Special Research Report #459: Use of Beneficial Microbes to Enhance Plant Growth and Improve Stress Tolerance in Ornamentals.

Post-production studies

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BACKGROUND

Most greenhouse floriculture crops are produced in containers in soilless substrates. Little is known about the beneficial microbial communities that exist within these containerized growing systems, or how to utilize these beneficial bacteria-plant interactions to improve floriculture crop quality. The rising costs of fertilizing and irrigating greenhouse crops have increased the interest in beneficial microbes (both bacteria and fungi) that can improve water and nutrient use efficiency, at the same time reducing leaching and potential environmental contamination. Plant growth promoting microbes improve plant growth and resilience, resulting in the production of a higher quality crop that is also more tolerant of the environmental stresses that plants may encounter during shipping and retailing. These postproduction stresses are most commonly caused by water and nutrient deficient conditions.

Plant growth promoting bacteria, which colonize plant roots, include many taxonomic groups with diverse plant hosts. In these beneficial plant-microbe interactions, the host plant secretes compounds that are used as a food source by the bacteria. In return, the bacteria stimulate plant growth and improve stress tolerance by increasing nutrient uptake and nutrient use efficiency. They can also produce hormones that modulate plant growth and stress responses. The genus *Pseudomonas* has been well studied for its ability to stimulate plant growth under drought and low-nutrient conditions, and it is a model root colonizer, making it an excellent system for studying beneficial plant-microbe interactions. The goal of this research was to screen a novel collection of *Pseudomonas* bacteria (referred to as the OSU *Pseudomonas* collection), to identify strains that promote growth and ornamental plant quality under drought and low-nutrient conditions.

MATERIALS & METHODS

The OSU *Pseudomonas* collection.

This collection includes 44 strains of bacteria in the genus *Pseudomonas*. The strains represent nine different species, and the bacteria were isolated from various soil, water and plant samples. Identities were determined by sequencing (Subedi et al., 2019). All of the bacteria were characterized through a series of laboratory screens and greenhouse trials to identify the strains that could confer growth promotion and stress tolerance to greenhouse crops (**Fig. 1**).

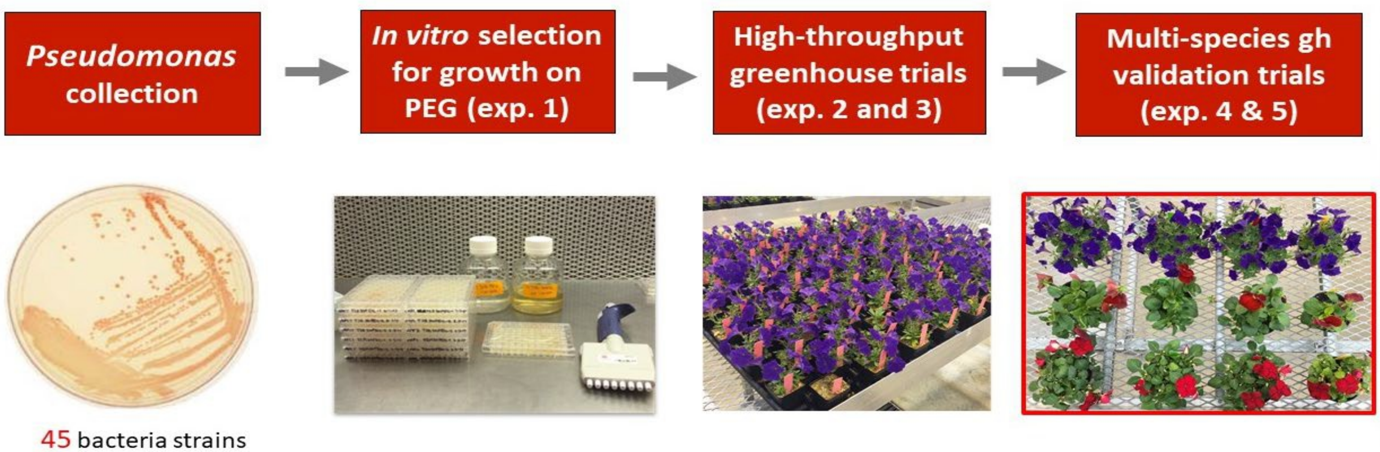
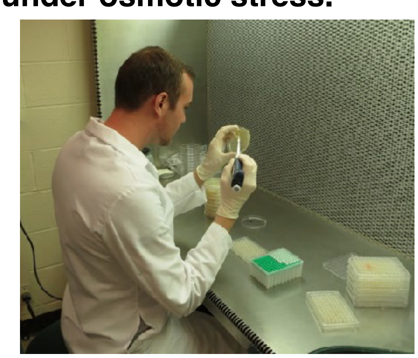


Fig. 1: Experimental pipeline. This project involved a series of experiments, beginning with laboratory screens of the 44 bacteria to identify strains that that could grow under osmotic stress (exp. 1). Ten strains were identified in the laboratory and then evaluated in high- throughput greenhouse trials where plants were subjected to drought stress (exp. 2) or grown under low-nutrient conditions (exp. 3). Lastly, a large-scale greenhouse trial (repeated in time) evaluated the top three bacteria strains in three different greenhouse crops (petunia, impatiens and pansy) subjected to drought stress (exp. 4) or grown under low-nutrient conditions (exp. 5).

Experiment 1. Identifying bacteria that can grow in the lab under osmotic stress.



- Screened for bacteria that will grow in media amended with 30% polyethylene glycol (PEG)
- PEG selects for osmoadaptive properties in bacteria
- PEG binds water molecules within liquid media, inducing osmotic stress
- Bacteria with osmoadaptive properties are more likely to confer drought tolerance to plants than those without

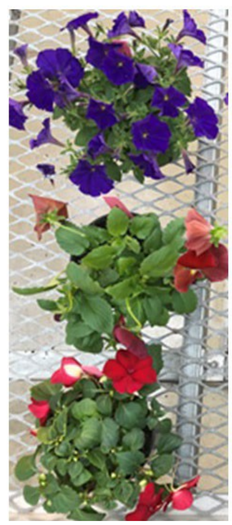
Experiment 2 & 3. High-throughput greenhouse trials to identify bacteria that promote shoot growth and flowering under drought and low-nutrient conditions.

- Evaluated 10 *Pseudomonas* strains selected from PEG assay (exp. 1)
- Used model plant Petunia 'Picobella Blue'
- 1:1 inert sand turface media in 2.5" pots
- Randomized Complete Block Design with 4 blocks and 4 replicates per block
- Bacteria cultures were diluted and drenched weekly on growing media

- **Exp. 2, Drought:** Irrigation was withheld three weeks after transplant until all plants were wilted. Irrigation and bacteria treatment were resumed and plants were grown to flowering
- **Exp. 3, Low-nutrient:** Plants were grown with 25 ppm N from 15-5-15 CaMg fertilizer
- Growth promotion under stress was assessed as shoot dry weight and flower number

Experiment 4 & 5. Multi-species greenhouse validation trials to identify bacteria that improve growth, flowering and plant quality under drought and low-nutrient conditions.

- Methods were similar to those previously described
- Evaluated the top bacteria selected from exps. 2 and 3 with three ornamental plant species grown in 4.5" pots of Pro-Mix PGX media
- Tissue nutrient analysis was conducted by the OSU STAR lab



Petunia × hybrida
'Picobella Blue'

Viola × wittrockiana
'Delta Pure Red'

Impatiens walleriana
'Super Elfin Ruby'

Pseudomonas strains:

P. fluorescens 90F12-2
P. poae 29G9

RESULTS

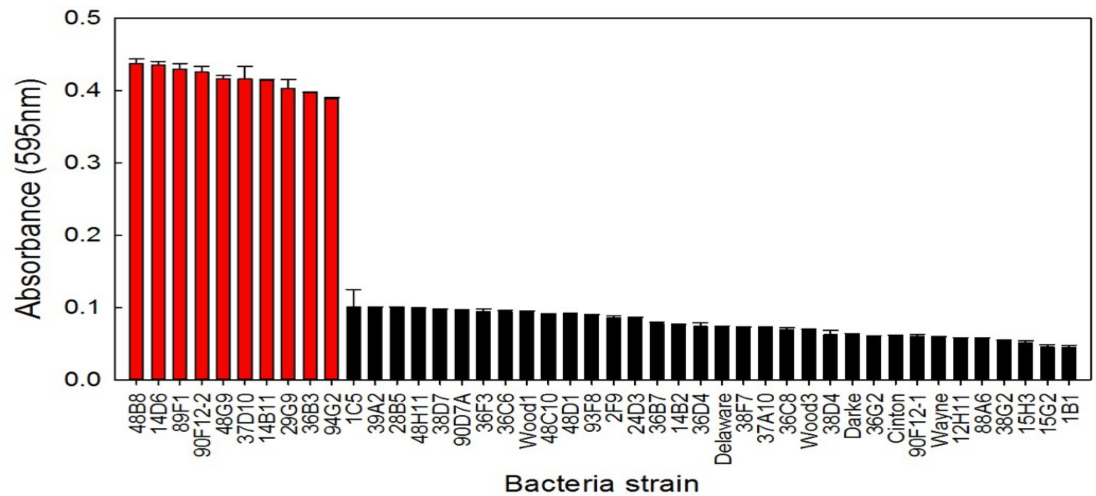


Fig. 2: Ten *Pseudomonas* strains were identified to grow under osmotic stress induced by 30% PEG.

Experiment 1. In lab assays identified osmoadaptive strains

Experiment 2. The high-throughput greenhouse trials identified seven *Pseudomonas* strains that result in greater petunia shoot biomass and flower number than untreated control plants after recovering from severe drought stress (Data not shown).

Experiment 3. The high-throughput greenhouse trials identified six *Pseudomonas* strains that result in greater petunia shoot biomass and flower number than untreated control plants when grown under low-nutrient conditions (25 ppm N from 15-5-15) (Data not shown).

Two *Pseudomonas* strains improved shoot biomass and flower number under both drought and low-nutrient stress. These two strains were used in exps. 4 & 5

Experiment 4: Multi-species greenhouse trial confirmed that two *Pseudomonas* strains improve plant recovery and growth after drought stress in a greenhouse system.

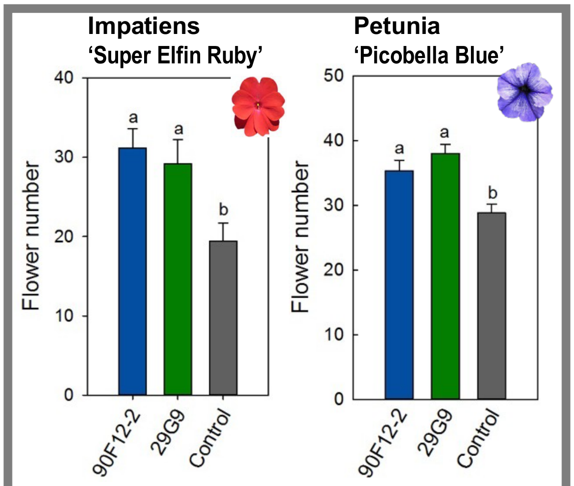


Fig. 3: Flower number was higher in impatiens and petunia plants after recovery from drought stress when treated with bacteria.

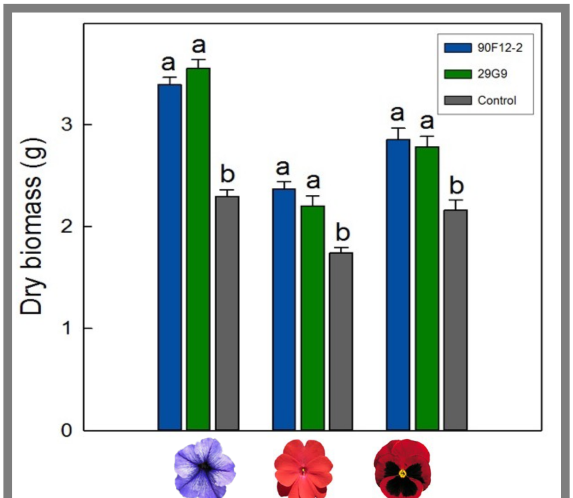


Fig. 4: Shoot growth (dry biomass) was greater in all three plant species after recovery from drought stress when treated with bacteria.

Experiment 5: Multi-species greenhouse trial confirms that two *Pseudomonas* strains improved plant growth under low-nutrient conditions in a greenhouse system.

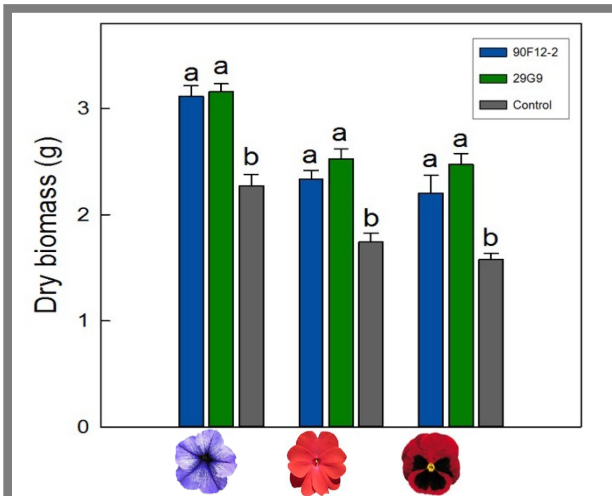


Fig. 6: Shoot growth (dry biomass) was greater in all three plant species grown under low-nutrient conditions when treated with bacteria.

Flower number was higher in impatiens grown under low-nutrient conditions when treated with *P. poae* 29G9 (data not shown).

Fig. 5: Plants were visibly of higher quality after recovery from drought stress when treated with bacteria.

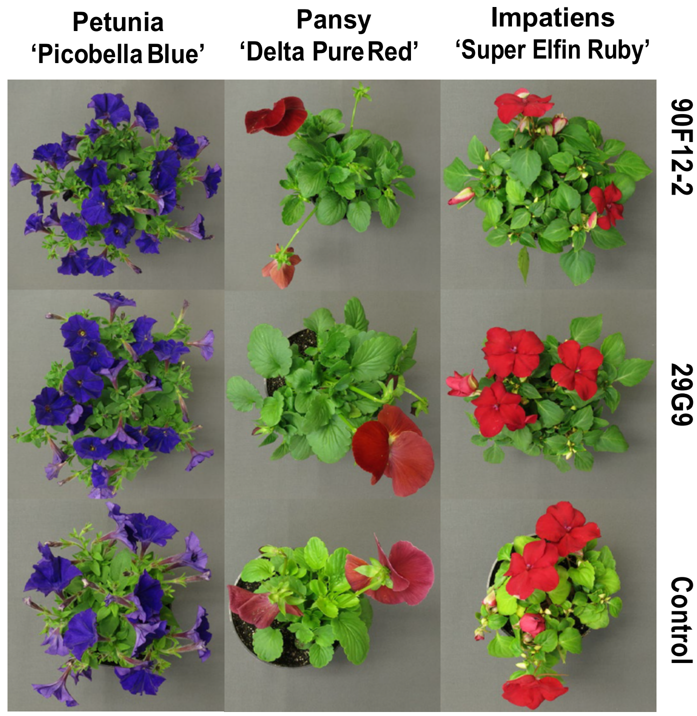


Fig. 7: Untreated impatiens and pansies were visibly chlorotic, while plants treated with bacteria were a dark green color.

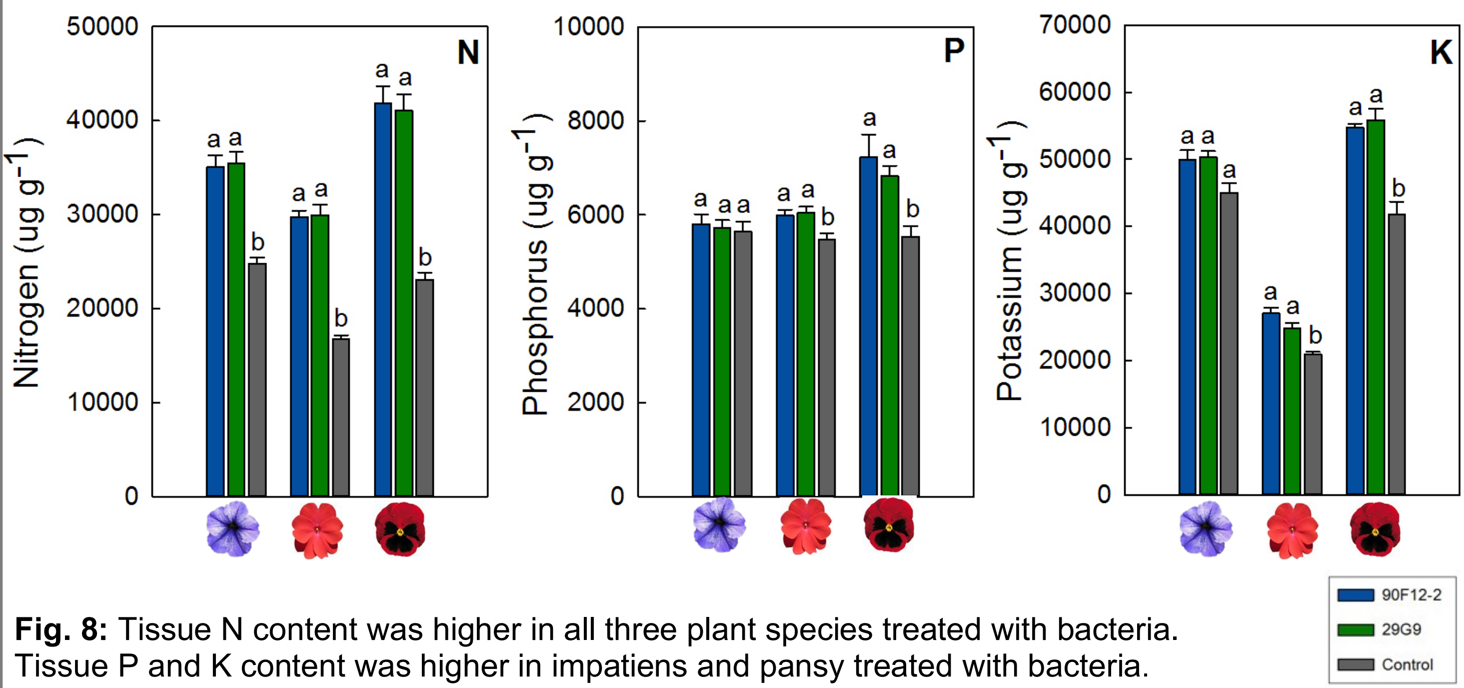


Fig. 8: Tissue N content was higher in all three plant species treated with bacteria. Tissue P and K content was higher in impatiens and pansy treated with bacteria.

CONCLUSIONS

- The lab and high-throughput greenhouse trials provided an efficient method to preliminarily evaluate bacteria for their ability to stimulate plant growth.
- This study has identified two elite bacteria strains that increase crop quality of economically-important greenhouse species subjected to different abiotic stresses.
- Application of beneficial bacteria can increase shoot biomass, flower number, and macronutrient content of plants that are grown under low-nutrient conditions.
- Application of beneficial bacteria can improve plant quality following drought stress by increasing shoot biomass, flower number and leaf greenness.

INDUSTRY IMPACT

- This research will contribute to the formulation of biostimulant products marketed for the horticulture industry.
- Microbial-based biostimulant products can be used to make existing greenhouse practices more economically and environmentally sustainable.
- Biostimulants provide a tool that growers can use to improve overall plant health and resilience.
- Beneficial bacteria can improve crop quality throughout all stages of production and marketing.
- Beneficial bacteria can reduce postharvest losses.
- High quality crops can be produced with reduced fertilizer inputs.

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