Dual Inoculation of Bacterial on Nutrients Uptake of In Vitro Banana Plantlets

Zuraida, A.R
Marziah, M.
Shamsuddin, Z.
Halimi, M.S

ABSTRACT

A experiments were carried out to observe the effects of dual inoculation of rhizobacterial and agrobacterial on nutrients of in vitro banana plantlets using MS basal medium. Dual inoculation of rhizobacteria (Azospirillum brasilense Sp7, Bacillus sphaericus UPMB10 and Microbacterium oxydens UPMB11) and agrobacteria (Agrobacterium rhizogenes strains AR9402 and A4) showed positive response in enhancing nutrients uptake of banana plantlets which, combination of UPMB10 with AR9402 or A4 treatments was the most effective treatment. The combined inoculation has advantages over the single inoculation especially in enhancing nutrients uptake of the plantlets. The results of the study clearly showed that combined inoculation of rhizobacteria and agrobacteria is a promising technique to enhance the nutrient uptake of in vitro banana plantlets.

Keywords: *Agrobacteria, banana, co-inoculation, in vitro, PGPR, Rhizobacteria*

Introduction

Bananas and plantains are among the most important crops in the world. In terms of gross value of production, they are the fourth most important global food crop. Bananas are the fourth most important export commodity in the developing countries and ranks first as a fruit. However, banana production had decreased due to shortage of land, labour and high input costs. The high cost of agriculture inputs such as fertilizers is one of the main constraints facing the banana industry in Malaysia (Siti Hawa, 1990). Co-inoculation is based on mixed culture inoculation, combinations of microorganisms that interact synergistically, or when rhizobacteria is functioning as a 'helper' bacterium to enhance the performance of other beneficial microorganisms (Bashan & Holguin, 1997). Co-inoculation of a *Pseudomonas* sp. with *Mesorhizobium* strain (Ca181) has shown a significant increase in nodule weight and shoot biomass of *Vigna radiata*, when grown in sterilized condition (Sindhu et al., 2002). Molla et al. (2000) showed that co-inoculation of *Azospirillum* with *Bradyrhizobium* significantly promoted nodulation in soybean. According to Belimov *et al.* (1995), Barley *cv. Belogorsky* inoculated with the mixture of *Azospirillum lipoferum* 137+*Agrobacterium radiobacter* 10 was significantly taller as compared to the uninoculated control and separate inoculation. A beneficial influence on straw yield was obtained after inoculation with *Azospirillum lipoferum* 137+*Arthrobacter mysorens 7*. The aims of this study are to determine the influence of co-inocuilation of rhizobacteria sp. and agrobacteria sp. on nutrients uptake of banana plantlets.

Materials and methods

Plant Materials

Banana plantlets cv. *Berangan* established in MS solid media as described by Marziah & Roslan (1995) were used. One-month-old plantlets were cultured in 30mL modified MS liquid medium at pH 5.7 using 100mL Erlenmeyer flasks. The cultures were incubated on an orbital shaker at 80

rpm and were exposed to continuous fluorescent light at 27 °C ± 1 °C for a month. The one-month duration was sufficient for plantlets to absorb all nutrients that were available in the media. The 30mL MS liquid medium was replenish with fresh medium at two week intervals.

Bacterial Cultures

Agrobacteria rhizogenes strains AR9402 and A4 were cultured on yeast extract media. The strains were then subcultured in 100mL Erlenmeyer flask on YEB liquid medium. Rhizobacteria: *Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10, *Microbacterium oxydens* UPMB11 were cultured on Okon media. The strains were then subculture in 100mL Erlenmeyer flask on Okon liquid medium. Both cultures were shaken continuously for 48 hours (150rpm, 28° C). The optical density (O.D) of each inoculum was recorded at 600 nm (UV Spectrometer UV-1201).

Dual Inoculation of Bacterial

Banana plantlets were inoculated with 1 mL inoculum containing respective strains of *A. rhizogenes* and rhizobacteria at $1x10^7$-$1x10^8$ cfu/mL (OD_{600}) concentrations. The non-inoculated plantlets and a single inoculated (*Bacillus sphaericus* UPMB10) were used as control. Co-inoculated plantlets and the control were placed on orbital shaker (80 rpm) at 27±1 °C under continuous florescent light. The experiments was set up with 10 replicates and repeated twice. Physiological data's were recorded one month after the inoculation. The N, P, K, Ca and Mg content in the plantlets were assayed after one month of culture.

Results and Discussion

The effect of combined inoculation on essential nutrient distribution in leaves and roots of banana plantlets is shown in Table 1. There was an increase in N, P, and K uptake in UPMB10 and UPMB11 treatments either combined with agrobacterial strains AR or A4 when compared to the single inoculation or the control. The combined inoculation of UPMB10 with agrobacterial strains AR9402 or A4 promoted the nutrient uptake (N, P, K, and Mg) of the host plant compared to single inoculation. The result showed that treatment with UPMB10+AR gave the highest N (6.71 mg/gdw), P (0.821 mg/gdw) and Mg (0.32 mg/gdw) uptake compared to other treatments. However, the K uptake was the highest in UPMB10+A4 treatment at 7.21 mg/gdw. However, all the treatments did not produce a significant change on Ca uptake of plants compared to the single inoculation.

Table 1: Nutrients Content(mg/gdw) of *in vitro* Banana Plantlets after One Month Culture in MS Liquid Medium Co-inoculated with Rhizobacteria and Agrobacteria.Values are Means of Ten Replicates with ± SD (P=0.05, n=10).

	N (mg/gDW)	P (mg/gDW)	K (mg/gDW)	Ca (mg/gDW)	Mg (mg/gDW)
Contro	4.50±0.23	0.08±0.05	6.78±0.40	0.19±0.10	0.27±0.02
UPMB10	4.52±0.45	0.62±0.02	5.14±0.35	0.89±0.08	0.26±0.01
UPMB10+A4	6.07±0.11	0.80±0.09	7.21±0.23	0.79±0.11	0.31±0.02
UPMB10+AR	6.71±0.56	0.82±0.05	6.16±0.46	0.81±0.07	0.32±0.02
UPMB11+A4	5.10±1.10	0.64±0.02	5.25±0.66	0.84±0.10	0.29±0.01
UPMB 11+A4	6.70±0.44	0.57±0.04	6.06±0.45	0.81±0.03	0.29±0.05
SP7+A4	4.50±0.24	0.52±0.05	5.01±0.13	0.67±0.09	0.14±0.05
SP7+AR	4.51±0.34	0.49±0.07	4.12±0.45	0.68±0.20	0.19±0.01

Treatment with Sp7 combined either with AR or A4 failed to show a significant change in N, P, K, Ca and Mg uptake of plantlet for 30 days of culture. The enhancement of nutrient uptake by co-inoculation was probably due in part to the release of hormone from the interaction compared to single inoculation. According to Plazinski & Rolfe (1985c), a combination of *A.brasilense* and K.*pneumoniae* produced high quantities of IAA and tryptophol. Moreover only very low level of IAA was produced by *K.pneumoniae.*

Reis et al. (2000) showed that the association of *Herhaspirillum seropedicae* and *A.brasilense* with rice seedling grown in monoaxenic agar cultures could contribute up to 54 and 32% nitrogen from fixation process. The association caused a significant increase in dry weight and total N accumulation of the host plant. Earlier findings by Yahalom et al., (1991) demonstrated that treatment with *Azospirillum*, when combined with low levels of *rhizobium* (10^{-1} – 10^{-2} cfu/mL), led to enhanced nodulation in *Medicago polymorpha* relative to treatment with *rhizobium* alone. Stimulation of nodulation in legumes co-inoculated with *Azospirillum* and *Rhizobium* has been reported (Iruthayathas et al., 1983; Plazinski & Rolfe 1985a)., although there have been reports at nodulation inhibition (Plazinski & Rolfe, 1985b).

Conclusion

The experiments indicate that the co-inoculation treatment of rhizobacteria (strain UPMB10 or UPMB11) and agrobacteria (AR9402 or A4) could enhanced nutrients uptake by plantlets when compared to the single inoculation. The combined inoculation has advantages over the single inoculation especially in enhancing nitrogen uptakes. Both combination of rhizobacteria strain UPMB10 and agrobacteria strains 9402 showed the most effective among the treatments. Thus, the combination suggested to be exploited as co-inoculants for better growth of in vivo banana plant in the future experiment.

References

Bashan, Y. & Holguin, G. (1997). *Azospirillum*-plant relationship: Environmental and physiological advances (1990-1996). *Canadian Journal Microbiology, 43*, 103-121.

Marziah & Roslan. (1995). *Mengkaji regenerasi pisang (Plantain* Musa *spp.) melalui teknik* in vitro. Tesis Sarjanamuda. Serdang: Universiti Pertanian Malaysia.

Molla, A.H., Shamsuddin, Z.H., Halimi, M.S., Marziah, M. & Puteh, A.B. (2000). Potential for enhancement of root growth and nodulation of soybean co-inoculated with *Azospirillum* and *Bradyrhizobium* in laboratory systems. *Soil Biology and Biochemistry, 33*, 457-463.

Sindhu, S.S., Suneja, S., Goel, A.K., Parmar, N. & Dadarwal, K.R. (2002). Plant promoting effects pf *Pseudomonas* sp. on coinoculation with *Mesorhizobium* sp. *Cicer* strain under sterile and "wilt sick" soil condition. *Applied Soil Ecology, 19*, 57-64.

Siti Hawa, J. (1990). Characterization, evaluation and utilization of the banana germplasm in Malaysia. *Prosiding Simposium Buah-buahan Kebangsaan,* (hlm. 315-329). Serdang.

ZURAIDA, A.R., Pusat Penyelidikan Bioteknologi, Ibu Pejabat MARDI, Peti Surat 12301 Pejabat Pos Besar 50774 Kuala Lumpur. azuraida@mardi.my

MARZIAH, M., SHAMSUDDIN, Z. & HALIMI, M.S., Universiti Putra Malaysia, 43300 Serdang Selangor.