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Efficience of dual-inoculation of Arbuscular mycorrhizal fungi and Phosphate solubilizing bacteria on the growth and tuber inulin content of Jerusalem artichoke (*Helianthus tuberosus* L.)

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Abstract

Jerusalem artichoke (*Helianthus tuberosus* L.) is an important agricultural crop. Inulin contained in its tuber is considered as a functional food for its positive effects on human health such as preventing obesity, reducing serum cholesterol and so the risk of heart disease. Nowadays, cultivation of Jerusalem artichoke has been performed by applying chemical and compost. No previous report employing biofertilizer have been conducted. Therefore, the effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and phosphate solubilizing bacteria (PSB) on the growth of Jerusalem artichoke cv. JA102xJA89 were conducted in pot trial under non-sterile soil condition. Two AMF species, *Glomus multisubtensum* (GM) and *Glomus* sp.1 (G) and PSB (*Klebsiella variicola*; KV) were used as inoculums for this study comparing with applied either rock phosphate (RP) or chemical fertilizer (15-15-15). Un-inoculated plant was used as the control. The experimental design was assigned by randomized complete block design (RCBD). We found that the inoculation with G+KV+RP could improve the height, leaf area, tuber fresh weight, weight individual of tuber, inulin accumulation of tuber and total dry mass of plants. While the SPAD chlorophyll meter reading (SCMR) value and number of tuber per plant were no significantly higher than those from un-inoculated control, these finding indicates that the promotion of the growth plant depends by the synergistic effect of dual inoculation with AMF and PSB together with RP.

Keywords: Arbuscular mycorrhizal fungi; Dual-inoculation; Growth promotion; Jerusalem artichoke; Phosphate solubilizing bacteria

1. Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.), a native plant in North America, belongs to the sunflower family. Jerusalem artichoke contains inert carbohydrates in the form of inulin. Synthetically, inulin type fructans are prepared from sucrose [1] and the numbers of fructose units vary from 2 to 60, indicating a combination of oligomers and

polymers [2]. Jerusalem artichoke has many health benefits. For example, it is considered a form of soluble dietary fiber, reducing the lipid content in blood and liver in saturated fat-fed rats and as a prebiotic. Inulin played an important role in the prevention and inhibition of colorectal, colon and breast cancers [3]. Jerusalem artichoke is widely planted in farmer's groups, helping farmers to increase their revenue. Jerusalem artichoke production depends mainly on chemical fertilizers as the source of plant nutrients. This practice not only increases production costs but also causes environmental pollution. Arbuscular mycorrhizal fungi (AMF) are ecologically important because they help plants to capture nutrients such as phosphorus, potassium, nitrogen and micronutrients from the soil [4]. Mycorrhizal plants of *Miscanthus sacchariflorus* were more effective than non-mycorrhizal plants at increasing growth and chlorophyll content of leaves [5]. Most agricultural crops can perform better and are more productive when well-colonized by AMF. AMF symbiosis increases the phosphorus and micronutrient uptake and growth of their plant host [6]. In case of phosphate solubilizing bacteria (PSB), they are an integral component of the phosphorus soil cycle to convert inorganic and organic soil P [7] into their bioavailable form, facilitating its uptake by plant roots. PSB have the ability to release several organic acid including citric, oxalic, fumaric, malic, formic, lactic, succinic etc. These organic acids are able to reduce pH of the surrounding soils and contribute to the solubilization of phosphate in the rhizosphere [8]. Arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) have a positive effect on plant productivity primarily through increasing phosphate availability. Simultaneous dual inoculation of plants with AMF and PSB in phosphate deficient soils has been shown to be more effective in growth promotion than single inoculation [9]. This dual inoculation may result in better utilization of phosphate fertilizer [10]. Kim et al. reported significantly increased production of oxalic acid citric acid in the rhizosphere of tomato plants inoculated simultaneously with AMF *G. etunicatum* and the PSB *Enterobacter agglomerans* compared to the un-inoculated control [11]. The increase in yield was higher and highest N and P uptake on mung bean in the presence of PSB and inoculation with a combination of PSB and AMF [12]. The beneficial properties of those microorganisms make them good substitutes to chemical fertilizers to promote the growth of Jerusalem artichoke. Therefore, the aim of this study was to determine the effect of co-inoculation of AMF and PSB on growth promotion and inulin accumulation of Jerusalem artichoke variety JA102xJA89 in non-sterile soil under pot trial.

2. Materials and methods

Microorganisms used in this study were isolated from rhizosphere soil surrounding of Jerusalem artichoke roots and showed the best result of promoting the growth of Jerusalem artichoke in the pot trial. Additionally, *Klebsiella variicola* could produce high level of IAA (Data not shown). Seedlings of Jerusalem artichoke variety JA102xJA89 were prepared in charred rice husk and then taken to the pots for transplanting. The treatments including (1) control (non-inoculated), (2) *Glomus multisubtensum* (GM), (3) *Glomus* sp.1 (G), (4) PSB (*Klebsiella variicola*; KV), (5) GM+KV, (6) G+KV, (7) GM+KV+Rock phosphate (RP) (100 kg ir^{-1}), (8) G+KV+RP, (9) RP, (10) chemical fertilizer (15-15-15) (25 kg ir^{-1}) were arranged in randomized complete block design (RCBD) with four replications per treatment. The experiment was conducted in the pot cultures of Jerusalem artichoke in a greenhouse at Faculty of Agriculture, Khon Kaen University, Thailand. In each pot (diameter 38 cm), 20 kg natural soil was used. Initial nutrient content and properties

of the soil was estimated. The inoculant species of AMF were multiplied by pot culture technique [13] which composed of 20 spores per gram soil. Twenty grams of soil inoculum was evenly mixed with soil before being used in a pot. Inoculation of PSB was carried out by injection of 10 ml of a 48-hr-old culture containing about 10^9 CFU ml^{-1} cell suspension in plant seedling root [14]. Pots were watered as necessary. The greenhouse culture lasted for 120 days. At harvest, the plant growth parameters including SPAD chlorophyll meter reading (SCMR) was measured by SPAD 502-plus, plant height, leaf area (LA) measured by LI-3100C Area Meter, total dry biomass (root stem and leaf dry weight) were determined after drying for 3 days at 80 °C. It was also counted the number of tubers per plant and tuber fresh weight. Besides, the inulin accumulation was determined followed by the method described by Saengkanuk et al. [15]. The pour plate technique was applied for the estimation of the PSB population in the rhizosphere soil [16]. The rhizosphere soil was collected by uprooting the plants. To estimate the extent of AM colonization, plant roots were cleaned, cut into small segments (1 – 2 cm $segment^{-1}$), rinsed and cleared for at least 12 h in 10% KOH solution an heated at 90 °C for 30 min, and then stained with 0.05% trypan blue in acetic glycerine solution [17]. Moreover, Spores were extracted from the soil sample by wet sieving and centrifugation method [18]. Some physicochemical properties of the experimental soils were measured; for instance, organic matter was determined by Walkley and Black method [19]. Total N was determined using Kjeldahl method [20]. Available P was determined by molybdenum blue colorimetric method after extraction by sodium bicarbonate, and Exchangeable K was determined by ammonium acetate extraction and flame photometric method [21]. Data were analyzed using Statistic software for Windows, version 8.0. All data were subjected to analysis of variance. Comparisons of means were made by Fisher's Least Significant Difference (LSD) ($P \leq 0.05$).

3. Results and Discussion

The soil properties from experimental pot were sandy texture with a pH of 5.27, 0.39% organic matter, 160.48 mg kg^{-1} total N, 4.80 mg kg^{-1} available P, and 35.74 mg kg^{-1} exchangeable K.

The results of plant growth parameters showed that plant inoculated with G+KV+RP (T8) gave rise the best value of the height, leaf area, tuber fresh weight, weight individual of tuber which was significantly difference compared to the control. However, these plant growth parameters from T8 had no significantly difference with plant inoculated with GM+KV+RP (T7) and GM alone (T2) ($P \leq 0.05$). Tuber fresh weight and weight individual of tuber showed the greatest value in chemical fertilizer treatment (T10), but had no significantly difference between plants inoculated AMF alone (T2 and T3) and dual cultured with PSB (T5 – T8). In addition, the plant inoculated with G+KV (T6) showed the number of tuber significantly higher than the other treatments. Moreover, the plant height showed the highest value in plant inoculated with GM+KV (T5) which was higher than the control. The SCMR value of all plants inoculated with microorganisms was not significantly difference from the control (Table 1). Therefore, the growth parameters of plants inoculated with microorganisms were significantly higher than those from non-inoculated control, particularly the treatment G+KV+RP (T8) presented the best values (Table 1).

Table 1 Means for height, SPAD chlorophyll meter reading (SCMR), leave area (LA), tuber fresh weight (TFW), number of tuber (NT) and weight individual of tuber (WIT) of Jerusalem artichoke variety JA102xJA89 treated with AMF and PSB evaluated at 120 days after transplanting under pots conditions.

T	Height (cm)	SCMR	LA (cm^3)	TFW (g)	NT	WIT (g)
1	66.57 d	30.77 a	886.20 cd	44.63 d	13.23 cde	3.42 d
2	94.0 bc	31.27 a	1049.00 ab	61.60 bc	12.07 e	5.09 bc
3	92.57 bc	33.43 a	848.30 d	68.07 bc	15.90 bc	4.30 c
4	92.73 bc	30.13 a	886.30 cd	54.93 cd	17.00 ab	3.24 d
5	103.23 a	31.93 a	861.40 d	69.20 b	15.40 bcd	4.67 bc
6	92.33 bc	32.90 a	799.00 d	62.67 bc	19.23 a	3.30 d
7	97.93 ab	32.03 a	1006.70 abc	66.87 bc	12.67 de	5.27 b
8	88.40 c	30.37 a	1093.50 a	67.57 bc	14.27 bcde	4.65 bc
9	91.77 bc	32.37 a	894.50 cd	56.40 bcd	12.00 e	4.69 bc
10	96.57 c	24.87 b	920.10 bcd	93.73 a	14.23 bcde	6.20 a
% CV	**4.15**	**9.37**	**9.08**	**12.38**	**11.81**	**10.34**
F-test	**	ns	*	**	*	**

Means followed by the same letter in the same column do not differ significantly according to LSD at P≤0.05; **, Significant at P≤0.01; *, Significant at P≤0.05; ns, non-significant. T1, Control; T2, GM; T3, G; T4, KV; T5, GM+KV; T6, G+KV; T7, GM+KV+RP; T8, G+KV+RP; T9, RP; T10, chemical fertilizer.

Most treatments inoculated with GM+KV (T5), G+KV (T6), GM+KV+RP (T7) and G+KV+RP (T8) had significantly higher inulin content than un-inoculated control except single inoculation with GM (T2), G (T3), KV (T4) RP (T9) and chemical fertilizer (T10) (Fig. 1).

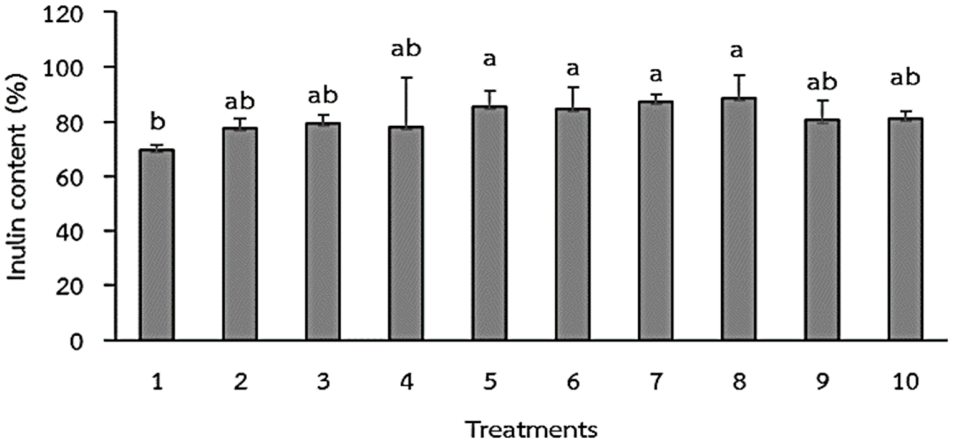

Fig. 1 Tuber inulin accumulation of Jerusalem artichoke variety JA102XJA89 inoculated with AMF and PSB cultivated in the pots at the harvesting stage. Means with the same letter are not significantly different at P≤0.05 when compared by LSD.

Treatments means are the average of four replications. G+KV+RP treatment (T8) showed a significant effect on dry mass production of plants higher than the control. Moreover, most treatments increased total dry mass of plants significantly higher than those from the non-inoculated control, except treatments inoculated with G, KV and RP alone. The maximum root (9.80 g) and leaf dry mass (9.40 g) was produced in the G+KV+RP treatment (T8) which revealed a significantly higher value in comparison with other treatments. The G+KV treatment (T6) showed better performance of stem dry mass (16.90 g) than the other (Fig. 2).

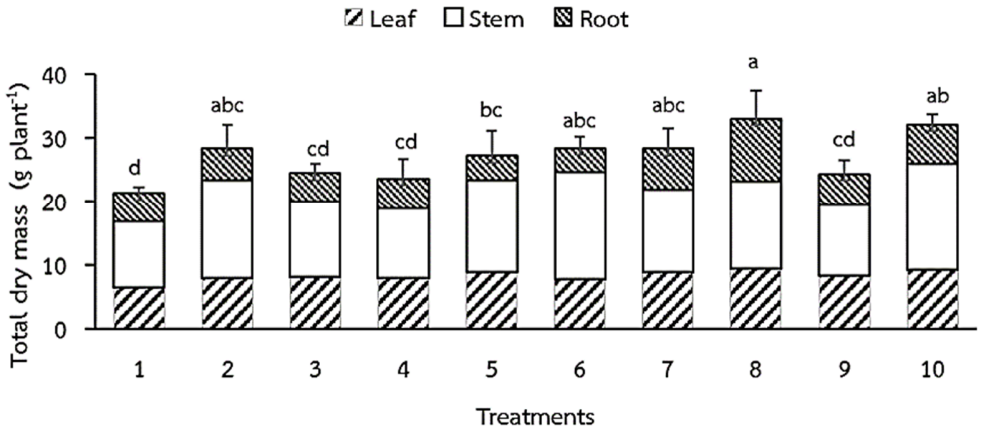

Fig. 2 Total dry mass production of plants from co-inoculation with AMF and PSB cultivated in the pots at the harvesting stage. Means with the same letter are not significantly different at P≤0.05 when compared by LSD. Treatments means are the average of four replications.

This result showed a significant increase in yields component due to inoculation with AMF and PSB with RP. This amendment includes greater of height, leaf area, tuber fresh weight, weight individual of the tuber, inulin accumulation of tuber and total dry mass of plants. The phosphorus solubilizing organisms dissolve unavailable forms of P by excreting organic acids and chelating substances [22, 23]. Toro et al. observed that when rock phosphate was added and *G. intraradices* with a PSB were co-inoculated in *Allium cepa*, the phosphorus absorbed by plants was preferentially the remaining phosphorus available from rock phosphate by the action of microorganisms [24]. The uptake of phosphate ions released from rock phosphate by the AMF mycelium, takes place in soil microhabitats where the rock particles are attacked by bacteria. This close contact would favor the persistence of the intimate relationship between PSB and AMF hyphae.

Several studies reported a synergistic effect on plant growth when specific combinations of AMF and bacteria were co-inoculated [25, 26]. Other studies with maize plants under greenhouse conditions showed that the application of *Glomus* species and bacteria, including a PSB strain of *Bacillus megaterium*, significantly increased plant growth [27]. With other plant hosts, *G. intraradices* + *B. megaterium* co-inoculation also increased plant biomass, although the combination of this bacterium with *Glomus constrictum* had the opposite effect [28].

Microscopic observations revealed that almost all root samples of plant in AMF, PSB alone and co-inoculated with AMF+PSB were root colonized by AMF significantly higher (P≤0.05) in comparison with the RP and control. The average colonization level of plants was 67 – 89% in different treatments (Fig. 3). The synergistic effect of two indigenous

PSB (*Pseudomonas fluorescens* BAM-4 and *Burkholderia cepacia* BAM-12) and arbuscular mycorrhizal (AM) fungus, *Glomus etunicatum* showed a significant increase in growth, yield and nutrient uptake of wheat plants was noticed and both strains of PSB interacted positively with AM fungus towards all growth parameters studied [29]. The population of PSB in the rhizosphere of Jerusalem artichoke was larger in the treatments which were inoculated with KV alone (T4), GM+KV+RP (T7) treatments, which were significantly higher than the control ($P \leq 0.05$) (Fig. 3). In addition, co-inoculated AMF and PSB plants (T5, T6 and T8) were taller than the un-inoculated with PSB ones (T1, T2, T3, T9 and T10) (Fig. 3).

This may have been due to high metabolic activities of PSB for a longer period in the rhizosphere of these plants due to inoculation with the AMF [30, 31]. Similarly, the number of spores of AMF in rhizosphere soil, a result showed the G alone (T3), co-inoculation with G+KV (T6), GM+KV+RP (T7) and G+KV+RP (T8) treatments were significantly higher spores of AMF than control and inoculated with RP and chemical fertilizer alone treatments ($P \leq 0.05$). So, this result indicate that dual inoculation have some synergistic effect to enhance the development of spores and root colonization of AMF and population of PSB in rhizosphere soil.

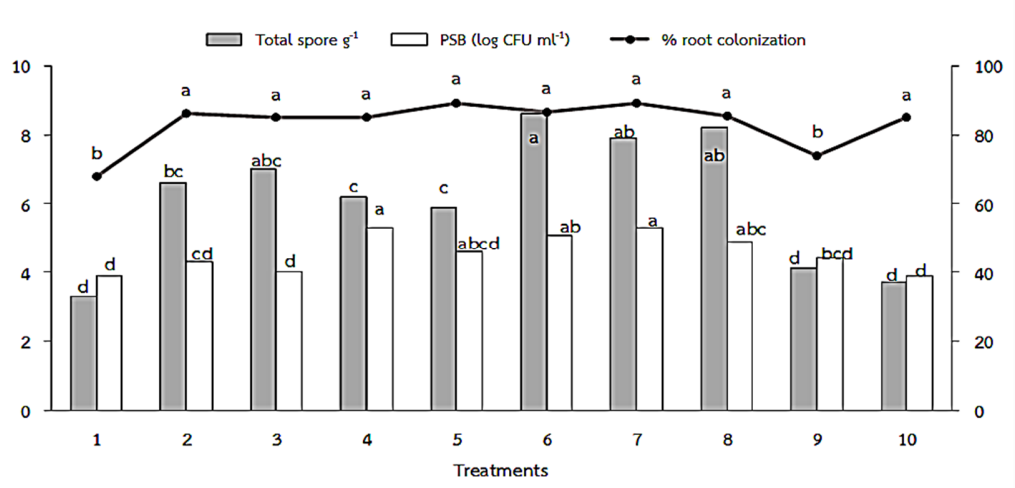

Fig. 3 Total spores and root colonization of AMF and the total count of PSB surround rhizosphere soil in the pots. Means with the same letter are not significantly different at $P \leq 0.05$ when compared by LSD. Treatments means are the average of four replications.

4. Conclusion

The present study was successful in application of plant growth promoting microorganisms for enhancement growth of Jerusalem artichoke under pots experiment. The results showed that height of plant, leaf area, tuber fresh weight, and individual weight of tubers can be improved by a combination of G+KV+RP (T8). In addition, the accumulation of tuber inulin was highly increased by dual inoculation of AMF and PSB. Whereas, some plant growth promoting microorganism treatments did not show a significant increase in plant growth and production when compared to the control, SCMR value and number of tuber. Therefore, the role of dual inoculation of AMF and PSB should be taken into consideration for the production of Jerusalem artichoke variety JA102xJA89 by farmers in Thailand.

5. Suggestions

The present study showed the great synergistic effect of AMF and PSB which was able to increase plant growth and production of Jerusalem artichoke grown on pot under the greenhouse experiment. However, their actual application are needed to further clarify under field condition.

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