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Lalrise Vita Improves Performance of French Marigolds Grown at pH 7.0 and Fertilized with Calcium Phosphate

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Abstract. **Calcium phosphate [$Ca_3(PO_4)_2$] is an insoluble form of phosphate that is not bioavailable for plant uptake. Phosphate-solubilizing bacteria can improve phosphorus (P) nutrition by solubilizing insoluble compounds such as $Ca_3(PO_4)_2$. French marigolds (*Tagetes patula* 'Durango Yellow') were grown in a peat-based substrate adjusted to pH 7.0, and P was provided weekly as $Ca_3(PO_4)_2$. Marigolds were treated weekly with Lalrise Vita or its active ingredient (*Bacillus velezensis*) for 5 weeks. Plant growth and health were measured weekly using digital phenotyping. Lalrise Vita– and *B. velezensis*–treated marigolds showed greater shoot digital biomass than untreated controls. Lalrise Vita– and *B. velezensis*–treated marigolds were healthier than untreated controls because they showed a low normalized pigment chlorophyll ratio index, plant senescence reflectance index, and proportion of red coloration on the canopy. The beneficial effect provided by Lalrise Vita was greater than the pure *B. velezensis*, which could be attributed to the additional components of the commercial biostimulant formulation.**

Phosphorus (P) is an essential nutrient taken up by plants as orthophosphate ($H_2PO_4^-$ and HPO_4^{-2}). Phosphate bioavailability depends on substrate pH. At a high pH, phosphate can be precipitated as calcium phosphate [$Ca_3(PO_4)_2$]. Phosphate-solubilizing bacteria (PSB) can solubilize insoluble compounds such as $Ca_3(PO_4)_2$, thereby increasing phosphate availability for plant uptake (Rawat et al. 2021). Bacterial phosphate solubilization mechanisms include production of organic acids, siderophores, or exopolysaccharides (De Zutter et al. 2022; Rawat et al. 2021).

Lalrise Vita (Lallemand, Milwaukee, WI, USA) is a commercial microbial-based biostimulant, and its active ingredient is *Bacillus velezensis*, a phosphate-solubilizing bacterium. Other *B. velezensis* strains have been reported to solubilize phosphate and increase crop yield (Afzal et al. 2023). Unfortunately, the lack of consistent biostimulant efficacy has limited the adoption of these technologies by growers (De Zutter et al. 2022).

PSB are reported to help plants grow in soil fertilized with rock phosphate or $Ca_3(PO_4)_2$ (Santos-Torres et al. 2021; Sharon et al. 2016). We evaluated the efficacy of *B. velezensis* in soilless culture. To accomplish this goal, we evaluated the performance of marigolds grown in a peat-based substrate adjusted to pH 7.0 that were fertilized with $Ca_3(PO_4)_2$ as their only source of P and were inoculated with the biostimulant Lalrise Vita or the strain *B. velezensis*.

Materials and Methods

French marigold (*Tagetes patula* 'Durango Yellow') seeds were double-sown into 6.5-cm^2 pots filled with a peat-based substrate (80% peat:20% perlite at pH 7). After 7 d, seedlings were thinned to one per pot.

A fertilizer solution devoid of P was prepared at 100 mg·L^{-1} nitrogen (N) from a 15N–0P–12.5K–2.9Ca–1.2Mg fertilizer (JR Peters Inc., Allentown, PA, USA). At 7 d, the fertilizer solution was supplemented with monopotassium phosphate (20 mg·L^{-1} P), and each plant received 25-mL aliquots of the solution. Monopotassium phosphate application happened only once during the experiment. Starting at 14 d, insoluble $Ca_3(PO_4)_2$ was supplemented into the fertilizer solution to deliver 160 mg·L^{-1} P weekly. Each plant received 25-mL aliquots of $Ca_3(PO_4)_2$-supplemented solution. All other days, plants were irrigated with fertilizer solution without P.

Lalrise Vita is a wettable powder that contains *B. velezensis* [2×10^9 colony-forming units (cfu) per gram] and was applied at a rate of 2 g·L^{-1} in ultrapure water. *Bacillus velezensis* was isolated directly from the product and was grown from individual colonies in 25 mL Luria Bertani media. The culture was incubated for 16 h at 28 °C and 200 rpm agitation. After incubation, the culture was centrifuged at 3000 g_n for 5 min at room temperature. The supernatant was discarded, and bacteria cells were resuspended in ultrapure water to an optical density at 595 nm (OD_{595}) of 0.1 (~5×10^6 cfu·mL^{-1}). Lalrise Vita and *B. velezensis* were applied as a 20-mL drench weekly starting 15 d after sowing. Untreated plants were drenched with 20 mL ultrapure water.

Plants were phenotyped weekly using the TraitFinder greenhouse phenotyping system (Phenospex, SG Heerlen, Netherlands). Digital biomass, hue angle, normalized pigment chlorophyll ratio index (NPCI), and plant senescence reflectance index (PSRI) were analyzed to evaluate plant performance. To exclude the influence of flowers in the NPCI and PSRI,

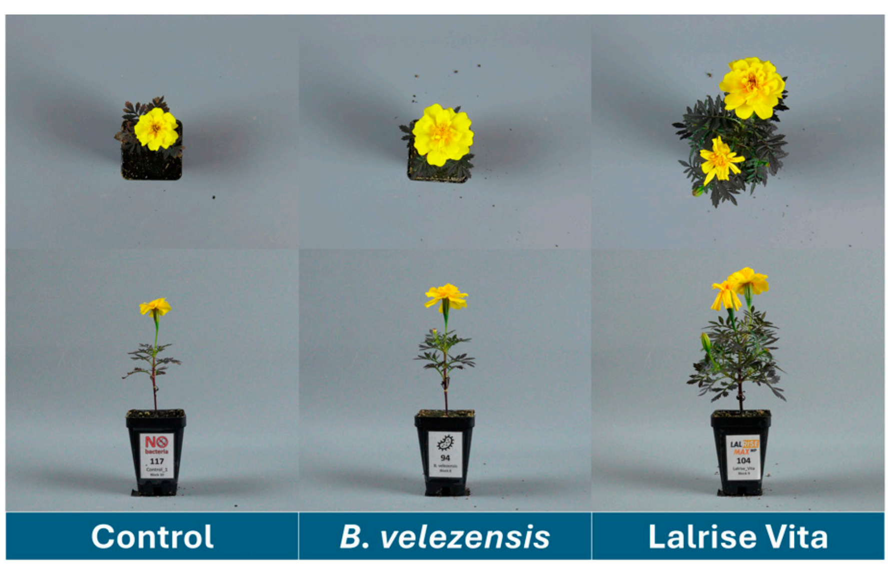

Fig. 1. French marigolds 'Durango Yellow', untreated or inoculated with phosphate-solubilizing bacteria, were grown in a peat-based substrate (pH 7.0); 4 mg phosphorus was applied weekly as calcium phosphate. Photos are 6 weeks after the initial inoculation.

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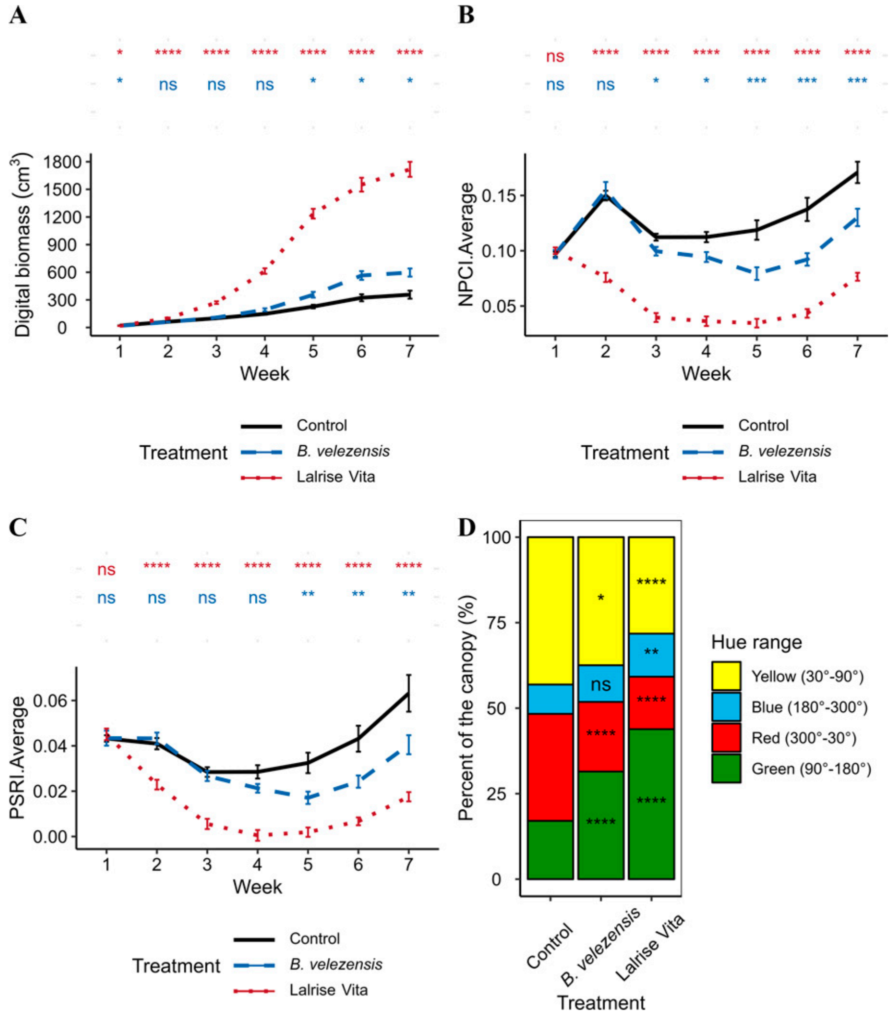

Fig. 2. Digital phenotyping [digital biomass (**A**), normalized pigment chlorophyll ratio index (NPCI) (**B**), plant senescence reflectance index (PSRI) (**C**), and canopy hue (**D**)] of French marigold 'Durango Yellow' untreated or inoculated with phosphate-solubilizing bacteria. Marigolds were grown in a peat-based substrate (pH 7.0), and 4 mg phosphorus was applied weekly as calcium phosphate. The line plots show means and standard errors (n = 15). The stacked bar plot represents the average proportion of the canopy classified into each hue range. The treatments were compared with the negative control using Dunnett's test. ns, *, **, ***, **** Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, or 0.0001, respectively.

the data were filtered using saturation (< 60%) (Quijia Pillajo et al. 2023). Hue angle was analyzed from scans taken after flowers were removed manually at the end of the experiment. Hue data are presented as the proportion of the canopy falling within hue ranges representing green (90°–180°), red (300°–30°), yellow (30°–90°), and blue (90°–180°).

The experiment was organized in a randomized complete block design (n = 15). Statistical analyses and data visualization were conducted using R ver.4.3.1 (R Foundation for Statistical Computing, Vienna, Austria). One-way analysis of variance was conducted according to the following model: Y = Block + Treatment. Residuals were tested for normality and homoskedasticity. The significance level was set at $\alpha = 0.05$. Treatments were compared against the control using Dunnett's test.

Results and Discussion

Bioavailability of phosphate is reduced at basic substrate pH because it can be precipitated with calcium. Phosphate deficiency symptoms include reduced growth and leaf purpling (de Bang et al. 2021). In marigolds receiving $Ca_3(PO_4)_2$ as the only P source, Lalrise Vita and *B. velezensis* promoted growth (Figs. 1 and 2A). Moreover, growth promotion observed in Lalrise Vita–treated marigolds occurred earlier and was larger than the effect observed in *B. velezensis*–treated marigolds (Figs. 1 and 2A).

TraitFinder measures the amount of red, green, blue, and near-infrared light reflected by the canopy, and light reflectance data are used to calculate vegetation indices commonly used to evaluate plant health. We used the NPCI, PSRI, and canopy hue angle to evaluate marigold health. The NPCI is a vegetation index that correlates negatively with chlorophyll (Peñuelas et al. 1994); the PSRI is an index used to measure senescence (Merzlyak et al. 1999). Healthy foliage usually shows NPCI and PSRI values closer to zero (Bazhenov et al. 2023). The NPCI and PSRI values increase in plants under abiotic stress (Merzlyak et al. 1999; Peñuelas et al. 1994). Accordingly, we observed that untreated plants showed greater NPCI and PSRI values than plants treated with Lalrise Vita or *B. velezensis* (Fig. 2B and C). Therefore, nutritional stress caused by the insoluble $Ca_3(PO_4)_2$ and high substrate pH was greater in untreated plants. The leaves of French marigold 'Durango Yellow' turn purple in response to P deficiency. Untreated marigolds showed a greater proportion of red canopy and a lesser proportion of green canopy than plants treated with Lalrise Vita or *B. velezensis* (Fig. 2D), indicating that treated plants had less P deficiency. A greater proportion of yellow canopy in untreated plants is indicative of early senescence, another symptom of P deficiency (Mengel and Kirkby 2001) (Fig. 2D).

The Lalrise Vita product outperformed the *B. velezensis* (pure culture) application. Further evaluations are needed to assess whether Lalrise Vita–treated marigolds perform like optimally grown marigolds. The formulation of commercial products aims to enhance longevity and performance of microorganisms in microbial-based biostimulants. The observed performance of Lalrise Vita could be explained by the additional ingredients in its formulation that enhance *B. velezensis* survival and establishment or by differences in the concentrations of the applied bacteria. Our results highlight the potential of PSB as a tool to improve P nutrition, and the importance of effective product formulation to boost the efficacy of beneficial microorganisms in the field or greenhouse.

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