

Short communication

Seed and Seedling Vigour of Winged Bean (*Psophocarpus tetragonolobus*) after Single and Dual Inoculation with *Rhizobium* sp. and *Bacillus cereus*

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The present study was conducted to evaluate the effect of single and dual inoculation of *Bacillus cereus* strain UPMLH24 and *Rhizobium* sp. strain AM2 on seed germination and seedling vigour of winged beans (*Psophocarpus tetragonolobus*). Treatments were as follows: Uninoculated control, *Rhizobium* sp. AM2, *Bacillus cereus* UPMLH24, and a combination of *Rhizobium* sp. AM2 and *B. cereus* UPMLH24. Present study found that inoculation with *B. cereus* alone and its combination had significantly increased ($p < 0.05$) root length, shoot length and vigour index of winged bean seedlings as compared to uninoculated control. However, present study indicated that single inoculation with *B. cereus* UPMLH24 was significantly higher than *Rhizobium* sp. AM2 + *Bacillus cereus* UPMLH24 inoculum in all parameters. In conclusion, single inoculation with *Bacillus cereus* UPMLH24 or in combination with *Rhizobium* sp. AM2 have the potential to enhance seed germination and seedling vigour of winged beans and might be suitable to be formulated as a biostimulant.

Key words: Combined inoculation, *Psophocarpus tetragonolobus*, rhizobacteria, single inoculation, winged bean

In recent years, several studies have been conducted to investigate the effectiveness of combined application of rhizobacteria on growth performance of several agricultural crops (Mathivanan *et al.*, 2014; Nadeem *et al.*, 2014; Xun *et al.*, 2015). The combined application of effective and beneficial rhizobacteria have seen as one of the promising approaches to improve plant productivity. It might be due to multifunctional plant growth-promoting properties that may be present as mixed inoculum. Winged bean (*Psophocarpus tetragonolobus*) is a potential source of protein

for the tropics and almost equivalent to protein content of soybean (Gross, 1983). However, information on winged bean-rhizobacteria association is still limited, thus allow more study should be conducted to unravel the association and their possible mechanisms. Moreover, the dormancy of seed of winged bean is hard to break due hard seed coat that acts as barrier to water uptake, gas exchange, or loss of chemical inhibitors (Finkelstein, 2010). Various methods have been employed to improve the seed germination rate of winged bean, including seed inoculation with

rhizobacterial inoculum (Kazaz et al., 2013; Mia et al., 2014). In the present study, *Rhizobium* sp. AM2 and *Bacillus cereus* UPMLH24 inocula were tested on seed germination and seedling vigour of winged bean (*Psophocarpus tetragonolobus*).

Pure cultures of *Rhizobium* sp. AM2 and *Bacillus cereus* UPMLH24 (GenBank accession number: HQ876004) were obtained from culture collection of Laboratory of Microbiology and Plant Pathology of Department of Crop Science, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Sarawak, Malaysia. Both strains were previously isolated from root nodule of *Acacia mangium* (unpublished data) and rhizosphere of *Piper nigrum* (Zakry et al., 2010), respectively. *Rhizobium* sp. AM2 was cultured and maintained on yeast extract mannitol agar medium while *Bacillus cereus* was cultured on tryptic soy agar medium. In the inoculum production, a loopful of pure culture from respective fresh agar medium was transferred to 100 ml of yeast extract mannitol broth for *Rhizobium* sp. AM2 and tryptic soy broth for *Bacillus cereus* UPMLH24. All the broth-filled conical flasks were shaken at 120 rpm for up to 48 hours under ambient temperature.

Winged bean seeds were surface sterilised with 95% alcohol for 5 minutes followed by 5.25% sodium hypochlorite for 5 minutes and then rinsed with sterile distilled

water six times. Subsequently, surface sterilised seeds were soaked in warm water (about 40°C) for 2 hours. The surface sterilised seeds were then ready for treatments. The treatments were as follows: 1) uninoculated sterilised broth serve as control, 2) *Rhizobium* sp. AM2, 3) *Bacillus cereus* UMPLH24, 4) a combination of *Rhizobium* sp. AM2 and *Bacillus cereus* UPMLH24. The sterile seeds were dipped into respective treatments for 30 minutes. Then the inoculated and uninoculated seeds were immediately sown into double-layered wet tissue in a tray and assigned in completely randomised design with four replicates. Seeds were daily monitored and sprayed with water to keep them moist.

After one week, the treated seeds with 1 cm radicle were considered germinated and the observation was recorded for percentage of germination and then allow for another one week for shoot and root proliferation. Fourteen days after inoculation, all seedlings were harvested and evaluated for root and shoot length (cm), vigour index, and number of lateral roots. All data were analysed by using analysis of variance. Means were compared by Duncan's New Multiple Range Test at 5% significance level (SAS version 9.3). Table 1 shows the effect of single and dual inoculations with *Rhizobium* sp. AM2 and *Bacillus cereus* UPMLH24 on seed germination and early growth of winged bean seedlings.

Table 1: The effect of co-inoculation of *Rhizobium* sp. AM2 and *Bacillus cereus* UPMLH24 on seed germination and early growth of winged bean seedlings

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index	No. of lateral roots
Uninoculated control	21.33c ± 1.33	11.51c ± 0.65	4.87c ± 0.34	368.5c ± 11.2	2.66c ± 0.33
<i>Rhizobium</i> sp. AM2	14.66d ± 1.33	6.14d ± 0.15	2.76d ± 0.28	129.6d ± 6.4	3.00c ± 0.51
<i>Bacillus cereus</i> UPMLH24	48.00a ± 2.31	27.99a ± 1.57	9.05a ± 0.28	1770.0a ± 21.1	10.44a ± 0.48
<i>Rhizobium</i> sp. AM2 + <i>Bacillus cereus</i> UPML24	32.00b ± 2.31	15.12b ± 1.53	6.84b ± 0.10	695.6b ± 9.0	5.22b ± 0.35

Means with the same letter(s) are statistically not significant according to Duncan's New Multiple Range Test (p>0.05).

Present study found that *B. cereus* UPMLH24 inoculation had the greatest increment ($p < 0.05$) in germination rate (48%), root length (28 cm), shoot length (9 cm), vigour index (1770) and number of lateral roots (10) of winged bean as compared to *Rhizobium* sp. AM2, combined inoculum and uninoculated control. However, seedlings inoculated with *Rhizobium* sp. AM2 was significantly inhibited ($p > 0.05$) on most of the plant parameters as compared to uninoculated control. This phenomenon possibly due to incompatibility of the *Rhizobium* cells with the host plant or the *Rhizobium* only effective in the subsequent growth stage where the root system is set to accept symbiotic relationship between *Rhizobium* and host plant. The incompatibility might produce an imbalance of phytohormone level thus delaying the seed germination and seedling emergence (Anuradha and Rao, 2001; Pudelski et al., 2001).

Based on vigour index, *B. cereus* UPMLH24 was the highest and followed by a combined inoculant. In the previous studies (Zakry et al., 2010; Aziz et al., 2012), *Bacillus cereus* UPMLH24 has demonstrated the ability to secrete phytohormone indole-3-acetic acid (IAA) and suggested that the ability might be one of the beneficial mechanisms used in stimulating plant growth. IAA has been reported as one of the potential mechanisms used by *B. cereus* UPMLH24 to stimulate the initiation of roots and its proliferation of pepper stem cuttings (Aziz et al., 2015) and also improving seed germination of mustard and mung bean (Aziz et al., 2012). However, IAA is possibly not the only phytohormone that involve in the regulatory network of seed germination and dormancy, it might be from the interaction with other phytohormone such as abscisic acid, gibberellins, brassinosteroids and ethylene (Ushahra et al., 2013). The germination rate as demonstrated in the

present study was still low, thus more opportunity for improvement in the future. Exploring for new application or reformulating the present treatment may be needed to enhance the seed germination.

In conclusion, the inoculation of *Bacillus cereus* is beneficial for winged bean at seed germination and early seedling growth stage. Further studies are needed to investigate the effect of single and dual inoculations on the subsequent plant growth and yield performance of winged bean.

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