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Effect of exopolysaccharide-producing *Azotobacter* and cow manure on nutrient uptake and root-to-shoot ratio of sorghum

Pengaruh *Azotobakter* penghasil eksopolisakarida dan kotoran sapi terhadap serapan hara dan rasio tajuk-akar tanaman sorgum

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ABSTRACT

Nitrogen-fixing *Azotobacter* synthesizes exopolysaccharide, which is important among other to improve aggregate stability and hence nutrients uptake. A pot experiment has been conducted to determine the effect of exopolysaccharide-producing *Azotobacter* and organic matter on nitrogen, phosphor, and potassium uptake by the shoot of sorghum (*Sorghum bicolor* (L.) Moench), and plant growth. The pot experiment was setup in randomized block design which test eight combination treatments of *Azotobacter* isolates (AS5, AS6, and AS5 + AS6) and organic matter application (with and without 20 t ha^{-1} of cow manure). The result showed dual inoculation of *Azotobacter* AS5 and AS6 inoculation combined with cow manure application increased N and P uptake. The dual inoculation treatment did not affect root length; but increased the shoot height and dry weight when accompanied by the application of cow manure. The ratio of root and shoot dry weight was not influenced by single or dual *Azotobacter* inoculation with or without organic matter.

INTRODUCTION

Marginal soil with a clay texture limits crop production because roots may not be able to easily penetrate the clayed loam horizons (Benhough et al., 2020). While the water content of the clay is low mainly in dry season (Severiano et al., 2013), it causes physical stress which inhibits the root elongation, thereby affecting plant growth and yield. Roots develop a response to facilitate root elongation in heavy soils via root exudation (Oleghe et al., 2017). The release of slough-off cells and exudates from the root tips thickens the roots to reduce soil density decrease around the root tips (Benhough et al., 2020).

Root exudates contain polysaccharides (Galloway et al., 2020) which are also produced by a number of *rhizobacteria*. The cell walls of the nitrogen-fixing *Azotobacter* is covered by a capsule composed of exopolysaccharide (Emtiazi et al., 2004; Hindersah et al., 2006; Ventorino et al., 2019) which naturally plays a role in avoiding oxygen pressure to nitrogenase (Sabra et al., 2000). Bacterial exopolysaccharides (EPS) improve aggregation, water retention and pore distribution (Guo et al., 2018) which facilitate nutrient uptake and subsequent root and shoot growth. Researchers had previously described that nutrient uptake is determined by pores, aggregates, available water and soil bulk density (Guidi et al., 2013; Yulina et al., 2018).

In addition to fixing nitrogen (N) and producing the EPS, *Azotobacter* produces phytohormones and siderophores which stimulate the plant growth (Rubio et al., 2013; Ahmad et al., 2008) and dissolve phosphates (Nosrati et al., 2014). In sustainable agriculture, rhizobacteria are widely used as the active substance of biofertilizer. *Azotobacter* inoculation in cereal cultivation was reported to increase the growth and the yield components

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such as the number of seeds per panicle and the weight of 1,000 seeds (Huthily et al., 2015; Mahato and Kafle, 2018).

The application of heterotrophic *Azotobacter* requires a simultaneous addition of organic matter to the soil. While *Azotobacter* uses organic matter as a source of carbon and energy, the organic matter is important in plant production to improve soil physical properties and nutrient sources (Wijanarko et al., 2012; Widodo and Kusuma, 2018). This greenhouse research aims to verify the changes in the uptake of the major macro nutrients by sorghum, and the sorghum root and canopy biomass upon the application of EPS-producing *Azotobacter* and the cow manure as organic fertilizer.

MATERIALS AND METHODS

The research was conducted in a greenhouse of Faculty of Agriculture, Universitas Padjajaran, Jatinangor Campus. The EPS-producing *Azotobacter* sp. isolates AS5 and AS6 were isolated from the local corn rhizosphere in Ultisols of Alas Selatan, Nusa Tenggara Timur. The pure cultures were maintained in N-free Ashby medium (10 g mannitol, 0.2 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, 0.2 g NaCl, 0.1 g $CaCO_3$, 10 mg Na_2MoO_4). The nitrogen fixation and exopolysaccharide production capacities of the AS5 and AS6 isolates were determined to be 0.25 and 0.1 µM/g/h, and 27.3 and 13.5 g/L, respectively.

Table 1. The combination treatments of *Azotobacter* isolates and cow manure (CM)

Code	Treatment
a_0b_0	Without *Azotobacter*; without CM*
a_0b_1	Without *Azotobacter*; with CM
a_1b_0	*Azotobacter* AS5 without CM
a_1b_1	*Azotobacter* AS5 with CM
a_2b_0	*Azotobacter* AS6 without CM
a_2b_1	*Azotobacter* AS6 with CM
a_3b_0	*Azotobacter* AS5 + AS6 without CM
a_3b_1	*Azotobacter* AS5 + AS6 with CM

The seed of Sorghum var 2.24 is a collection of the Plant Breeding Laboratory of of the Faculty of Agriculture, Universitas Padjajaran. Ultisols taken from Ciparanje village in Jatinangor were used as the growing media for sorghum with the clay texture and acidity of 5.7. The soil was low in organic C (1%) and total N (0.1%), very high in potential P but low in available P, K, cation exchange capacity and base saturation. The cow manure (C organic 38.78%, total N 1.68%, P_2O_5 1.26%, K_2O 0.41%, Cation Exchange Capacity 12.36 cmol/kg) was obtained from the Waste Treatment Plant of the Faculty of Animal Husbandry, Universitas Padjajaran.

Experimental Setup

The pot experiment was carried out in a factorial randomized block design with two treatment factors and three replications. The treatments were shown in Table 1.

The soil was taken at a depth of 0-20 cm, dried for seven days, ground and filtered using a sieve with a diameter of 2 mm. A total of 5 kg of soil was put into the individual polybag without drainage holes. The cow manure as much as 50 g polybag (equal to 20 t/ha) was added and mixed evenly. The *Azotobacter* application was carried out by pouring 50 mL of inoculant in the N-free Ashby broth with a density of 10^8 CFU/mL evenly onto the soil surface in the polybag prior to homogenization. The potted soils were watered by the ground water to optimize the field capacity and incubated for seven days.

One sorghum seed was planted in each polybag and kept in a greenhouse for six weeks. Inorganic urea, SP-36 and KCL fertilizer were added at the recommended dosage seven and 21 days after planting. The dosages of the fertilizers were 130 kg/ha N (0.1625 g/polybag), 30 kg/ha SP-36 (0.0375 g/polybag) and 100 kg/ha KCl (0.125 g/polybag). After chemical fertilizer amendment, the content of N, P and K have not been analyzed.

Research Parameters and Statistical Analysis

The level of N, P and K in the sorghum shoots were determined at 6 weeks after planting (WAP) by AOAC methods (AOAC, 2012). The plant height, root length, a well as shoot and root dry weight and were measured at 6 WAP. The, N, P and K uptake was counted by multiplying the level of nutrient with the shot dry weight. All data were analyzed by analysis of variance (F test, $p < 0.05$) and continued by using Duncan's Multiple Range Test ($p < 0.05$) if the sum squares of F test were significant.

RESULTS AND DISCUSSIONS

Nutrient Uptake

The analysis of variance showed that *Azotobacter* and cow manure affected N and P uptake, but did not change the K uptake by the sorghum shoot. As shown in Table 2, the result suggests that *Azotobacter* AS5 + AS6 inoculation followed by cow manure application increased the N and P uptake compared to the control treatment.

This study observed that while the dual *Azotobacter* inoculation combined with cow manure increased N and P uptake, both the single inoculation of *Azotobacter* sp. did not show any apparent role in the uptake of both major nutrients.

Nitrogen fixation is the major *Azotobacter* mechanisms to provide and hence increase N uptake (Kizikaya, 2009). More recently the ability of *Azotobacter* to solubilize the soil inorganic phosphore to become available phosphate for plant uptake has been reported (Nosrati et al., 2014). However, the potassium solubilizing mechanisms of *Azotobacter* has not been reported.

Table 2. The effect of *Azotobacter* isolates and cow manure (CM) on nutrient uptake of the 6-week-old sorghum

Treatment	Nutrient uptake (mg/plant)		
	Nitrogen	Phosphor	Potassium
Without *Azotobacter*; without CM (Control)	0.25 a	0.50 ab	0.22 a
Without *Azotobacter*; with CM	0.40 a	0.68 ab	0.28 a
Azotobacter AS5 without CM	0.34 a	0.56 ab	0.23 a
Azotobacter AS5 with CM	0.44 a	0.52 ab	0.27 a
Azotobacter AS6 without CM	0.29 a	0.45 a	0.27 a
Azotobacter AS6 with CM	0.38 a	0.69 ab	0.28 a
Azotobacter AS5 + AS6 without CM	0.24 a	0.48 ab	0.21 a
Azotobacter AS5 + AS6 with CM	0.51 b	0.78 b	0.32 a

Numbers followed by the same letter were not significantly different based on the Duncan Test p <0.05

Table 3. The growth traits and the biomass partition of the 6-week-old sorghum grown in clay soil after *Azotobacter* and cow manure (CM) application

Treatment	Root length (cm)	Shoot height (cm)	Root DW (g)	Shoot DW (g)	R/S*
Without *Azotobacter*; without CM (Control)	56.53 b	138.73 ab	3.6 a	13.60 a	0.30 a
Without *Azotobacter*; with CM	63.07 b	147.57 b	3.4 a	13.09 a	0.29 a
Azotobacter AS5 without CM	50.83 a	126.87 a	3.8 a	13.00 a	0.28 a
Azotobacter AS5 with CM	52.10 a	137.13 ab	5.2 ab	14.68 b	0.36 a
Azotobacter AS6 without CM	64.83 b	130.90 a	5.2 ab	14.32 a	0.36 a
Azotobacter AS6 with CM	64.03 b	144.27 b	5.4 ab	18.91 c	0.28 a
Azotobacter AS5 + AS6 without CM	56.27 b	131.57 a	4.3 b	12.74 a	0.34 a
Azotobacter AS5 + AS6 with CM	68.00 b	145.57 b	5.1 ab	16.90 bc	0.31 a

*Numbers followed by the same letter were not significantly different based on the Duncan Test p <0.05; *R/S: root to shoot ratio*

Positive effect of dual inoculation on N and P uptake might be resulted by synergistic interaction between both isolates to increase their population and hence function in N fixation, P solubilization and EPS production. The EPS have a prominent role in soil aggregation (Guo et al., 2018) and determine nutrient uptake (Guidi et al., 2013).

The result showed that organic matter increases the N and P uptake of sorghum that treated by double inoculation. It has been reported previously that the organic matter plays an important role in the formation of soil mesopores (Widodo and Kusuma, 2018), which facilitates the movement of the nutrients and water, leading to facile absorption of nutrients by the plants (Bodhinayake et al., 2004). The animal manure in this study contains the significant amounts of N, P and K, the three nutrients which can be readily absorbed by the plants. This result agrees with the increase of macronutrient uptake with cereal grown using organic matters and chemical fertilizer reported by others (Minardi et al., 2016; Puli et al., 2017).

Plant Biomass

Based on the statistical analysis, the resulting data presented in Table 3 shows that *Azotobacter* inoculation with cow manure application affected the shoot height as well as root and shoot biomass. However, the treatment did not change the root lenght and R/S. The biomass traits were depend on the treatments but in general, dual inoculation combined with cow manure consistently increased shoot height as well as root and shoot dry weight (as given in Table 3). Consistent with the finding presented in Table 1, this increase was likely caused by the significant increase in N and P uptake after the organic matter amendment with introducing AS5 and AS6 isolates. This experiment explains that the effect of the application of *Azotobacter* and the cow manure was not significant to affect the root-to-shoot (R/S). For all treatments the R/S were less than 1 (as given in Table 3). The shoot growth was more pronounce than roots which is then be contribute to better photosynthates production to support sorghum yield.

Ultisols used in this experiment contained a high level of total P but low in available P as discribed in Materials and Methods Section. Organic matter amendment usually resulted in the release of available P catalyzed by phosphate solubilizing microbes (Alori et al., 2017). The increase of available P and hence P uptake provide the sufficient chemical energy (Adenosine tri phosphate, ATP) to boost the shoot growth (Kim et al., 2006). In this pot experiment the cow manure that contained N, P_2O_5 and K_2O increased the plant biomass significantly but did not affect the root-shoot ratio (Table 3). Increased in N uptake following dual inoculation and cow manure amendment (Table 2) facilitate plant biomass synthesis since N is a major nutrient during vegetative plant growth.

The absence of the single inoculation of *Azotobacter* effect was due partly to the fact that plant growth promoting activities of *Azotobacter* is supported by multistrain in soil. Actually, the soil already contained about 4.1 log_{10} CFU/g but unlike *Azotobacter* AS5 and AS6 (as described in Material and Methods section), biological properties of indigenous *Azotobacter* was unknown. After the experiment, the count of total *Azotobacter* in the rhizosphere around 9 log_{10} CFU/g but the highest population (9.2 log_{10} CFU/g) was recorded in the rhizosphere of sorghum received dual inoculation combined with cow manure. However, that value was only significant with control treatment. It is likely that dual inoculation of exogenous *Azotobacter* ensure their function to promote plant growth include through EPS production.

The significant role dual inoculation over single inoculation on nutrient uptake and growth traits of sorghum has been showed in this experiment. The positive effect of EPS-producing *Azotobacter* on N and P uptake, as well as plant biomass indicated that the *Azotobacter* in Jatinangor Ultisols had performed its function for the N fixation and EPS production during the six weeks. The results showed the adaptation of both *Azotobacter* isolates to different conditions from the isolated site. This suggests that exogenous *Azotobacter* enable to proliferate and to eventually affect the nutrient uptake and sorghum growth. However, *Azotobacter* AS6 or AS5 with cow manure gave the highest Shoot dry weight compared to the control. Cow manure has multifunction to support plant growth; they improve mainly soil physics, supply beneficial microbes and provide organic carbon source for microbial proliferation irrespective manure amendment. In order to determine the *Azotobacter* effectivity in sorghum cultivation, a field research is needed.

CONCLUSIONS

Dual inoculation of EPS-producing *Azotobacter* isolates combined with cow manure consistently increased the N and P uptake of the shoot, and the sorghum biomass at 6 WAP over single inoculation. Combination treatments of EPS-producing *Azotobacter* and organic matter has not change the R/S of the plant in comparison with the control. In general, the positive effect of cow manure on shoot growth was demonstrated when they applied along with *Azotobacter* AS5 and AS6 in single or mixed inoculation.

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DECLARATION

The authors have no conflicts of interest to declare that are relevant to the content of this article.

REFERENCES

Ahmad, F., Ahmad, I., & Khan, M.S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiology Research, 163(2):173-181.

Alori, E., Glick, B.R., & Babalola, O.O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Frontier in. Microbiology, 8(971): 1-8

AOAC. (2012). Official methods of analysis, Association of official analytical chemist 19th edition, Washington D.C., USA

Bengough, A.G., McKenzie, B.M., Hallett, P.D., & Valentine, T.A. (2011). Root elongation, water stress, and mechanical impedance: a review of limiting stresses and beneficial root tip traits. Journal of Experimental Botany 62(1):59–68.

Bodhinayake, W., Cheng Si, B., & Xiao, C. (2004). New method for determining water conducting macro- and mesoporosity from tension infiltrometer. Soil Science Society American Journal, 68:760-769.

Emtiazi, G., Ethemadifar, Z., Habibi, M.H. (2004). Production of extracellular polymer in *Azotobacter* and biosorption of metal by exopolymer. African Journal of Biotechnology, 3:330-333.

Galloway, A.F., Akhtar, J., Marcus, S.E., Fletcher, N., Field, K., & Knox, P. (2020). Cereal root exudates contain highly structurally complex polysaccharides with

soil-binding properties. The Plant Journal, 103(5): 1666-1678.
Guo, Y-S., Furrer, J.M., Kadilak, A.L., Hinestroza, H.F., Gage, D.J., Cho, Y.K., & Shor, L.M. (2018). Bacterial extracellular polymeric substances amplify water content variability at the pore scale. Frontier in Environmental Science, 6:93, pp 13.
Guidi, P., Falsone, F., Mare, B.T., Simoni, A., Gioacchini, P., & Vianello, G. (2013). Relating loss of soil fertility to water aggregate stability and nutrient availability in agricultural calcaric soils. Environmental quality/Qualité de l'Environnement/Qualità ambientale, 11: 01-16.
Hindersah, R., Arief, D.H., Soemitro, S., & Gunarto, L. (2006). Exopolysaccharide extraction from rhizobacteria *Azotobacter* sp. Proc. International Seminar IMTGT. Medan, 22-23 June 2006. pp 50-55.
Huthily, K.H., Maje, H.R., Al-deen Gaze, E.A., & AL-Qadisiyah. (2015). The effect of bio-fertilization in growth and yield four two types of sorghum (*Sorghum bicolor* (L.) Moench). Journal of Agriculture Sciences, 5(2):96-105. Abstract in English
Kim, S.Y., Sivaguru, M., & Stacey, G. (2006). Extracellular ATP in plants. Visualization, localization, and analysis of physiological significance in growth and signaling. Plant Physiology, 42(3):984-92.
Kizikaya R. (2009). Nitrogen fixation capacity of *Azotobacter* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. Journal of Environmental Biology, 30(1):73-82.
Mahato, S., & Kafle, A. (2018). Comparative study of *Azotobacter* with or without other fertilizers on growth and yield of wheat in Western hills of Nepal. Annals of Agrarian Science, 16(3), 250–256.
Minardi, S., Harieni, S., Anasrullah, A., & Purwanto, H. (2016). Soil fertility status, nutrient uptake, and maize (*Zea mays* L.) yield following organic matters and P fertilizer application on Andisol. IOP Conf. Series: Materials Science and Engineering, 193: 012054
Nosrati, R., Owlia, P., Saderi, H., Rasooli, I., & Ali Malboobi, M. (2014). Phosphate solubilization characteristics of efficient nitrogen fixing soil *Azotobacter* strains. Iranian Journal of Microbiology, 6(4), 285–295.
Oleghe, E., Naveed, M., Baggs, E.M. et al. (2017). Plant exudates improve the mechanical conditions for root penetration through compacted soils. Plant & Soil, 421:19–30
Puli, M.R., Prasad, P.R.K, Jayalakshmi, M., & Rao, B.R., (2017). Effect of organic and inorganic sources of nutrients on NPK uptake by rice crop at various growth periods. Research Journal of Agricultural Sciences, 8(1): 64-69,
Rubio, E.J., Montecchia, M.S., Tosi, M., Cassán. F.D., Perticari, A., & Correa, O.S. (2013). genotypic characterization of Azotobacteria isolated from Argentinean soils and plant-growth-promoting traits of selected strains with prospects for biofertilizer production. Science World Journal, 2013: 519603.

Sabra, W., Zeng, A.P., Lünsdorf, H., & Deckwer, W.D. (2000). Effect of oxygen on formation and structure of *Azotobacter* vinelandii alginate and its role in protecting nitrogenase. Applied and Environmental Microbiology, 66(9):4037-44.
Severiano, E.C., Oliveira, G.C., Dias Junior, M.S., Curi, N., Costa, K.A.P., & Carducci, C.E. (2013). Preconsolidation pressure, soil water retention characteristics, and texture of Latosols in the Brazilian Cerrado. Soil Research, 51:193-202.
Susilowati, A., Puspita, A.A., & Yunus, A. (2018). Drought resistant of bacteria producing exopolysaccharide and IAA in rhizosphere of soybean plant (Glycine max) in Wonogiri Regency Central Java Indonesia. IOP Conference Series: Earth and Environmental Science, 142:012058
Ventorino, V., Nicolaus, B., Di Donato, P., Pagliano, P., Poli, A., Robertiello, A. et al. (2019). Bioprospecting of exopolysaccharide-producing bacteria from different natural ecosystems for biopolymer synthesis from vinasse. Chemical and Biological Technology in Agriculture, 6:18.
Wijanarko, A., Purwanto, B.H., Shiddieq, Dj., & Indradewa, D. (2012). Pengaruh kualitas bahan organik dan kesuburan tanah Terhadap mineralisasi nitrogen dan serapan N oleh tanaman ubikayu di Ultisol. Jurnal Perkebunan dan Lahan Tropika, 2(2):1-14
Widodo, K.H., & Kusuma, J. (2018). Pengaruh kompos terhadap sifat fisik tanah dan pertumbuhan tanaman jagung di Inceptisol. Jurnal Tanah dan Sumberdaya Lahan, 5(2):959-967.
Yulina, H., Devnita, R., & Harryanto, R. (2018). Respon air tersedia dan bobot isi tanah pada tanaman jagung manis dan brokoli

terhadap kombinasi terak baja dan bokashi sekam padi pada andisol, Lembang. Jurnal Agrikultura, 29 (2): 66-72.