SYNTHETIC V NATURAL GROWTH PROMOTING HORMONES

The nursery industry has been using synthetic auxins such as indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) to assist in the development of adventitious roots on plant cuttings for many decades. This Nursery Paper provides an overview of research by Masters student Apriwi Zulfitri to determine if inoculation of plant cuttings with indole-3-acetic acid (IAA)-producing plant growth promoting rhizobacteria (PGPR) may be a cost-effective alternative to using synthetic auxins in the propagation of ornamental plants.

Summary

- The effect of *Azospirillum brasilense* Sp245 on N uptake could not be detected in this experiment.
- *Azospirillum brasilense* Sp245 bacteria were shown to produce the most bacterial IAA after three days incubation in the presence of tryptophan.
- This research indicates there is plant specificity in PGPR-plant interaction and different sensitivity of ornamental species to IAA.
- Commercially available peat and freeze-dried microorganism inoculants generate insufficient IAA production to stimulate root growth of *Lavandula stoechas* cuttings.

POTENTIAL BENEFITS OF IAA-PRODUCING PGPR

Vegetative propagation is used in the nursery industry to preserve essential ornamental properties such as flower colour, productivity, disease resistance and also to prevent plant variations that may result from seed propagation.

Successful ornamental cutting production relies on adventitious root growth, which is often stimulated using the synthetic plant hormone, auxin. Auxin is naturally synthesised by plants, mainly as indole-3-acetic acid (IAA), and is distributed throughout plant parts.

The nursery industry currently uses auxin products that are produced outside plants, such as synthetic indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA), to enhance cutting root growth.

Inoculating plant cuttings with IAA-producing PGPR may be a cost-effective alternative to using synthetic auxins. Inoculation of agricultural crops with rhizobacteria *Azospirillum*, one of the most well-studied IAA-producing PGPR, has been shown to improve water and mineral uptake by developing root systems.

Previous studies where carnation cuttings and chrysanthemum seedlings were inoculated with *Azospirillum* strains demonstrated significantly increased growth parameters and flower yield compared to the uninoculated and also reduced the requirement for fertiliser. These studies indicate the potential for using *Azospirillum* in the ornamental industry to reduce the use of both growth hormone and fertiliser.

FORMULATIONS AND CARRIERS

Once a PGPR-plant association is demonstrated, similar to the *Azospirillum brasilense* Sp245 and *Lavandula stoechas* (Lavender avonview) in this study, the bacterial formulation needs an effective carrier to enable commercial use.

The carrier must support the bacterial growth and survival during production and storage and effectively distribute the bacteria to the target host. In doing so, the inoculant must maintain the desirable biological, chemical and physical properties during production, be easy to manufacture, easy to handle, non-toxic, and environmentally friendly.

A highly successful example of this is the rhizobial inoculants used in legume crop production. These crop-specific bacteria formulations are available as peat, liquid or broth, freeze-dried and granular products with proven efficacy as live inoculant in the field.

Nursery & Garden Industry Australia

This project has been funded by Horticulture Innovation Australia Limited using the Australian Nursery Industry levy and funds from the Australian Government.

Horticulture Innovation Australia

THE RESEARCH

STUDY OBJECTIVES

The overall aim of the study was to evaluate the effectiveness of PGPR on the growth and development of ornamental plants of high value to the nursery industry and determine if PGPR can substitute synthetic root growth hormone applied to ornamental plants thereby reducing input costs.

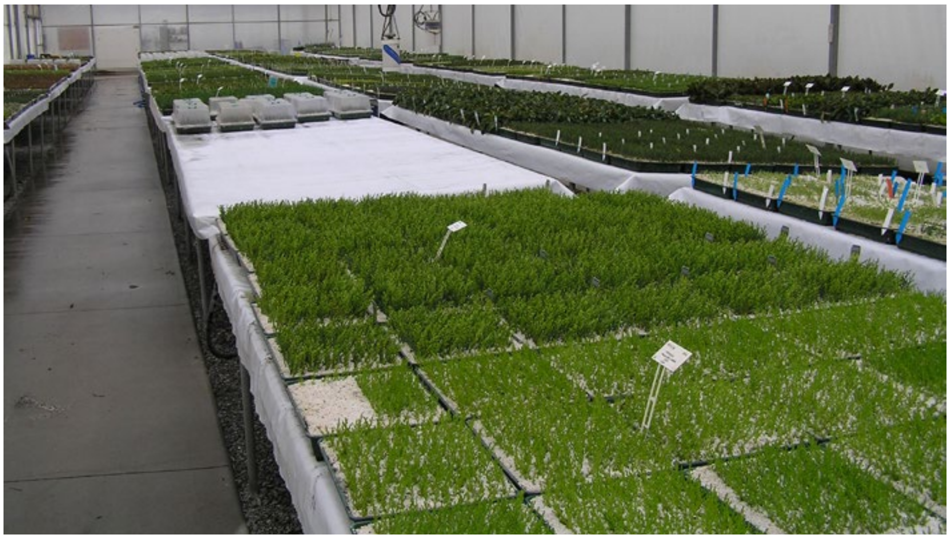
The specific aims of the project were to:

1. Determine the most effective way of growing ornamental plants to observe a PGP effect.
2. Determine the most effective ornamental plant host-PGPR interaction by screening a range of organisms and plants.
3. Measure changes in plant growth and development and nutrient composition after application of PGPR.
4. Investigate the most effective inoculation formulation to promote plant growth.

PLANT–BACTERIA SYMBIOTIC RELATIONSHIP

One of the first objectives of this study was to identify plant growth promoting rhizobacteria (PGPR) species that produce sufficient IAA hormone to initiate a beneficial effect on commonly grown ornamental plant cuttings.

The PGPR strains used in the project were selected based on their ability to produce IAA in liquid medium. The PGPR isolates tested in the study were:



- *Citrobacter freundii* 3C
- *Pseudomonas fluorescens* 1N
- *Azospirillum brasilense* Sp7
- *Azospirillum brasilense* Sp7-S
- *Azospirillum brasilense* Sp245

These PRPR where tested on cuttings from three ornamental plants:

- *Argyranthemum* sp. (Marguerite daisy)
- *Lavandula stoechas* (Lavender avonview)
- *Osteospermum* sp. (The African daisy)

The adventitious root growth in the cuttings was measured in terms of root formation, the number of main roots and root length. This initial screening experiment identified *Azospirillum brasilense* Sp245 as the most prolific IAA producer and *Lavandula stoechas* (Lavender avonview) as the most responsive ornamental plant associate.

Importance of tryptophan

As part of the screening experiment, all five PGPR cultures were assessed for their ability to produce IAA in a defined liquid medium, with and without the addition of tryptophan, the biochemical precursor of auxin (including IAA) production in bacteria.

In the presence of tryptophan, all strains significantly increased their production of IAA compared with the same strains grown in the absence of tryptophan. The IAA production was found to be up to ten times higher in the presence of tryptophan indicating that tryptophan was important for IAA production of all PGPR strains. This experiment also indicated that tryptophan rapidly induces the synthesis of bacterial IAA production, especially in *A. brasilense* strains.

Best combination to test

PGPR inoculation was first trialed on pansy seedlings; however, the effects on seedling growth were not detectable, possibly due to competition between inoculated PGPR and microorganisms already present in the seedling potting media.

Once *A. brasilense* Sp245 was chosen as the best potential inoculant due to its ability to produce the highest amount of IAA in the liquid medium, cuttings of the three ornamental species—*Argyranthemum* sp. (Marguerite daisy), *Lavandula stoechas* (Lavender avonview) and Osteospermum sp. (The African daisy) were inoculated.

Inoculation of *L. stoechas* cuttings with *A. brasilense* Sp245 resulted in positive responses such as adventitious root formation, high number of Sp245 cell recovery from the cuttings and no endemic *Azospirillum* populations. Accordingly, this pairing was used in further experiments to evaluate PGPR effectiveness in ornamental plant cutting propagation techniques and to compare their effectiveness to that of commercially available root growth regulators.

The main study

The aims of the experiments in this study were to investigate the growth promoting effects of *A. brasilense* Sp245 on adventitious root growth of *L. stoechas* cuttings in different plant growth media and to determine if inoculation can stimulate nutrient uptake efficiency. The efficacy of different inoculant formulations containing *A. brasilense* Sp245 was also tested and compared with a commercially available biofertiliser, TwinN.

Treatments compared were:

- Sterile water
- 0.003% IAA
- Commercial plant rooting hormone (IBA) Rootex-L (Bass laboratories, Australia) contains 0.4% indole-3-butyric acid (IBA)
- Supernatant of Sp245 grown without tryptophan
- Supernatant of Sp245 grown with tryptophan
- Culture of Sp245 grown without tryptophan
- Culture of Sp245 grown with tryptophan
- Peat culture of Sp245
- Commercial biofertiliser (TwinN)

Cutting were immersed in the inoculant treatments for six hours then grown in sand medium. The experiment was replicated in water medium to allow observations of root growth.

There was a positive relationship between IAA concentration in immersion solution and root growth parameters of *L. stoechas* cuttings. In general, root development was better when cuttings were grown in sand than in water, however, growth parameters increased with increased IAA concentration in both growth media.

In sand medium, IAA concentration could explain 62% of the variation in root formation, 81% of the variation in the number of main roots and 79% of variation in root length. In comparison, IAA concentration accounted for 79% of variation in root formation, 58% of variation in number of roots and 64% of root length in water. This indicates that treatment with IAA consistently affects root formation and subsequent growth of roots (number and length) is affected by plant growth medium. Propagation of cuttings in water may reduce root growth responses to IAA compared with sand because of dilution.

Influence on nitrogen status of *L. stoechas* shoots

The final experiment was conducted to determine the growth and nitrogen (N) uptake of *L. stoechas* shoots after treated cuttings were transferred from sand to potting media.

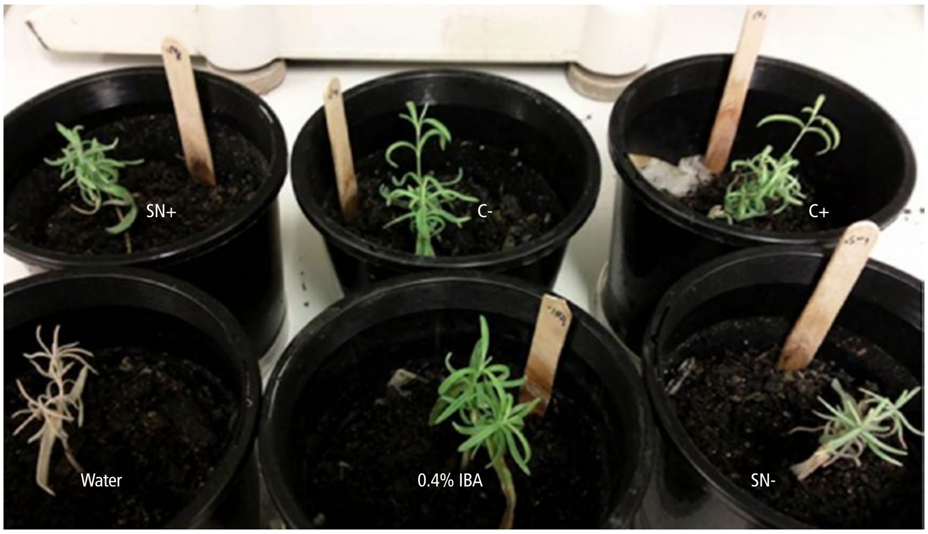
Cuttings were treated with the previously described immersion solutions including water, commercial rooting hormone, supernatant of *A. brasilense* Sp245 (with and without tryptophan) and culture of *A. brasilense* Sp245 (with and without tryptophan). Peat culture of *A. brasilense* Sp245 and commercial biofertiliser were not included in this experiment as their effects on root development were less significant than broth cultures of *A. brasilense* Sp245 grown with tryptophan.

Sand-grown cuttings were transferred to potting mix after 30 days to investigate the survival of cuttings that had developed roots and the N uptake of the new plants. The cuttings were harvested after 30 days of growth in potting mix and the shoots were dried, then ground for determination of the N content.

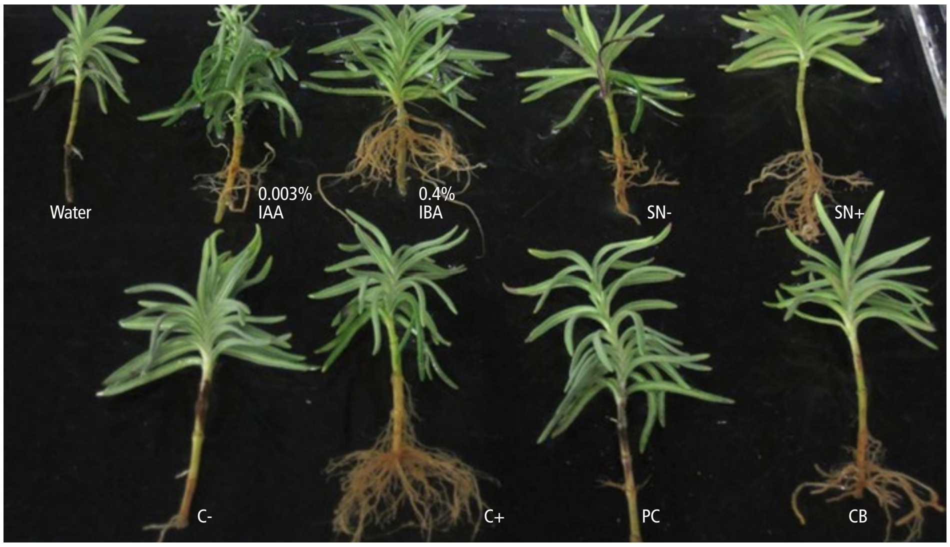
The differences in the root abundance and appearance of the adventitious roots due to treatments before transfer to potting mix were similar to that observed in the earlier experiment to assess root development as a result of the different treatments. The cuttings treated with commercial rooting hormone had the most abundant roots, followed by supernatant and culture of *A. brasilense* Sp245 grown with tryptophan. The lowest root growth resulted from the water treatment.

After 30 days further growth in potting media, the shoots of water treated cuttings were dry whereas cuttings from other treatments displayed better vigour and survival. Cuttings treated with commercial rooting hormone had the healthiest overall appearance. Cuttings treated with *A. brasilense* Sp245 cultures and supernatant containing tryptophan were similar to each other in appearance and cuttings treated with supernatant from cultures without tryptophan were the least healthy.

Of all the treatments, cuttings dipped in commercial rooting hormone had the highest N content in the shoots; however, there was no significant effect that could be attributed to the immersion solutions. The researcher suggests a longer growth period before harvesting may have provided better information regarding the influence of *A. brasilense* Sp245 inoculation on nitrogen uptake.



L. stoechas cuttings at 30 days after transfer to potting media. SN: Supernatant of Sp245, C: culture of Sp245. With/without tryptophan (-/+).



The difference of root abundance and appearance of *L. stoechas* cuttings at 30 days after being immersed with various solutions. SN: supernatant of Sp245, C: culture of Sp245, PC: peat culture of Sp245, CB: commercial biofertiliser. Notion -/+ means without/with tryptophan.

POTENTIAL FOR NEW PRODUCT DEVELOPMENT

In general, root development of cuttings treated with *A. brasilense* Sp245 cultures was not significantly different compared to cuttings treated with commercial rooting hormone.

There was a general increase in root formation, number of main roots and total length of adventitious root over the water control in both sand and water medium in response to *A. brasilense* Sp245 cultures grown with tryptophan treatment. The increase of root growth parameters positively correlated to increasing IAA concentration in the immersion solutions.

The presence of high levels of IAA, together with *A. brasilense* Sp245 cells in the immersion solution, is essential to obtain maximum effects of PGPR inoculation on adventitious root growth of *L. stoechas* cuttings. Commercially available inoculant technology such as peat and freeze-dried microorganism cultures are not likely to be effective as stimulators of root growth of *L. stoechas* cuttings because of the low IAA production in these formulations. Therefore, new formulations of *A. brasilense* Sp245 would need to be developed that stimulate IAA production as well as satisfy other criteria essential for the practical application of PGPR microbial inoculants.

IMPLICATIONS OF THE FINDINGS FOR THE NURSERY INDUSTRY

This research project has demonstrated through a single plant–bacteria combination that plant growth promoting rhizobacteria (PGPR) can be an effective replacement for synthetic hormone preparations used to propagate cuttings in the ornamental plant nursery industry.

For PGPR to be a practical alternative, the nursery industry will rely on the identification of other beneficial plant–bacteria partners and the development of suitable carrier technologies. The beneficial nature of such relationships has been exploited in many other agricultural industries, perhaps the most notable being the legume–rhizobia symbiotic relationships that greatly reduce the nitrogen requirement of 'infected' legume plants.

In addition to nutrient management properties, PGPR also have the untapped potential to assist with disease management and water use efficiency.

The differences in adventitious root morphology and appearance of L. *stoechas* cuttings at 30 days were most obvious when observed between commercial rooting hormone and *A. brasilense* Sp245 culture with tryptophan treatments. Commercial rooting hormone treatment stimulated high number of but short main roots whereas *A. brasilense* Sp245 culture with tryptophan cuttings developed fewer but longer and more branched roots.

LINKS TO RESOURCES

The efficacy of IAA-producing PGPR in different formulations as plant growth regulators by Apriwi Zulfitri, Faculty of Agriculture and Environment, The University of Sydney.

Nursery paper Sept 2015 – Roots, Hormones and in-between - Back to Fundamentals *[http://www.ngia.com.au/Attachment?Action=Download&Attachment_id=1902]*

'Nursery Environmental & Technical Research, Development and Extension 11/12' project (NY11000) *http://www.ngia.com.au/Attachment?Action=Download&Attachment_id=349*

This research topic was funded as part of the 'Nursery Environmental & Technical Research, Development and Extension 11/12' project (NY11000) funded by Horticulture Innovation Australia Limited using the Australian Nursery Industry levy and funds from the Australian Government, and was undertaken by Masters student Apriwi Zulfitri.
The study investigated the efficacy of IAA-producing PGPR in different formulations as plant growth regulators.