Malaysian Journal of Microbiology, Vol 8(1) 2012, pp. 47-50
http://dx.doi.org/10.21161/mjm.33711

SHORT COMMUNICATION

Variable responses on early development of shallot (*Allium ascalonicum*) and mustard (*Brassica juncea*) plants to *Bacillus cereus* inoculation

Zakry Fitri Abdul Aziz[1,3]*, Halimi Mohd Saud[2], Khairuddin Abdul Rahim[4] and Osumanu Haruna Ahmed[3]
[1]Laboratory of Plantation Crops, Institute of Tropical Agriculture,
[2]Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[3]Department of Crop Science, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus Sarawak, 97008 Bintulu, Sarawak, Malaysia.
[4]Agrotechnology and Biosciences Division, Malaysian Nuclear Agency, Bangi, 43000 Kajang, Selangor, Malaysia.
E-mail: zakry@btu.upm.edu.my

Received 20 June 2011; received in revised form 31 October 2011; accepted 31 October 2011

ABSTRACT

Aim: Auxin, a phytohormone secreted by plant growth-promoting rhizobacteria is one of the direct mechanisms vital for plant growth promotion. A laboratory experiment was conducted to observe the effect of IAA-producing and non-IAA-producing diazotroph *Bacillus cereus* strains on early growth of shallot (*Allium ascalonicum*) and mustard (*Brassica juncea*) plants.
Methodology and Results: Treatments evaluated were as follows: Control = uninoculated, no inoculation, UPMLH1 = IAA-producing *B. cereus* UPMLH1, and UPMLH24 = non-IAA-producing *B. cereus* UPMLH24. Inoculation with IAA-producing *B. cereus* UPMLH1 significantly increased shallot adventitious roots (root number and length) and shoot growth (19 to 54% increment). Inoculation of non-IAA-producing *B. cereus* UPMLH24 did not significantly improve growth of adventitious roots of shallot as compared to uninoculated control, except its shoot (up to 40% increase). However, primary roots and shoot growth of mustard plants significantly increased through inoculation with IAA-producing and non-IAA-producing strains (14 to 73% increment).
Conclusion, Significance and Impact of Study: The results indicated that exogenous IAA secreted by *B. cereus* UPMLH1 might have play an important role in inducing roots of shallot bulbs and it may have a variable promotional effect depending on plant species.

Keywords: Indole-3-acetic acid, inoculation, mustard, plant growth-promoting rhizobacteria, shallot

INTRODUCTION

Auxin, a phytohormone auxin production is one of the several beneficial mechanisms of several bacterial species associated with plant rhizosphere. These bacterial species are commonly known as plant growth promoting rhizobacteria (PGPR) and belong to the genera *Azospirillum*, *Bacillus*, *Burkholderia*, and *Pseudomonas* (Vessey, 2003). The plant growth promoting effects of PGPR are mainly derived from morphological and physiological changes of the inoculated plant roots and their functions, and the enhancement of water and mineral uptake (Vessey, 2003).

In recent years, IAA-producing diazotrophic PGPR have been highlighted because of their versatility in fixing N_2 biologically and produce phytohormone indole-3 acetic acid (IAA) in promoting plant growth (Pedraza *et al*., 2004). However, other plant growth-promoting mechanism such as siderophore production, biocontrol agent, and phosphate solubilisation could possibly be part of the process (Dobbelaere *et al*., 2003). These beneficial rhizobacteria have drawn attention for manipulation probably because of their adaptability to stress and adverse environment (Vessey, 2003). The ability of diazotrophic PGPR to secrete IAA appears to be widespread and has been confirmed in several genera including *Azospirillum*, *Burkholderia*, *Herbaspirillum*, and *Pseudomonas* (Pedraza *et al*., 2004). IAA-producing diazotrophic PGPR may also benefit host plants in a multiple ways including improved nutrient cycling or accumulation by increased root surface and elongation or potentially delay pathogenic spread.

B. cereus is able to form endospores that enable it to survive for extended periods of time under adverse environmental conditions (Jensen *et al*., 2003). This is one of the potential characteristics which can be exploited as the most suitable and consistent PGPR in the field. Vessey and Buss (2002) reported that *B. cereus* strain UW85 had little to no positive effects on pea and common bean symbioses and they also suggested that the UW85 inoculation indirectly increased nodulation by increasing root growth but not stimulating the nodulation process per se. Zhao *et al*. (2010) reported that *B. cereus* inoculation could enhance tanshinone production and root growth of

***Corresponding author**

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Salvia miltiorrhiza. According to Hassen and Labuschagne (2010), *B*. *cereus* (KFP9-F) inoculation resulted in significant increase in root and shoot fresh weight, root dry weight and total root length in tomatoes. They also suggested that indoleacetic acid production, phosphate solubilisation and siderophore secretion were possible mechanisms by which the bacterial isolate KFP9-F enhanced plant growth. Based on our knowledge, little is known of the mechanisms involved in plant growth promotion by *B. cereus* strains. The objective of the present study was to observe the effect of IAA-producing and non-IAA-producing *B*. *cereus* strains on early growth of shallot (*A*. *ascalonicum*) and mustard (*B*. *juncea*) plants.

MATERIALS AND METHODS

Two bacterial strains used in this study, namely, UPMLH1 and UPMLH24 were isolated from the rhizosphere of *Piper nigrum* L. and were characterised as PGPR from their beneficial capacity to biologically fix N_2 (UPMLH1 and UPMLH24) and secrete IAA (UPMLH1) under *in vitro* conditions (Zakry *et al*., 2010). The present study classified UPMLH1 as IAA-producing diazotrophic strain and UPMLH24 as non-IAA-producing diazotrophic strain. These bacteria were identified based on 16S rRNA sequence analysis and both belong to *B*. *cereus* (unpublished data). 16S rRNA sequences of *B. cereus* strain UPMLH1 and *B. cereus* UPMLH24 have been deposited to the GenBank with accession number HQ876003 and HQ876004, respectively.

Shallot bulbs and seeds of mustard (*B*. *juncea*) were used as test plants to evaluate the effect of *B. cereus* strain UPMLH1 and UPMLH24 inoculation on root formation and shoot growth. Fresh shallot bulbs were surface-sterilised with 70% ethanol for 30 s, 10% sodium hypochlorite for 30 min, and then thoroughly rinsed five times with sterile distilled water. Surface-sterilised shallot bulbs were dipped into broth culture of the respective diazotrophic PGPR isolates (> 10^9 CFU/mL broth) for five min. The inoculated bulbs were placed in sterilised white sand and kept moist. In the case of the uninoculated control, sterilised inoculum was used. Treatments were arranged in a completely randomised design with seven replicates. Data on root number, root length, shoot height, fresh weight (root and shoot) and dry weight (root and shoot) were recorded at 14 days after inoculation.

Seeds of mustard (*B. juncea*) were surface-sterilised as mentioned above. Surface-sterilised mustard seeds were bacterised with more than 10^9 CFU/mL in for 30 min. Germination tests were carried out by the paper towel method (ISTA, 1993). Twenty seeds for each treatment with five replicates were evaluated in a completely randomized design under laboratory condition at 28±1 °C. After seven days, the number of germinated seeds, root length and shoot height of individual seedling were measured and recorded. Vigour index of individual seedlings was calculated based on the following formula (Abdul Baki and Anderson, 1973):

$$\text{Vigour index} = (\text{mean root length} + \text{mean shot height}) \times \text{\% seed germination}$$

Data on root number, root length, shoot height, root fresh and dry weight, shoot fresh and dry weight of shallot bulbs, and also percentage of germination, primary root length, shoot height and vigour index of mustard seedlings were subjected to analysis of variance (ANOVA) to detect treatment differences. Duncan's New Multiple Range Test (DNMRT) at a probability level 0.05 was used to separate treatment means when the ANOVA indicated a significant effect of the treatments. Statistical analysis was performed using SAS Software (SAS Institute, 1998).

RESULTS

Inoculation of *B. cereus* strain UPMLH1 significantly increased (p<0.05) adventitious root number and length by 52% and 19%, respectively, and shoot height (54% increase) of shallot bulbs over the uninoculated controls. Inoculation with PGPR strain UPMLH1 also significantly increased (p<0.05) adventitious root (114% increase) and shoot (57% increase) fresh weight of shallot bulbs, as compared to the uninoculated controls. Concomitantly, adventitious root (48% increase) and shoot (68% increase) dry weight improved significantly after inoculation with *B. cereus* UPMLH1 as compared to uninoculated controls. Shallot adventitious root number, root length, and also root and shoot fresh and dry weight were not significantly (p>0.05) affected by the inoculation of *B. cereus* strain UPMLH24, except for shoot height which increased significantly (p<0.05) to 21.75 cm (40% increase) as compared to uninoculated controls at 15.53 cm (Table 1).

The percentage germination of the mustard seeds showed significant differences after inoculation with *B. cereus* strain UPMLH1 and UPMLH24, with UPMLH24 having the highest effect (97.5%). UPMLH1 inoculation significantly enhanced (p<0.05) root length and shoot height of mustard seedlings (6.07 cm i.e. 73% increase) and (3.77 cm, i.e. 15% increase), respectively. Likewise, UPMLH24 inoculation significantly increased (p<0.05) root length (5.71 cm, i.e. 63% increase) and shoot height (3.71 cm, i.e. 14% increase) of mustard seedlings as compared to uninoculated controls. Overall performance of inoculation on early growth of mustard seedlings was interpreted based on vigour indexes. Inoculation with *B. cereus* UPMLH24 improved vigour index at the highest level of 920 followed by *B. cereus* UPMLH1 (853) and uninoculated controls (510) (Table 2).

DISCUSSION

The present study was conducted with the aim of observing the effect of IAA-producing and non-IAA-producing *B. cereus* strains on early growth of shallot (*A. ascalonicum*) and mustard (*B. juncea*) plants. Additionally, the study also aimed to gather more evidence on plant growth-promoting potential by PGPR and to properly understand the mechanism involved, i.e. IAA production. Based on a previous study, diazotroph *B. cereus* strain

ISSN (print): 1823-8262, ISSN (online): 2231-7538

UPMLH1 which was originally isolated from the rhizosphere of *P. nigrum* L., has the ability to produce IAA *in vitro*. However, *B. cereus* UPMLH24 has no such ability (Zakry *et al*., 2010).

Data on growth performance of shallot bulbs show that IAA-producing diazotroph *B. cereus* UPMLH1 significantly improved the overall early growth

Table 1: Effect of inoculation with two PGPR *B*. *cereus* strains, UPMLH1, and UPMLH24 on early growth of shallot (*A*. *ascalonicum*) bulbs.

PGPR strain	Root number	Root length (cm)	Shoot height (cm)	Fresh weight (g)		Dry weight (g)	
				Root	Shoot	Root	Shoot
Control	35.14^{b}	6.44^{b}	15.53^{c}	0.238^{b}	2.624^{b}	0.023^{b}	0.250^{b}
UPMLH1	53.43^{a}	7.63^{a}	23.90^{a}	0.581^{a}	4.116^{a}	0.034^{a}	0.419^{a}
UPMLH24	33.14^{b}	7.00^{ab}	21.75^{ab}	0.211^{b}	3.059^{b}	0.022^{b}	0.267^{b}

Different letters within the same column indicate significant differences according to Duncan's New Multiple Range Test (p<0.05).

Table 2: Effect of inoculation with two PGPR *Bacillus cereus* strains, UPMLH1, and UPMLH24 on early growth of mustard (*Brassica juncea*) seedling.

PGPR strain	Germination (%)	Root length (cm)	Shoot height (cm)	Vigour index
Control	75.0^{c}	3.51^{b}	3.27^{b}	510^{b}
UPMLH1	86.7^{b}	6.07^{a}	3.77^{a}	853^{a}
UPMLH24	97.5^{a}	5.71^{a}	3.71^{a}	920^{a}

Different letters within the same column indicate significant differences according to Duncan's New Multiple Range Test (p<0.05).

performance of the test plants. This could be due to the effect of indole-3-acetic acid released by the strain. According to Volkmar and Bremer (1998), PGPR inoculation can promote density and length of root hairs, increase root surface area, and consequently improve plant uptake potential of water and nutrients. These improvements were related to the capacity of the bacteria to produce auxin IAA (Vessey, 2003). Adventitious root formation arises from any part of the plant other than normal root development. Several plants have the ability of adventitious root formation and but not on certain plants, especially woody plants (Koyuncu and Balta, 2004). However, no significant difference was found in shallot bulbs inoculated with non-IAA-producing *B. cereus* UPMLH24 as compared to the uninoculated controls. Its ability to fix N_2 was unable to promote the early growth of shallot bulbs. Therefore, this observation indicates that the exogenous bacterial IAA plays an important role in promoting adventitious root growth and shoot in shallot bulbs. It is known that exogenous IAA is needed to induce adventitious rooting (Rahman *et al*., 2003) and high amount of exogenous IAA may be needed by shallot bulbs because of existing rooting cells at the base of bulb, out of which a part may be not viable or may have dead cells or tissue. The absence of exogenous IAA could delay the emergence of roots.

Data from early growth performance of mustard seedlings show that there were significant differences among the treatments. Both strains increased seedling vigour index with non-IAA-producing strain of UPMLH24 emerging the best. Inoculation with IAA-producing strain UPMLH1 was slightly lower than non-IAA-producing strain UPMLH24, especially at seed germination. This could be due to the effect of substantial auxin IAA produced by the IAA-producing strain UPMLH1. IAA does not affect the germination process directly, although, IAA can stimulate ethylene biosynthesis (Yoshii and Imaseki, 1981). Ethylene can enhance seed germination; thus, small increase in ethylene levels could change the ratio between ethylene and gibberellin. Changes in the ratio between ethylene and gibberellin can affect the germination process negatively (Bleecker *et al*., 1988; Karssen *et al*., 1989). Non-IAA-producing diazotroph *B. cereus* UPMLH24 associated with the mustard seeds, which stimulated germination and improvement of seedling health could be due to N_2 fixation activity. As discussed earlier, the findings of this study suggest that exogenous IAA do not directly affect germination process, but could negatively affect this process. Thus, only seed germination is affected by endogenous IAA (Slavov *et al*., 2004). This observation is consistent with that of Gholami *et al*. (2009) who also reported that PGPR inoculation improved seed germination and seedling vigour of maize. In conclusion, IAA-producing diazotroph *B. cereus* strain UPMLH1 played an important role in adventitious rooting of shallot bulbs. Non-IAA-producing diazotroph *B. cereus* UPMLH24 is effective in germinating the seeds. Both PGPR *B. cereus* strains, UPMLH1 and UPMLH24, may be useful in the stimulation of seed germination and seedling growth. Therefore, the present study suggests the use of both strains as biostimulants in planting material production in organic nursery.

ACKNOWLEDGEMENTS

We wish to thank the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus Sarawak for research facilities and the Malaysian Pepper Board for financial support.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

REFERENCES

Abdul Baki, A. A., and Anderson, J. D. (1973). Vigour determination in soybean seed by multiple criteria. *Crop Science* **13, 630-633.**

Bleecker, A. B., Estelle, M. A., Somerville, C., and Kende, H. (1988). Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. *Science* **241, 108-1089.**

Dobbelaere, S., Vanderleyden, J., and Okon, Y. (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. *Critical Reviews in Plant Sciences* **22, 107-149.**

Gholami, A., Shahsavani, S., and Nezarat, S. (2009). The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *World Academy of Science*, *Engineering and Technology* **49, 19-24.**

Hassen, A. I., and Labuschagne, N. (2010). Root colonization and growth enhancement in wheat and tomato by rhizobacteria isolated from the rhizoplane of grasses. *World Journal of Microbiology and Biotechnology* **26, 1837-1846.**

ISTA. (1993). Proceedings of the International Seed Testing Association, International Rules for Seed Testing. *Seed Science and Technology* **21, 25-30.**

Jensen, G. B., Hansen, B. M., Eilenberg, J., and Mahillon, J. (2003). The hidden lifestyles of *Bacillus cereus* and relatives. *Environmental Microbiology* **5, 631-640.**

Karssen, C. M., Zagórski, S., Kepczynski, J., and Groot, S. P. C. (1989). Key role for endogenous gibberellins in the control of seed germination. *Annals of Botany* **63, 71-80.**

Koyuncu, F., and Balta, F. (2004). Adventitious root formation in leaf-bud cuttings of tea (*Camellia sinensis* L.). *Pakistan Journal of Botany* **36, 763-768.**

Pedraza, R. O., Ramirez, A., Xiqui, M. L., and Baca, B. E. (2004). Aromatic amino acid aminotransferase activity and indole-3-acetic acid production by associative nitrogen-fixing bacteria. *FEMS Microbiology Letters* **233, 15-21.**

Rahman, M. M., Amin, M. N., Azad, M. A. K., Begum, F., and Karim, M. Z. (2003). *In vitro* rapid regeneration of plantlets from leaf explant of native-olive (*Elaeocarpus robustus* Roxb.). *Online Journal of Biological Sciences* **3, 718-725.**

Slavov, S., van Onckelen, H., Batchvarova, R., Atanassov, A., and Prinsen, E. (2004). IAA production during germination of *Orobanche* spp. seeds. *Journal of Plant Physiology* **161, 847-853.**

Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* **255, 571-586.**

Vessey, J. K, and Buss, T. J. (2002). *Bacillus cereus* UW85 inoculation effects on growth, nodulation, and N accumulation in grain legumes: Controlled-environment studies. *Canadian Journal of Plant Science* **82, 283-290.**

Volkmar, K. M., and Bremer, E. (1998). Effects of seed inoculation with strain of *Pseudomonas fluorescens* on root growth and activity of wheat in well-watered and drought-stressed glass-fronted rhizotrons. *Canadian Journal of Plant Science* **78, 545-551.**

Yoshii, H., and Imaseki, H. (1981). Biosynthesis of auxin-induced ethylene. Effects of indole-3-acetic acid, benzyladenine and abscisic acid on endogenous levels of 1-aminocyclopropane-l-carboxylic acid (ACC) and ACC synthase. *Plant and Cell Physiology* **22, 369-392.**

Zakry, F. A. A., Halimi, M. S., Abdul Rahim, K. B., Osumanu, H. A., Wong, S. K., Franklin, R. K., Stephen, L. C. T., and Make, J. (2010). Isolation and plant growth-promoting properties of rhizobacterial diazotrophs from pepper vine (*Piper nigrum* L.). *Malaysian Applied Biology* **39, 41-45.**

Zhao, J. L., Zhou, L. G., and Wu, J. Y. (2010). Promotion of *Salvia miltiorrhiza* hairy root growth and tanshinone production by polysaccharide-protein fractions of plant growth-promoting rhizobacterium *Bacillus cereus*. *Process Biochemistry* **45, 1517-1522.**

ISSN (print): 1823-8262, ISSN (online): 2231-7538