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Full Length Article

Effect of Nutrient Concentration, PGPR and AMF on Plant Growth, Yield, and Nutrient Uptake of Hydroponic Lettuce

Nurul Aini[1*], Wiwin Sumiya Dwi Yamika[1] and Bachrul Ulum[2]
[1]*Department of Agronomy, Faculty of Agriculture, Brawijaya University, Malang, East Java, Indonesia*
[2]*Undergraduate Program of Agroecotechnology, Department of Agronomy, Faculty of Agriculture, Brawijaya University, Malang, East Java, Indonesia*
[*]For correspondence: nurulrulyaini@gmail.com

Abstract

This study determined the effect of three nutrient concentration level and inoculation of PGPR, AMF and consortium PGPR+AMF on plant growth, yield, and nutrient uptake in hydroponic romaine lettuce. Data were recorded for changes in plant growth, yield, nutrient uptake, and dynamics of microorganism population. The results showed that three level nutrient concentration with inoculation of AMF and/or consortium PGPR+AMF greatly changed the leaf anatomical traits, linked to increasing leaf thickness and leaf area that was positively correlated with increasing of plant fresh biomass. Three level nutrient concentration with inoculation of AMF and/or consortium PGPR+AMF also increased root colonization, and macro-nutrient uptake, but decreasing population of rhizospheric bacterial. That phenomenon was also has positively correlated with increasing of plant fresh biomass. In crux, the result of this study showed that nutrient concentration with EC 0.9–1.8 dS m^{-1} combined with inoculation of AMF and/or consortium PGPR+AMF may be recommended for production or cultivation of romaine lettuce, particularly using hydroponic substrate culture systems. Though with the consequence that reducing 50% nutrient concentration from EC value 1.8 to 0.9 dS m^{-1} take 14 days more to achieve minimum harvest fresh weight. © 2019 Friends Science Publishers

Keywords: PGPR; AMF; Microorganism dynamics; Nutrients; Lettuce

Introduction

Increasing plant production may be done through efficient and environmentally-friendly cultivation techniques. The technique of cultivation by hydroponics is one of the alternative intensified efforts that may be implemented in order to increase quality and quantity of plant products as well as efficiency in the use of land, water, and nutrients (Barbosa *et al*., 2015; Pamungkas and Yuliando, 2015). Hydroponics is a soilless plant cultivation technique that in principle supplies a solution of nutrients to plants according to their needs in a regular manner (Susila and Koerniawati, 2004).

Management of plant nutrition becomes the key factor in the success of cultivation by hydroponics. The action taken as part of this management is the regulation of nutrient concentration. The right nutrient concentration will increase the effectiveness and efficiency of nutrient absorption by plants. In addition to the regulation of nutrient concentration, inoculation of nutritive agents such as Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth Promoting Rhizobacteria (PGPR) become an alternative solution to increase efficiency of nutrient usage as well as absorption of nutrients by plants. Inoculation of AMF and PGPR is beneficial for the growth and developments of plants. The positive effects of PGPR inoculation include the provision and mobilization or facilitation of the absorption of various nutrients in soil, synthesis and changing in concentration of various growth-inducing phytohormones. Application of PGPR also suppress of pathogenic activity by the production of various compounds or metabolites such as antibiotics and siderophores (Nadeem *et al*., 2014; Katiyar *et al*., 2016; Hossain *et al*., 2017). Meanwhile, the positive effects of AMF inoculation include production of phytohormones and secondary metabolic products such as vitamins, amino acids etc. In addition, AMF also increased solubilization of minerals, increased absorption of macro and micro-essential nutrients, increased water absorption efficiency, thereby increasing resistance to environmental stresses such as drought, salinity and contamination by heavy metals; production of osmolytes; and improvement of soil structure (Nadeem *et al*., 2017).

The aims of introducing or inoculating biological agents into hydroponic cultivation (soilless culture) are to increase plant resistance toward biotic and abiotic threats, as well as to increase the absorption of macro- or micro-

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nutrients that affect plant growth and yields (Alsanius and Gertsson, 2004; Alsanius *et al*., 2004; Deniel *et al*., 2006). The aims of this research are to study the effects of nutrition solution concentration and inoculation of biological agents (AMF and PGPR) on a hydroponic substrate culture on plant growth and yields, and the absorption of primary macro-nutrients (N, P and K) of romaine lettuce plants (*Lactuca sativa* L. var. *longifolia*).

Materials and Methods

Experimental Location

The research was conducted at the Greenhouse Agrotechnopark of Brawijaya University, Jatikerto Village, Kromengan Sub-District, Malang Regency, at a height of 321 m above sea level with average yearly temperatures of 23.9℃, monthly rainfall of 133.75 mm, and relative humidity of 81.67%. The research took place from May to June of 2017. The romaine lettuce (*Lactuca sativa* L. var. *longifolia*) seedlings used for the research were of Tiberius variety. The seedlings were dispersed on rockwool medium with a size of approximately 6.25 cm^2. Transplantation was performed 21 days after sowing, or when the seedlings had developed at least 3–4 veined leaves. Polybags were filled each with one seedling and planting medium composed of a mixture of fine sand, charcoal husk, and compost; 3:1:1; estimation EC value of planting medium by PourThru Extraction is ≈ 3.1 dS m^{-1} and/or by Saturated Media Extraction is ≈ 2.2 dS m^{-1} amounting to 4537.3 cm^3 or 80% of the total polybag size (5671.625 cm^3). The romaine lettuce seedlings were cultivated using a hydroponic substrate culture and watering with drip irrigation system which have an average discharge of 3.6 L h^{-1}. The estimated water needs for the plants were based on climatology data from Karangkates Station from 2016, plant coefficient (Kc), and actual evapotranspiration (ET_0). The equation for the calculation of ET_0 utilizes that of the Penmann-Monteith method (Allen *et al*., 1998) and the scheduling of irrigation duration is based on the Savva and Frenken (2012) equation. The following are the results of estimation for plant water needs and drip irrigation duration (Table 1).

The experimental design used in the research was a Nested Design composed of two factors. The first factor (main plot) was the concentration of nutrient solution, composed of three levels: 100% ≈ 1.8 dS m^{-1}, 75% ≈ 1.4 dS m^{-1}, and 50% ≈ 0.9 dS m^{-1}. The second factor (sub-plot) is the inoculation of biological agents, composed of four levels: without inoculation/control, PGPR, AMF, and PGPR+AMF.

Hydroponics

The formulation of hydroponic nutrients was based on the nutritional needs of the plants (Resh, 2013 with modification). The needs for lettuce were (mg L^{-1}): NO_3 (165); NH_4 (25); P (50); K (210); Ca (200); Mg (40); S (111); Fe (5); Mn (0.5); Cu (0.5); Zn (0.18); B (0.26); and Mo (0.007). Calculation of chemical salt needs for nutrient formulation was performed using the Hydrobuddy 1.50 software. Calculation of chemical salts are appropriate to the needs of the plants: stock A mixture was 1074 g $5Ca(NO_3)_2.NH_4NO_3.10H_2O$, 109 g KNO_3, and 33 g Fe-EDTA, while stock B mixture was 222 g KH_2PO_4, 271 g K_2SO_4, 408 g $MgSO_4.7H_2O$, 68 g $(NH4)_2SO_4$, and 29 g Librel® BMX.

Application of the nutrient concentration treatment was done by dissolving the nutrient formulations with clean water (EC ≈ 0.38 dS m^{-1}) to obtain (concentrated) nutrient stock solutions, each with a volume of 5 liters. Dilution was then carried out by adding water to each nutrient stock solution until a dilution volume of 100 liters was obtained. The estimation of the electrical conductivity and needs for each hydroponic nutrient stock solution used for each treatment refers to Genuncio *et al.* (2012) as presented in Table 2.

Plant Growth Promoting Microorganisms

The biological agents used in this research were AMF and PGPR. Their isolates belong to the collection of the Laboratory for Plant Diseases, Department of Pests and Plant Diseases, Faculty of Agriculture, Brawijaya University. The utilized PGPR formulation is composed of a consortium of several strains of non-phytopathogenic *Azotobacter chroococcum*, *Azospirillum brasilense*, *Pseudomonas flourescens*, and *Bacillus subtilis*, bacteria as well as non-phytopathogenic *Aspergillus niger* fungus. The density or colony-forming unit (CFU) of each bacterial isolate in the PGPR formulation is 10^8 CFU mL^{-1} (optical density (OD) was adjusted to ≈ 0.6) (Roesti *et al*., 2006). Meanwhile, the utilized AMF is an isolate of *Glomus* spp. (Endomycorrhiza) and had a spore density of 5 spores g^{-1}.

Inoculation of AMF was done once during the transplanting by adding AMF granules as much as 10 g or ± 50 AMF spores (Tahat *et al*., 2008). AMF was added near the roots of the plant or in the planting hole (Musfal, 2010). PGPR inoculation was done four times, during the transplanting and 7, 14 and 21 (days after planting) DAP (Khaeruni *et al*., 2016). Inoculation during the transplanting was done by immersing the plant roots for 30 min (Pedraza *et al*., 2009). While the application at 7, 14 and 21 DAP was done by watering the planting medium with a suspended PGPR solution which had been diluted with clean water to create a suspended solution ready for inoculation with a dose of 10 mL L^{-1}. Before watering (inoculation), a pit was first created around the plant with a depth of ± 3 cm at a distance of 3 cm from the plant (Amaria and Wardiana, 2014). And then the formulated PGPR solution was

Table 1: Plant water needs and drip irrigation duration

Month	Dates	Phase	K_C	ET_C	Plant Water Needs	Frequency of Application [(1)]	Duration of Irrigation
				mm day^{-1}	ml plant^{-1}	appl. day^{-1}	minutes appl.$^{-1}$
May	11-20	Deve.	0.72	2.87	287	5[(1)]	1
May	21-31	Deve.	0.81	3.16	347	6[(2)]	1
June	1-10	Deve.	0.91	3.45	345	6[(2)]	1
June	11-20	Mid.	0.97	3.61	361	6[(2)]	1
June	21-23	Late	0.97	3.64	364	6[(2)]	1

[(1)] Frequency of drip irrigation application is continuous and of the same interval each day
[(2)] Interval of 4 h & 48 min application^{-1}
[(3)] Interval of 4 h application^{-1}

Table 2: Estimation of electrical conductivity and needs for each hydroponic nutrient stock solution

Treatment	Needs for Stock A (mL 100 L^{-1})	Needs for Stok B (mL 100 L^{-1})
K1 (100% ≈ 1.8 dS m^{-1})	500	500
K2 (75% ≈ 1.4 dS m^{-1})	375	375
K3 (50% ≈ 0.9 dS m^{-1})	250	250

poured amounting to 30 mL plant^{-1} (total bacterial density in the suspension ≈ 10^6 CFU mL^{-1}) (Kohler *et al*., 2008; Gul *et al*., 2011).

Harvesting

Harvesting was done when the plant reached the minimum fresh weight, *i.e*., 300–400 g plant^{-1}. The harvesting procedure carried out in this research comprised removal of the crown as well as the attached roots. The measured observation parameters included total fresh weight, leaf thickness with the fast approximation of leaf thickness method (Perez-Harguindeguy *et al*., 2013), leaf area estimation with the leaf area meter (LI-3100C Area Meter), population density of rhizospheric bacteria, AMF spore density, root infection by AMF, and absorption of the nutrients N, P and K.

Observation of the population density of rhizospheric bacteria was conducted using the plate count method (Olsen and Bakken, 1987). While observation of the density of mycorrhiza/AMF spores was conducted by a spore extraction method (wet sieving and decanting) (Gerdemann and Nicolson, 1963) and observation of root infection by mycorrhiza/AMF was conducted by the root staining method (Phillips and Hayman, 1970).

Analysis of the N-plant content was done by the Kjeldahl method (Kelley *et al*., 1946; Cavell, 1954), while for the P-plant content used the method of wet digestion, where the results of the plant sample destruction was measured for the contents using a colorimeter (Kelley *et al*., 1946; Cavell, 1954). Content of K-plant was analyzed using the same method as for P, which was wet digestion followed by measuring the plant sample destruction results for its contents by using a flame photometer (Cavell, 1954). Estimation of the absorption of nutrients was calculated based on the results of analysis for N, P and K contents by an equation taken from Adeli *et al*. (2005).

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA; Gomez and Gomez, 1984). Before performing ANOVA, the data were tested for normality using Shapiro-Wilk test, and homogeneity by the Bartlett test. Data, that were not normally distributed, were transformed into a logarithmic from ($\log_{x+1}$). When the combined ANOVA showed a significant difference, the Tukey's Honest Significance Difference (HSD) test was applied at a rate of 5% ($\alpha = 0.05$).

Results

Change in Plant Growth, Yield, Nutrient Uptake and Microorganism Dynamics with Nutrient Concentration, PGPR, and AMF

Growth and yield: Total fresh weight, leaf thickness, and leaf area due to the treatments of nutrient concentrations showed a significant difference for all observation periods. Nutrient concentration with EC 1.8 and 1.4 dS m^{-1} show the same results for total fresh weight, leaf thickness, and leaf area. In contrast, Nutrient concentration with EC 0.9 dS m^{-1} significantly reduced total fresh weight, leaf thickness, and leaf area. Inoculation of biological agents showed significant differences on the variables of total fresh weight and leaf thickness, but not for that of leaf area. Though there was not a significant difference, inoculation of biological agents was able to increase leaf area compared to the treatment of without inoculation/control.

Inoculation of biological agents showed significant differences on the variables of total fresh weight and leaf thickness, but not for that of leaf area. Though there was not a significant difference, inoculation of biological agents was able to increase leaf area compared to the treatment of without inoculation/control. Inoculation of PGPR, AMF, and consortium of PGPR+AMF increased the total fresh

Table 3: Effect of nutrient concentration and plant growth promoting microorganism on total fresh weight, leaf thickness, and leaf area

Treatments	Total fresh weight (g plant^{-1})		Leaf thickness (µm)		Leaf area (cm^2 plant^{-1})	
	28 DAP	42 DAP	28 DAP	42 DAP	28 DAP	42 DAP
Nutrient concentration (K)						
1,8 dS m^{-1}	390.23±37.77b	428.79±31.35b	109.68±11.87b	120.13±13.10b	2655.47±169.71b	3482.96±113.26b
1,4 dS m^{-1}	396.32±61.76b	429.52±55.07b	120.13±13.10b	131.18±24.14c	2600.53±253.93b	3300.08±222.28b
0,9 dS m^{-1}	238.64±31.25a	311.02±25.02a	115.55±15.87b	87.06±9.65a	2071.10±236.60a	3077.52±181.10a
Plant growth promoting microorganisms (A)						
Control	304.93±62.44a	358.00±53.58a	91.87±12.25a	99.26±15.11a	2338.00±402.49	3239.53±178.68
PGPR	329.77±81.90ab	384.74±67.50ab	101.49±15.85ab	110.10±22.15ab	2477.11±366.92	3313.01±227.78
AMF	363.00±98.37b	406.27±76.48b	110.44±20.88b	121.70±31.85b	2479.54±313.01	3285.43±353.37
PGPR+AMF	369.23±95.72b	410.10±71.62b	111.00±18.47b	120.11±25.49b	2474.81±326.62	3309.44±204.47

Mean ± standard deviation. Values sharing same letters differ non-significantly (P>0.05). DAP = days after planting

weight and leaf thickness compared to the treatment of without inoculation. Meanwhile, total fresh weight and leaf thickness for the treatment of PGPR inoculation showed insignificant results compared to the treatment of without inoculation/control (Table 3).

Microorganism dynamics: Inoculation of only PGPR as well as inoculation of a consortium of PGPR+AMF at all nutrient concentration levels significantly decreased the population density of rhizospheric bacteria compared to without inoculation/control. Nutrient concentration (EC 1.4 dS m^{-1}) with inoculation of consortium of PGPR+AMF showed the lowest population density of rhizospheric bacteria compared to all other treatments (Table 4).

Inoculation of only AMF as well as inoculation of a consortium of PGPR+AMF at all nutrient concentration levels significantly increased the density of AMF spores compared to without inoculation/control. Nutrient concentration (EC 1.4 dS m^{-1}) with inoculation of only AMF showed the highest density of AMF spores compared to all other treatments (Table 5). Inoculation of AMF as well as inoculation of consortium of PGPR+AMF also significantly increased the density of AMF spores compared to without inoculation. The application of different nutrient concentrations did not show significant differences in AMF root colonization (Table 6).

Nutrient uptake: Nutrient concentration (EC 1.4 dS m^{-1}) with inoculation of AMF and application all nutrient concentrations with inoculation of consortium of PGPR+AMF significantly increased nitrogen (N) uptake compared to without inoculation. The use of 1.4 dS m^{-1} $EC_{solution}$ with inoculation of AMF showed a highest N uptake compared to other treatments (Table 7). Inoculation of PGPR or AMF, and consortium of PGPR+AMF significantly increased P uptake compared to without inoculation. The application of different nutrient concentrations did not show significant differences in P uptake. Meanwhile, the application of different nutrient concentrations and inoculation of biological agents did not show significant differences in potassium uptake (Table 8).

Table 4: Effect of nutrient concentration and plant growth promoting microorganisms on rhizospheric bacteria population density

Treatments	Rhizospheric bacterial population density (10^7 CFU g^{-1}) (*)		
	1.8 dS m^{-1}	1.4 dS m^{-1}	0.9 dS m^{-1}
Control	1.39±0.08c C	0.74±0.07b A	1.07±0.08c B
PGPR	0.38±0.05b A	0.97±0.08c C	0.49±0.06b B
PGPR+CMA	0.13±0.02a A	0.09±0.02a A	0.38±0.05a B

Mean ± standard deviation. Values sharing same small letter in each column and same capital letter in each row differ non-significantly (P>0.05). (*) Data were transformed into log (x+1) for analysis

Table 5: Effect of nutrient concentration and plant growth promoting microorganisms on AMF spore density

Treatments	AMF spores density (spores 20 g^{-1})		
	1.8 dS m^{-1}	1.4 dS m^{-1}	0.9 dS m^{-1}
Control	11.00±2.20a A	12.00±2.40a A	10.00±2.00a A
AMF	29.00±5.80c A	33.00±6.60c B	28.00±5.60b A
PGPR+AMF	21.00±4.20b A	29.00±5.80b B	26.00±5.20b B

Mean ± standard deviation. Values sharing same small letter in each column and same capital letter in each row differ non-significantly (P>0.05)

Table 6: Effect of nutrient concentration and plant growth promoting microorganisms on AMF root colonization

Treatments	AMF root colonization (%)
Nutrient concentration (K)	
1,8 dS m^{-1}	38.33±19.44
1,4 dS m^{-1}	43.33±19.75
0,9 dS m^{-1}	41.67±23.23
Plant growth promoting microorganism (A)	
Control	16.67±3.84a
AMF	60.00±11.28c
PGPR+AMF	46.67±8.47b

Mean ± standard deviation. Values sharing same letters differ non-significantly (P>0.05)

Discussion

Trejo-Tellez and Gomez-Merino (2012) stated that nutrient concentration level are closely related with total amount of dissolved and EC. The Nutrient concentration with EC 1.8 dS m^{-1} and 1.4 dS m^{-1} proven to accelerate harvest time compared to 0.9 dS m^{-1}. This can be seen from the total fresh weight data which showed that upon observation at 28 DAP, the total fresh weight of romaine lettuce plants given the treatment nutrient concentration with EC 1.8 dS m^{-1} (390.23 g plant^{-1}) and 1.4 dS m^{-1} (396.32 g plant^{-1}) have achieved the minimum harvest fresh weight criteria, which is between 300–400 g plant^{-1}. Meanwhile the total fresh

Table 7: Effect of nutrient concentration and plant growth promoting microorganisms on nitrogen uptake

Treatments	Nitrogen uptake (mg plant^{-1})		
	1.8 dS m^{-1}	1.4 dS m^{-1}	0.9 dS m^{-1}
Control	61.19±17.01a A	69.36±28.73a A	54.84±18.49a A
PGPR	89.63±28.53ab A	80.46±14.60a A	80.09±33.75ab A
AMF	87.00±23.76ab A	131.74±48.04b B	78.87±37.42ab A
PGPR+AMF	104.43±35.25b A	117.49±28.16b A	91.29±27.79b A

Mean ± standard deviation. Values sharing same small letter in each column and same capital letter in each row differ non-significantly (P>0.05)

Table 8: Effect of nutrient concentration and plant growth promoting microorganism on phosphorus and potassium uptake

Treatments	Phosphorus uptake (mg plant^{-1})	Potassium uptake (mg plant^{-1})
Nutrient concentration (K)		
1,8 dS m^{-1}	32.77±5.22	146.81±41.04
1,4 dS m^{-1}	33.44±6.96	152.55±50.17
0,9 dS m^{-1}	29.12±7.38	119.09±43.75
Plant growth promoting microorganisms (A)		
Control	24.87±4.75a	117.38±38.01
PGPR	32.67±4.82b	132.86±39.38
AMF	35.16±6.93b	167.78±54.71
PGPR+AMF	34.41±5.10b	139.32±43.36

Mean ± standard deviation. Values sharing same letters differ non-significantly (P>0.05)

Table 9: Pearson correlation coefficient between each observation variable

Variable	SD	RC	N-Upt.	P-Upt.	TFW	
					28 DAP	42 DAP
SD	1	---	---	---	---	---
RC	0,951**	1	---	---	---	---
N-Upt.	0,779**	0,708**	1	---	---	---
P-Upt.	0,855**	0,798**	0,880**	1	---	---
TFW-28 DAP	0,424**	0,307	0,694**	0,692**	1	---
TFW-42 DAP	0,463**	0,337*	0,704**	0,745**	0,988**	1
LT-28 DAP	0,605**	0,487**	0,826**	0,818**	0,962**	0,971**
LT-42 DAP	0,554**	0,420*	0,790**	0,772**	0,969**	0,967**
LA-28 DAP	0,329	0,232	0,599**	0,615**	0,947**	0,921**
LA-42 DAP	0,382*	0,278	0,597**	0,652**	0,944**	0,943**

*;** Significant correlation at P < 0,05 and 0,01, respectively. N = 36; SD = AMF spores density; RC = AMF root colonization; N-Upt. = Nitrogen uptake; P-Upt. = Phosporus uptake; TFW = Total fresh weight; LT = Leaf thickness; LA = Leaf area; DAP = Days after planting

weight of romaine lettuce plants given a 0.9 dS m^{-1} (311.02 g plant^{-1}) nutrient concentration achieved the minimum harvest fresh weight criteria upon observation at 42 DAP. Result of this study indicated that three levels nutrient concentration may be recommended for production or cultivation of romaine lettuce using the hydroponic technique, though with the consequence that reducing nutrient concentration with EC 1.8 to 0.9 dS m^{-1} takes 14 days more to achieve minimum harvest fresh weight. In contrast, Karimaei *et al*. (2004), Cresswell (1991) stated that 2.2 dS m^{-1} and 2.0 dS m^{-1} were optimal EC for hydroponically grown lettuce.

Longstreth and Nobel (1980) stated that nutrient levels had an effect on several leaf properties, *e.g.*, cotton (*Gossypium hirsutum* L. var. Acala SJ-2) leaves from plants developing under nutrient stress were thinner than those developing under normal concentrations. Also, lower photochemistry or biochemistry of photosynthesis was leading under low nutrient treatments. Several leaves properties were had positively related with total plant biomass (fresh weight), especially leaf mass growth; where the leaf mass growth is the sum of mass increase for leaf area growth and leaf thickening (Weraduwage *et al*., 2015). Thick leaves would have substantially greater photosynthesis than thin leaves (Yun and Taylor, 1986), while leaf area contributes for canopy development. As the leaf area increases, a greater photosynthetic active surface area becomes available and increased growth (Kang and Iersel, 2004; Al-Tahir, 2014). Based on the result, leaf thickness and leaf area show positively correlations with total fresh weight for all observation periods, each correlation coefficient are r = 0.962 and r = 0.947 (28 DAP); r = 0.967 and r = 0.943 (42 DAP) (Table 9). If the increase in leaf thickness and leaf area goes parallel with the increase in total fresh weight, so every 1% (1 µm) leaf thickness increment would have leads to increase 2.42–4.02% of total fresh weight, while 1% (1 cm^2 plant^{-1}) increment of leaf area would have leads to increase 0.05–0.09% of total fresh weight (Fig. 1).

Inoculation of AMF or consortium PGPR+AMF as well as proper use of EC solution resulted more thicker leaves compared with control treatment, where this phenomenon also goes parallel with total fresh weight increment. Many research suggested that plant growth promoting (PGP) effect on several plant species. That caused by inoculation of AMF or consortium PGPR+AMF such as *Stevia rebaudiana* (Vafadar *et al*., 2013); *Fragaria vesca* (Roger *et al*., 2013); wheat (Pérez de Luque *et al*., 2017); *Solanum lycopersicum* var. *cerasiforme* (Candido *et al*., 2013); and many more. Developed and well-differentiated vascular tissues (transport tissues or other tissues) are more efficient on plants inoculated with only AMF. Moreover, consortium of PGPR+AMF increases of synthesis of growth-promoting plant hormones, especially cytokinin. Cytokinin is one class of plant hormones, which has the function of enhancing leaf growth. More developed and well-differentiated vascular tissues also improve the translocation and uptake of nutrients, which helps to increase plant growth and yields (Yaseen *et al*., 2018). Increasing of nutrient uptake would also increase plant growth and yield. Positive correlation coefficients between nutrient uptake (N and P) with leaf thickness and total fresh weight are shown in Table 9.

Inoculation PGPR or consortium PGPR+AMF significantly reduces the density of rhizosphere bacteria population. This maybe indicates the temporary antagonistic effects of each inoculant or their consortium to the population of indigenous rhizosphere bacteria, both pathogenic and non-pathogenic ones. The negative phenomenon of the reduction/decrease of the population of indigenous rhizosphere bacteria indicates the

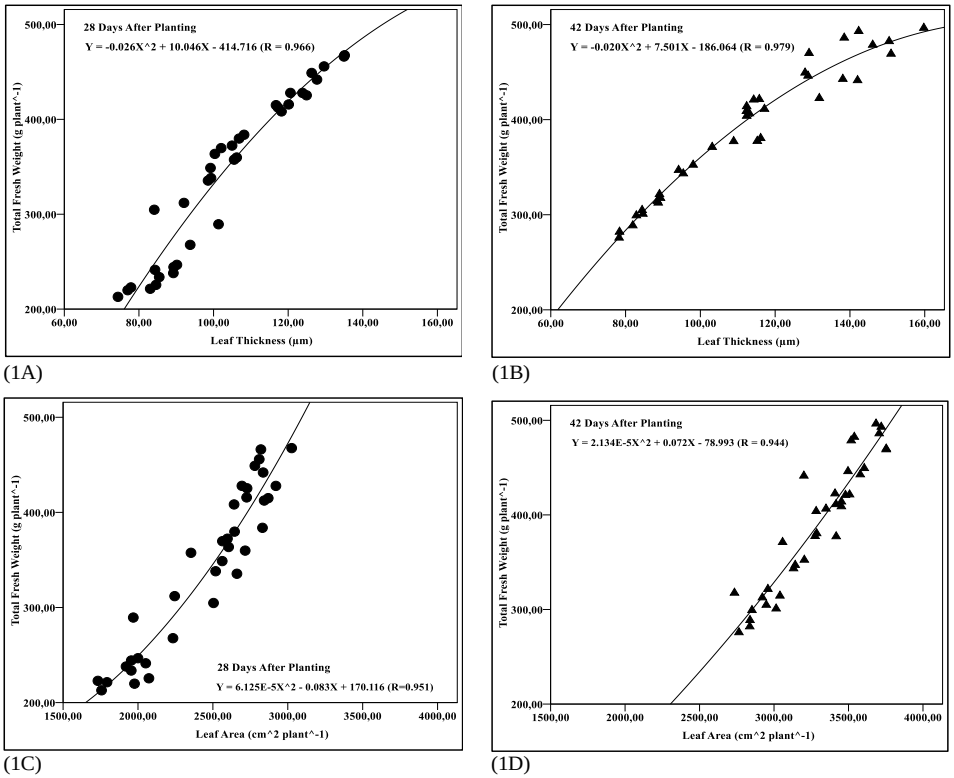
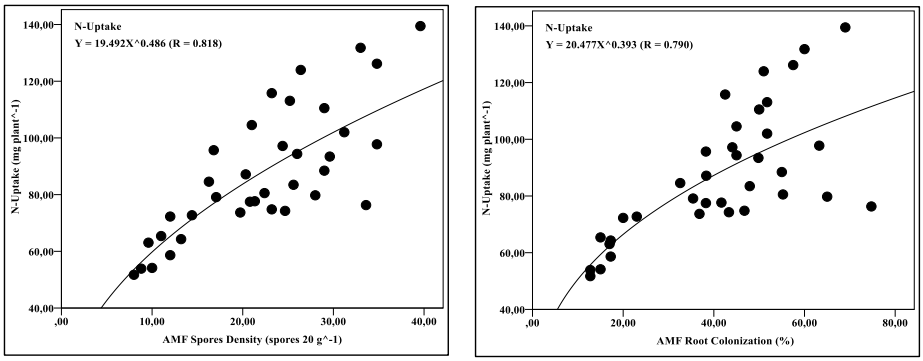


Fig. 1: Total fresh weight as a function of leaf thickness (1A-1B) and leaf area (1C-1D); symbols are measurements for each PGPR, AMF, and consortium; lines are quadratic regression of total fresh weight against leaf thickness and leaf area; for observation periods, separatel

instability of biological agent inoculants when applied on the field (Ramos *et al.,* 2003; Garcia *et al.,* 2004). However, a modification in the bacterial community structure caused by a temporary disturbance, such as a PGPR or consortium PGPR+AMF treatment, could be buffered by ecosystem resilience, which is driven by the level of diversity and interactions of the agroecosystem. The loss of certain bacterial species may also not change the functioning of the system, as different bacterial species can carry out the same function, a phenomenon defined as the bacterial redundancy. In line with Roesti *et al*. (2006), who stated even if the equilibrium of the bacterial community had been modified, the yield and grain quality remained unaffected. As suggested by Ciccillo *et al*. (2002), this result could mean that the negative effect caused by a modification of the bacterial community equilibrium was overcome by the beneficial effects of the bio-inoculants. This is also in line with the research results of Barriuso *et al.* (2008) where it was found that the inoculation of *Arthrobacter* sp. BB1 bacteria decreased the diversity of rhizospheric microorganisms, but also increased the performance of mycorrhiza and triggered the growth of *Pinus pinea* plants. In addition, Probanza *et al.* (2001) stated that the combined inoculation of the *B. licheniformis* CECT 5106 and *B. pumitus* CECT 5105 bacteria with the *Pisolithus tinctorus* fungus decreased the diversity of rhizospheric bacteria, yet positively triggered the growth of *P. pinea* seedlings.

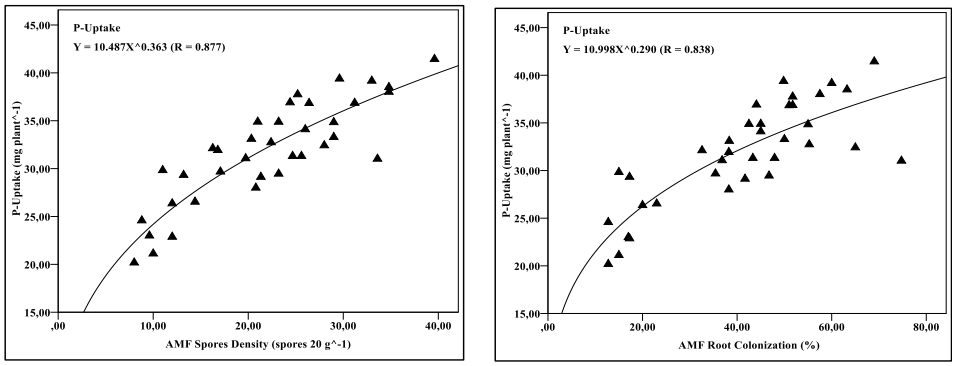
The rhizosphere microbial communities can be affected by a wide range of factors including plant type, plant age, distance from the soil to the root, soil characteristics, agronomic practices (e.g. intensive use of fertilizers), and mycorrhizal infection (Roesti *et al*., 2006; Candido *et al*., 2013). Nutrient concentrations also affect the efficacy and efficiency of biological agent inoculants; Shakori and Sharifi (2016) stated that the efficiency of inoculation with PGPR will increase when it will be combined with the 75% recommended dose of synthetic P fertilizer.

Nutrient level and inoculation of AMF and/or consortium PGPR+AMF increased the AMF activity and N and P uptake. Efficiency of the AMF inoculation increased as the level of nutrient concentration decreases, even more as the P level decreased. The level of P in the utilized nutrient solutions are 50 mg L^{-1} (100%), 37.5 mg L^{-1} (75%), and 25 mg L^{-1} (50%), in order. In a similar study, Richardson *et al*. (2011) found that the highest efficacy of AMF in general was found for soil/planting medium with low P content; in addition, Grant *et al.* (2005) stated that AMF activity is very much affected by the availability and



(2A) (2B)

Fig. 2: Nitrogen uptake as a function of AMF spores density (2A) and root colonization (2B); symbols are measurements for each nutrient concentration; lines are power regression of nitrogen uptake against AMF spores density and root colonization; for AMF spores density and root colonization, separately



(3A) (3B)

Fig. 3: Phosporus uptake as a function of AMF spores density (3A) and root colonization (3B); symbols are measurements for each nutrient concentration; lines are power regression of phosporus uptake against AMF spores density and root colonization; for AMF spores density and root colonization, separately

solubility of the P. AMF activity increased when the nutrient concentration was decreased to the 75% level, where at this level the concentration has a P content of 37.5 mg L^{-1}, but AMF activity decreased when the nutrient concentration was increased to the EC 1.8 dS m^{-1} (50 mg L^{-1} P) or decreased to the 0.9 dS m^{-1} (25 mg L^{-1} P) level. Inoculation of AMF and/or consortium PGPR+AMF also affected on AMF colonization. Constantino *et al*. (2008) also found that inoculation with only AMF increased the root colonization/infection compared to inoculation of a mixture of AMF with *A. brasilense* or *A. chrococcum.*

Meng *et al*. (2015) found that a single or dual inoculation of *Bradyrhizobium japonicum* bacteria SH212 and *Glomus mossae* fungus increased N absorption in a polyculture of corn with soybeans. The presence of AMF facilitates absorption of the N through plant transport activation; in particular N in the form of ammonium (NH_4^+). This may become a new paradigm, considering that the presence of the mycorrhiza (AMF) fungus is able to increase the efficiency of N absorption. Kavatagi and Lakshman (2014) found that the inoculation of only the *G. fasciculatum* fungus, or the inoculation of a mixture of the *G. fasciculatum* fungus with *A. chrococcum* and *P. fluorescens* bacteria significantly increased P absorption compared to the control treatment for tomato plants.

Based on the result, AMF activity showed positive correlations with N and P uptake (Table 9). Each correlation coefficient are r = 0.779 (spores density vs. N uptake); r = 0,855 (spores density vs. P uptake); r = 0.708 (root colonization vs. N uptake) and r = 0.798 (root colonization vs. P uptake). If an increase in AMF activity goes parallel with an increase in macro-nutrient uptake, every 1% (1 spore 20 g^{-1}) increase in spores density would have increased 78,27% N uptake and 77,62% of P uptake, while 1% increment of root colonization would have leads to increase 84.00% of N uptake and 81.78% of P uptake (Fig. 2 and 3).

Conclusion

Nutrient concentration with EC 0.9–1.8 dS m^{-1} combined with AMF and/or PGPR+AMF consortium can be used

for growing romaine lettuce. Particularly using hydroponic substrate culture systems, though with the consequence that reducing 50% nutrient concentration with EC 1.8 to 0.9 dS m^{-1}, it takes 14 more days to achieve minimum fresh weight. Nutrient concentration level combined with inoculation of AMF and/or consortium PGPR+AMF substantially improving romaine lettuce productivity, through indirect mechanism which are changes of leaf anatomical traits by increasing leaf thickness and leaf area, also increasing root colonization and macro-nutrient uptake. But, in other hand decrease the rhizospheric bacterial population.

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