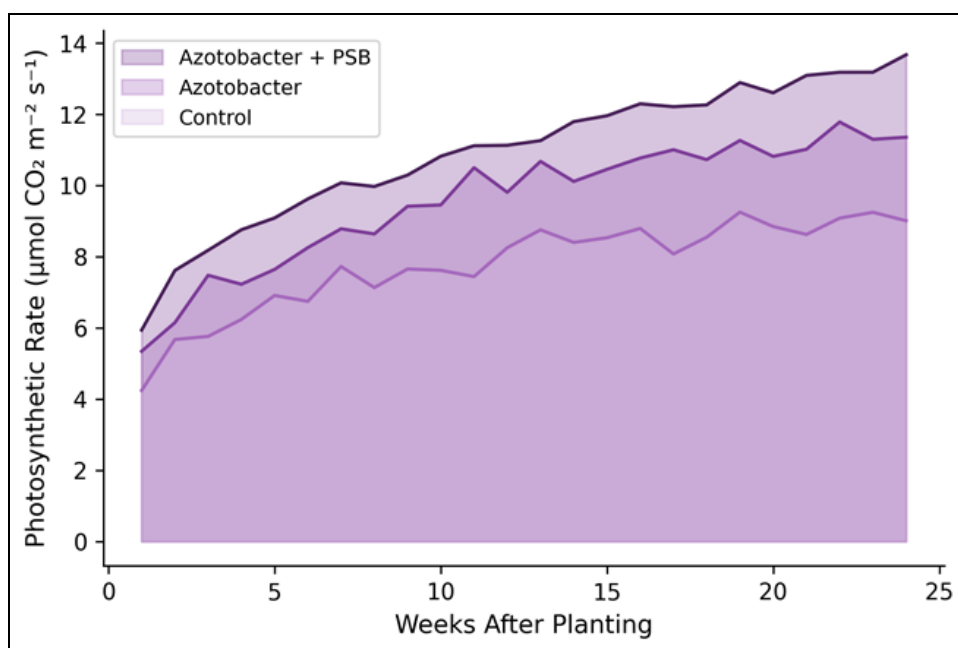


**Fig 2:** Area plot of photosynthetic rate trends over 24 weeks for selected treatments.

### Comprehensive Interpretation

Pearson's correlation between bulb diameter and Pn was  $r = 0.87$  ( $P < 0.001$ ), confirming that photosynthetic vigour during early growth directly predicts final bulb size. This correlation offers a practical screening tool: a single mid-season gas-exchange measurement could flag underperforming plots in commercial tulip fields, allowing targeted corrective action.

### Discussion

The synergistic growth response to combined Azotobacter + PSB mirrors findings in wheat<sup>[13]</sup> and chickpea<sup>[14]</sup>, where dual inoculation enhanced both N and P availability simultaneously, creating a balanced nutrient supply that single organisms cannot provide. The 27 percent gain in bulb diameter is agronomically meaningful because bulb size governs both forcing quality and market price — Dutch

auctions discount undersized bulbs steeply<sup>[15]</sup>.

Photosynthetic gains likely resulted from higher leaf chlorophyll content driven by improved N nutrition, since Azotobacter supplies both ammonium and indole-3-acetic acid to the root zone<sup>[16]</sup>. The PSB component would then ensure adequate P for ATP regeneration in the Calvin cycle, removing a second bottleneck. That both Pn and gs were elevated under combined treatment suggests stomatal limitation was partially relieved, perhaps because better-hydrated roots under the biofertilizer-modified rhizosphere maintained turgor signals.

The volatile profile enrichment is a novel finding for tulip. Linalool and geraniol are terpenoid pathway products; their increased abundance may reflect higher substrate availability (GPP, FPP) when N and P nutrition is optimised<sup>[17]</sup>. For the fragrance-flower market, this translates into a perceptible improvement in scent quality without any

chemical input.

Seed quality improvements are consistent with the source-sink theory: larger bulbs accumulate more starch reserves, which then support more vigorous embryo development in the subsequent generation<sup>[18]</sup>. The vigour index gain of 30 percent in the combined treatment could shorten nursery cycles by one season if confirmed under field conditions. Deficit irrigation at 60% FC did not appear to negate biofertilizer benefits, which is encouraging for water-scarce environments like the Ob Basin.

### Conclusion

Combined inoculation with *Azotobacter chroococcum* and a PSB consortium proved the most effective biofertilizer strategy for tulip under deficit irrigation. Bulb diameter, photosynthetic rate, and volatile fragrance compounds were all highest in the dual-inoculant treatment, and seed vigour index improved by 30 percent over the control. The strong correlation between photosynthetic capacity and bulb size provides a practical, non-destructive indicator for field monitoring. GC-MS results showed that biofertilizers not only increase yield but also improve sensory quality by enriching floral volatiles. These outcomes support the adoption of combined biofertilizer inoculants as a cost-effective, environmentally sound input for tulip production in water-limited continental basins.

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#### Contributions Not Qualifying for Authorship

Mr. A. Rahman assisted with GC-MS sample preparation and Ms. N. Ismail contributed to statistical analysis.

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