Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2017 14(3): 653-661. **OPEN ACCESS**

Effect of *Azotobacter* and Urea on the nitrogen uptake enhancement and yield of maize (*Zea mays* L.)

Didik Hariyono[1*], Nur Winda Rachmadhani[1], Mudji Rahaju[2]

[1]Department of Agronomy, Faculty of Agriculture, Brawijaya University, Jl. Veteran, Malang 65145, **Indonesia**
[2]Indonesian Legumes and Tuber Crops Research Institute (ILETRI), Jl. Raya Kendal Payak, Malang 65101, **Indonesia**

.
***Correspondence:didikharyono@yahoo.com**, **ddh_sing@yahoo.com** **Accepted:**16 Aug. 2017 **Published online:** 30 Sep. 2017

Maize (*Zea mays* L.) requires high nitrogen during its life cycle. Farmers often apply urea fertilizer to fulfill nitrogen needs of these plants. On the other side, urea has a low level of efficiency, in which the loss of the nitrogen content of urea on the dry land are between 60% up to 70%. The experiment was using randomized block design. The treatment consist of two factors. The first factor was biofertilizer treatment using *Azotobacter* inoculant, consist of three levels of inoculant doses including 0 mL L^{-1} (A_0) or without bio fertilizer, 10 mL L^{-1} (A_{10}) and 20 mL L^{-1} (A_{20}). The second factor was inorganic fertilizer tretments using urea fertilizer, consist of five levels of urea doses including 0 kg ha^{-1} (U_0) or no urea treatment, urea 75 kg ha^{-1} (U_{75}), urea 150 kg ha^{-1} (U_{150}), urea 225 kg ha^{-1} (U_{225}) and urea 300 kg ha^{-1} (U_{300}). Therefore, it needs biological input such as rhizobacteria to improve the efficiency of urea fertilization, and one of them is by utilizing *Azotobacter*. The results showed that generally there were not significant interaction between the dose of *Azotobacter* and urea, either on the growth and yield parameters, or on the plant nitrogen uptake. The inoculation of *Azotobacter* did not give any significant effect on the plant nitrogen uptake. Nitrogen uptake of maize reached the optimum value when the dose of urea supplied was 263.50 kg ha^{-1}. The conclusion, inoculation of *Azotobacter* was able to increase the maize yield. However, increasing the dose of *Azotobacter* more than 10 mL L^{-1} did not affect to increase the maize yield. Maize yield reached the optimum value when the dose of urea supplied was 153.50 kg ha^{-1}

Keywords: *Azotobacter sp.*, Biofertilize, Inoculation, Nitrogen uptake, *Zea mays* L

INTRODUCTION

In Indonesia, maize (*Zea mays* L.) is an important cereal crop after rice, which is cultivated in wide areas of the country. Maize is the multipurpose commodity because it used as source of food, livestock and raw materials for industry. In this recent years, the maize consumption in Indonesia has increased because of the increasing of population, human needs, maize milling industry and livestock industry. The production and consumption of maize is unbalanced. The total production of maize in Indonesia from 1983 to 2012 was 295,206,310 tons, but the total consumption of maize was 303,567,000 tons (Bachtiar, 2014).

Yield of maize is depends on plant variety, location and also the availability of essential factors such as soil nutrient status and application of fertilizers. Nitrogen is an important plant nutrient and a major yield determining factor required for maize production. If the availability of nutrients in the soil is not sufficient, it will lower plants nitrogen uptake and will affect the growth and yield. This will be known at the components of growth and yield which both of them will be experiencing stagnation, stunted, or develop abnormally (Muyasir, 2013).

Farmers use inorganic fertilizer to fullfill nitrogen requirement of crops including maize. Urea is the most important inorganic fertilizer applied to maize. Intensive farming activities has encourage the increasing use of inorganic fertilizers. However, yields obtained is not proportional to the input of fertilizer that has been given. It happens because the low efficiency of urea caused by the mobile characteristic of this element, so it could be lost easily through the evaporation or leaching process. The loss level of urea nitrogen content on the dry land is 60% up to 70% (Jamilah and Safridar, 2012; Danapriatna, 2010). Therefore, the nitrogen uptake of plants become low, because the availability of nitrogen in the soil is less.

Biofertilizer is an alternative fertilizer material that usefull to agricultural production to nutrient supply particularly on nitrogen and phosphate. Bacteria of the genus *Azotobacter* is recognized to play a multiple role in helping plants to their optimum growth potential, yield and maintenance of soil health for sustainable agriculture. *Azotobacter* has ability to synthesize auxins, cytokinins, and GA-like substances, and these growth materials are the primary substance to controlling the growth of crops. These hormonal substances could affect the growth of the closely associated plants. This bacteria has been reported from the rhizosphere of crops such as rice, maize, sugarcane, and plantation crops (Arun, 2007). *Azotobacter* has the ability to uptake nitrogen from the atmosphere, which is able to supply nitrogen to the soil as much as 20 kg ha^{-1} yr^{-1} (Baral and Adhikari, 2013). The population of *Azotobacter* is generally low in rhizosphere around the crops and in the uncultivated soils. Therefore, the inoculation of *Azotobacter* is expected, that the nitrogen needs of maize can be obtained and the use of urea can be saved. Hindersah, Sulaksana and Herdiyantoro (2014) also stated that *Azotobacter* inoculation is able to increase the efficiency of inorganic fertilizers up to 30% in dry land ecosystems. The objective of this study was to determine the use of *Azotobacter* and urea fertilizer to nitrogen uptake and corn production.

MATERIALS AND METHODS

The field experiment was conducted during rainy season of February - June 2016 at Donowarih Village. The place is located at Karangploso, Malang District, East Java Province, Indonesia. Materials were used in this experiment consist of maize seed (DK 771 variety), *Azotobacter* inoculants with a concentration of 10^8 cfu mL^{-1}, urea fertilizer, SP-36 fertilizer, KCl fertilizer, soil, composted goat manure and polybags. The experiment was using randomized block design. The treatment consist of two factors. The first factor was biofertilizer treatment using *Azotobacter* inoculant, consist of three levels of inoculant doses including 0 ml l^{-1} (A_0) or without bio fertilizer, 10 ml l^{-1} (A_{10}) and 20 ml l^{-1} (A_{20}). The second factor was inorganic fertilizer tretments using urea fertilizer, consist of five levels of urea doses including 0 kg ha^{-1} (U_0) no urea treatment, urea 75 kg ha^{-1} (U_{75}), urea 150 kg ha^{-1} (U_{150}), urea 225 kg ha^{-1} (U_{225}) and urea 300 kg ha^{-1} (U_{300}). There were 15 treatments in combination *with 3 repetition*s, so the total were 45 experimental units.

Maize was planted 3 seeds per polybag, which contains of soil mix (soil and manure). Each polybag was placed in the field plots, inserted into the soil whole with planting distance between polybags 0.45 m x 0.10 m. The polybag diameter was ± 30 cm, so that the plant spacing was 0.75 m x 0.40 m. The seedling was remained growing one plant in a polybag. Both urea and *Azotobacter* inoculant were applied into polybags in three times.

Observations of plant growth was done destructively and non destructively. Number of leaves and leaf area were observed non destructively at 21, 31, 41, 51 and 61 days after planting (dap). Dry weight of plant was observed destructively at the maximum vegetative phase (60 dap). Yield and the component of yields were observed at 120 dap, including the number of seeds per plant, dry weight of seeds per plant, dry weight of seeds per plot and dry weight of seeds per hectare. Nitrogen uptake of plant was analized at 60 dap. Data obtained from the observations were analyzed by using analysis of varian (F-test) at the level of 5%. If there are significantly different, it will be followed by the LSD test at the level of 5% to determine the differences among the treatments.

RESULTS

The results of variance analysis showed that there was a significant interaction between the dose of *Azotobacter* and urea on the number of leaves (Table 1) and leaf area (Tables 3) at 61 dap.

Table 1. Number of leave on Maize due to the Interaction of *Azotobacter* and Urea

***Azotobacter* Doses**	**Number of Leaves at 61 dap**				
	Urea Doses				
	0 kg ha^{-1}	75 kg ha^{-1}	150 kg ha^{-1}	225 kg ha^{-1}	300 kg ha^{-1}
0 mL L^{-1}	8.89 ab	9.33 abc	9.33 abc	11.33 de	11.89 e
10 mL L^{-1}	8.34 a	9.89 bc	12.22 e	12.22 e	11.56 e
20 mL L^{-1}	9.22 abc	10.22 cd	12.00 e	11.55 e	12.56 e
LSD 5%	1.28				

Description: Numbers followed by the same letters showed no significant difference based on LSD 5%; dap = days after planting; LSD = Least Significant Difference

Table 2. Number of leaves on Maize due to Application of *Azotobacter* and Urea

Treatments	**Number of Leaves at . . . Time Observations**			
	21 dap	**31 dap**	**41 dap**	**51 dap**
***Azotobacter* Doses**				
0 mL L^{-1}	5.53	6.30 a	7.44 a	8.16
10 mL L^{-1}	5.73	7.27 b	7.62 ab	8.36
20 mL L^{-1}	5.50	7.20 b	7.93 b	8.36
LSD 5%	ns	0.68	0.44	ns
Urea Doses				
0 kg ha^{-1}	5.17	5.61 a	6.48 a	7.55 a
75 kg ha^{-1}	5.50	6.22 a	7.07 b	7.78 ab
150 kg ha^{-1}	5.61	7.22 b	7.96 c	8.41 bc
225 kg ha^{-1}	5.94	7.83 b	8.33 c	8.74 c
300 kg ha^{-1}	3.72	7.72 b	8.48 c	8.96 c
LSD 5%	ns	0.88	0.57	0.66

Description: Numbers followed by the same letters in the same column showed no significant difference based on LSD 5%; dap = days after planting; LSD = Least Significant Difference; ns = not significant

Table 3. Leaf Area of Maize due to Interaction Effect of *Azotobacter* and urea

***Azotobacter* Doses**	**Leaf Area (cm^2) at 61 dap**				
	Urea Doses				
	0 kg ha^{-1}	75 kg ha^{-1}	150 kg ha^{-1}	225 kg ha^{-1}	300 kg ha^{-1}
0 mL L^{-1}	2991.51 abc	3448.54 abc	3478.84 abc	5116.03 e	5377.10 e
10 mL L^{-1}	2718.47 a	3847.38 bc	5254.66 e	5896.98 e	4924.22 de
20 mL L^{-1}	2939.88 ab	3953.24 cd	5581.88 e	5312.08 e	5802.83 e
LSD 5%	988.91				

Description: Numbers followed by the same letters showed no significant difference based on LSD 5%; dap = days after planting; LSD = Least Significant Difference

Table 4. Leaf Area of Maize due to Application of *Azotobacter* and Urea

Treatments	Leaf Area (cm^2) at . . .Time Observations			
	21 dap	**31 dap**	**41 dap**	**51 dap**
***Azotobacter* Doses**				
0 mL L^{-1}	165.36	661.21 a	1402.49 a	2844.11 a
10 mL L^{-1}	192.00	753.44 ab	1722.58 b	3160.54 ab
20 mL L^{-1}	183.70	891.52 b	1895.24 b	3329.37 b
LSD 5%	ns	154.50	281.27	349.07
Urea Doses				
0 kg ha^{-1}	106.80 a	397.33 a	801.57 a	1902.59 a
75 kg ha^{-1}	156.46 ab	563.93 a	1175.17 b	2562.48 b
150 kg ha^{-1}	214.75 bc	814.68 b	1752.79 c	3309.39 c
225 kg ha^{-1}	237.53 c	1122.21 c	2404.45 d	3998.26 d
300 kg ha^{-1}	186.25 bc	945.48 bc	2233.21 d	3783.99 d
LSD 5%	67.38	199.45	363.12	450.64

Description: Numbers followed by the same letters in the same column showed no significant difference based on LSD 5%; dap = days after planting; LSD = Least Significant Difference; ns = not significant

It showed that urea on level of 150 kg ha^{-1}, application of *Azotobacter* with doses of 10 mL L^{-1} and 20 mL L^{-1} were able to produce both number of leaves and leaf area higher than *Azotobacter* dose 0 mL L^{-1}. Inoculation of *Azotobacter* 10 mL L^{-1} and 20 mL L^{-1} in the treatment of urea with level of 150 kg ha^{-1}, were able to increase the growth of maize plants. So that the maize plants had an average number of leaves and leaf area, that were not significantly different compared to maize with higher level of urea, namely 225 kg ha^{-1} and 300 kg ha^{-1}.Based on the results, there was no significant interaction between the dose of *Azotobacter* and urea on the number of leaves (Table 2) and leaf area (Tables 4) at 21 dap until 51 dap. Eventhough there was no significant interaction between the two factors, but separately, the dose of *Azotobacter* had any significant effect on the number of leaves and leaf area. Generally, the inoculation of *Azotobacter* had significant effect on these both parameters on 31 dap up to 41 dap. The inoculation of *Azotobacter* with dose of 20 mL L^{-1} had higher value compared to treatment without *Azotobacter* (0 mL L^{-1}), but this value was not significantly different when compared to the treatment of *Azotobacter* with dose of 10 mL L^{-1}. On the other side, urea with level of doses 225 kg ha^{-1} and 300 kg ha^{-1} had higher average number of leaves and leaf area when compared to the other treatments

Based on the results of variance analysis on Table 5 showed that there was no significant interaction between the dose of *Azotobacter* and urea on the dry weight of leaves, stem, roots and plant. But, the inoculation of *Azotobacter* had any significant effect on the leaves, stem and plant dry weight. Generally, the inoculation of *Azotobacter* with dose of 20 mL L^{-1} had higher average value of leaves, stem and plant dry weight when compared to the other treatment.

The application of urea had significant effect on the dry weight of leaves, stem, roots and plant. The urea level of 150 kg ha^{-1}, 225 kg ha^{-1} and 300 kg ha^{-1} had higher average leaves, stem and plants dry weight when compared to the other treatments. The dry weight of roots in the urea levels of 225 kg ha^{-1} and 300 kg ha^{-1} had higher average value when compared to the other treatments.Table 6 showed, there were no significant interaction between the treatments of *Azotobacter* and urea on the nitrogen uptake of leaves, stem, root and plant. Inoculation of *Azotobacter* had no significant effect also on those parameters. But on the other side, the treatment of urea had any significant effect on the nitrogen uptake of leaves, stem, roots and plant. Generally, the treatment of urea with level of 225 kg ha^{-1} and 300 kg ha^{-1} had higher average values of nitrogen uptake when compared to the other treatments, eventhough their values were not significantly different with the level of 150 kg ha^{-1}. Based on the observation of maize yield and the components of yield including number of seeds per plant, dry weight of seed per plant, dry weight of seed per plot and dry weight of seed per hectare (Table 7), there was no significant interaction between the dose of *Azotobacter* and urea.

Table 5. Dry Weight of Vegetative Organs on Maize Plant to Application of Azotobacter and urea

Treatments	Dry Weight (g) at 60 dap			
	Leaves	Stem	Roots	Plant
***Azotobacter* Doses**				
0 mL L^{-1}	24.15 a	51.91 a	28.01	104.07 a
10 mL L^{-1}	25.51 a	57.27 ab	25.63	108.41 ab
20 mL L^{-1}	29.10 b	64.54 b	32.66	126.31 b
LSD 5%	3.47	11.37	ns	20.60
Urea Doses				
0 kg ha^{-1}	14.94 a	28.92 a	11.67 a	55.53 a
75 kg ha^{-1}	21.79 b	45.89 b	21.32 ab	89.00 b
150 kg ha^{-1}	30.49 c	67.91 c	27.62 bc	126.02 c
225 kg ha^{-1}	31.51 c	73.17 c	41.17 cd	145.85 c
300 kg ha^{-1}	32.53 c	73.66 c	42.06 d	148.25 c
LSD 5%	4.49	14.68	13.68	26.60

Description: Numbers followed by the same letters in the same column showed no significant difference based on LSD 5%; dap = days after planting; LSD = Least Significant Difference; ns = not significant

Table 6. Nitrogen Uptake of Maize due to Aplication of *Azotobacter* and urea

Treatments	Nitrogen Uptake (g) at 60 dap			
	Leaves	Stem	Roots	Plant
***Azotobacter* Doses**				
0 mL L^{-1}	0.40	0.86	0.48	1.74
10 mL L^{-1}	0.42	0.97	0.42	1.81
20 mL L^{-1}	0.46	1.05	0.54	2.05
LSD 5%	ns	ns	ns	ns
Urea Doses				
0 kg ha^{-1}	0.24 a	0.50 a	0.20 a	0.94 a
75 kg ha^{-1}	0.35 ab	0.72 ab	0.34 a	1.41 ab
150 kg ha^{-1}	0.49 bc	1.10 bc	0.46 ab	2.04 bc
225 kg ha^{-1}	0.54 c	1.29 c	0.71 b	2.54 c
300 kg ha^{-1}	0.52 bc	1.19 bc	0.68 b	2.40 c
LSD 5%	0.17	0.49	0.30	0.88

Description: Numbers followed by the same letters in the same column showed no significant difference based on LSD 5%; dap = days after planting; LSD = Least Significant Difference; ns = not significant

Table 7.Seeds Yield of Maize (Number and Dry Weight) due to Application of *Azotobacter* and urea

Treatments	Number of Seeds(per Plant)	Seeds ($g.plant^{-1}$)	Seeds (kg/plot of 1.2 m^2)	Seeds ($t.ha^{-1}$)
***Azotobacter* Doses**		.........................Dry Weight		
0 ml l^{-1}	316.43 a	140.63 a	0.56 a	3.99 a
10 ml l^{-1}	459.13 b	190.72 ab	0.76 ab	5.41 ab
20 ml l^{-1}	465.17 b	194.15 b	0.78 b	5.50 b
LSD 5%	125.27	51.59	0.21	1.46
Urea Doses		.........................Dry Weight		
0 kg ha^{-1}	203.61 a	87.12 a	0.35 a	2.47 a
75 kg ha^{-1}	**311.78 a**	**138.13 a**	**0.55 a**	**3.91 a**
150 kg ha^{-1}	502.44 b	209.68 b	0.84 b	5.94 b
225 kg ha^{-1}	532.89 b	220.50 b	0.88 b	6.25 b
300 kg ha^{-1}	517.17 b	220.40 b	0.88 b	6.25 b
LSD 5%	161.73	66.61	0.27	1.89

Description: Numbers followed by the same letters in the same column showed no significant difference based on LSD 5%; LSD = Least Significant Difference

Even though there was no significant interaction between those two factors, but separately, its had any significant effect on those all parameters. Generally, the inoculation of *Azotobacter* with dose of 20 mL L^{-1} had higher average value when compared to the other treatment, even though its value was not significantly different with the dose of 10 mL L^{-1}. The treatment of urea had any significant effect on those all parameters. The result showed that the treatment of urea with level of 150 kg ha^{-1}, 225 kg ha^{-1} and 300 kg ha^{-1} had higher average values when compared to the other treatments.

DISSCUSSION

Results of these experiment indicated that biofertilizer such as *Azotobacter* can be used to increase nitrogen uptake on maize. The bacterial inoculant has been used by previous researchers. There was no significant interaction effect between *Azotobacter* and urea treatments on plant growth, seeds yield and nitrogen uptake. However, the treatments had signifcantly interaction on number of leaves and leaf area at the maximum vegetative development phase of maize.

Table 1 and Table 3 showed that the inoculation of *Azotobacter* with 10 mL L^{-1} and 20 mL L^{-1} also application of urea 150 kg ha^{-1} may increasing the number of leaves and leaf area when compared to uninoculated or *Azotobacter* 0 mL L^{-1}. These values were not significantly different when compared to urea doses with higher levels 225 kg ha^{-1} and 300 kg ha^{-1}. It can be happen because *Azotobacter* application into the soil can supply nitrogen for maiz, it is associated with the ability of *Azotobacter* to fix the free nitrogen from the atmosphere (Rao, 2010; Syam'un, et al, 2012).

The increase in number of leaves and leaf area of maize are closely related to the activity of cell division, cell enlargement and cell differentiation. One of the factors that influence these activities is the availability of nitrogen. Nitrogen is an important nutrient for plant metabolism as it functions of protein, nucleic acids, and chlorophyll biosynthesis, being necessary since the early stages of plant development (Basso and Ceretta, 2000; Widiyawati et al, 2014). Espinoza and Ross (2013) also stated that the nitrogen is needed by maize plants in the early growth up to the maximum vegetative phase, in which the nitrogen uptake of maize in the maximum vegetative phase has reached 70% of the total nitrogen requirements. Chlorophyll is a light catcher pigment that plays a role in photosynthesis. If the nitrogen needs are met properly, plants will have an optimal leaf chlorophyll content, so that they are able to absorb the light optimally, which in turn the rate of photosynthesis can be done optimally.

Azotobacter has the ability to produce plant growth regulators such as Indol Acetic Acid (IAA) (Usharani, Sujitha and Sivasakthi, 2014). This ability explains the beneficial influence of *Azotobacter* inoculation in connection with the IAA's role which able to improve the plant growth and cell division, so it can increase the growth of maize, such as the number of leaves and leaf area. So that, in the urea treatment with level of 150 kg ha^{-1}, the adding of *Azotobacter* with dose of 10 mL L^{-1} and 20 mL L^{-1} were able to produce averages number of leaves and leaf area were higher when compared to the treatment of *Azotobacter* with dose of 0 mL L^{-1}.

Based on the results, there was a tendency that the inoculation of *Azotobacter* to the soil with dose of 10 mL L^{-1} and 20 mL L^{-1} were able to increase the growth of maize plants when it added to the treatment of urea with level of 150 kg ha^{-1}. At the high level of urea (225 kg ha^{-1} or 300 kg ha^{-1}), inoculation of *Azotobacter* was not able to increase the number of leaves (Table 1) and leaf area (Table 3). This is presumably because the nitrogen fertilization in large amounts will cause the activities of rhizo bacteria i.e *Azotobacter* is low, which will cause the inoculation of rhizobia become less effective (Rosmarkam and Yuwono, 2002). Salhia (2010) and Saribay (2003) also stated that the high nitrogen content in the media will disrupt the lag phase (adaptation phase) and regeneration phase of *Azotobacter*, which will cause the *Azotobacter* inoculation become ineffective in conducting nitrogen fixation.

In the urea treatments with low level (0 kg ha^{-1} and 75 kg ha^{-1}) the fertilization using *Azotobacter* also was not able to increase the number of leaves and leaf area. This is presumably because the nitrogen content in the soil have not been able to meet the needs of plant. Basically, plant still needs another source of nitrogen despite the planting media inoculated with *Azotobacter.* It is because plants need nitrogen fertilizer as a starter for their growth (Rao, 2010).

Based on the Table 5 shows that the higher the dose of *Azotobacter* or urea would increase the total dry weight of maize. *Azotobacter* inoculation and urea fertilization aim to increase the availability of nitrogen contents in the soil that can be utilized by maize plants. Plant dry weight is the accumulation of organic compounds synthesized by plants through photosynthesis. Gardner, Pearce and Mitchell (2003), stated that the photosynthesis process will produce assimilates that are accumulated as plant dry matter. Therefore, the dry matter production of plant is closely related to the rate of photosynthesis. The nitrogen is successfully fixed by *Azotobacter* or the nitrogen derived from urea, both of them will increase the supply of nitrogen in the planting media. The higher the supply of nitrogen for the plant, it will increase the chlorophyll content of leaves, which in turn will increase the rate of photosynthesis. The higher the rate of photosynthesis, then the assimilates produced will increase, so it will increase the total dry weight of plant.

Based on the Table 6 indicates that the improvement of plant nitrogen uptake in line with the increasing doses of *Azotobacter.* However, the improvement of nitrogen uptake generated by an increase in the dose of *Azotobacter* is relatively small, so based on the statistical analysis did not show any significant effect. These results is supported with the research of Baral and Adhikari (2013), which stated that the ability of *Azotobacter* to tie up nitrogen from the atmosphere is only 20 kg ha^{-1} yr^{-1}. Rao (2010), stated that based on the the result of a research carried out in temperate regions, the fixation of nitrogen in the soil that is inoculated with *Azotobacter* will not be more than 10 up to 15 kg ha^{-1} yr^{-1}. These results are relatively small when it compared to the nitrogen needs of maize plant that is high enough, so that the inoculation of *Azotobacter* was not provide significant effect on the nitrogen uptake of plant.

Table 6 shows that the higher level of urea, it would increase the nitrogen uptake of maize plants. The adding of urea to the planting media will increase the availability of nitrogen contents in the soil. This is because urea is single fertilizer which containing nitrogen by 46% (Gordon, 2014). Plant nitrogen uptake is influenced by the availability of nitrogen in the soil. If the availability of nitrogen in the soil increases, it will increase the nitrogen uptake of maize. The increasing of nitrogen uptake by plant, it will increase the nitrogen content inside the plant (Widiyawati et al, 2014).

Based on the Figure 1, shows that regression between doses of *Azotobacter* with seeds dry weight (t ha^{-1}) were 97,53% (R^2 = 0,9753).

The analysis of variance shows that there was no significant interaction between the doses of *Azotobacter* and urea on the number of seeds per plant, dry weight of seed per plant, dry weight of seeds per plot and dry weight of seeds per hectare. But separately, the doses of *Azotobacter* or urea had any significant effect on these all parameters. The higher dose of *Azotobacter* or urea increased the number of seeds per plant (Table 7). However, the inoculation of *Azotobacter* with doses of 10 ml l^{-1} or 20 ml l^{-1} were not increased the maize yield significantly. Similarly, urea with level of 150 kg ha^{-1}, 225 kg ha^{-1} and 300 kg ha^{-1}, were not affect the yield significantly.

According to Huber *et al.* (1994) there are four characteristics which determine the response of maize yield to nitrogen availability including root uptake, vegetative storage capacity, recycling efficiency from vegetative tissues to developing seeds, also dynamics and strength of the seeds sink. The research showed that the higher level of urea was increased the nitrogen uptake of maize plant (Table 6).

The beneficial effects of Azotobacter is providing the supply of available nitrogen to the maize plants tissue by enhancing nitrogen fixation. The ability of maize to maintain nitrogen uptake during grain filling may be related to the fertilizer supply to the roots. Grain seeds such as maize, its seeds contain gluten components. The main component of gluten is a protein (Rosmarkan and Yuwono, 2002). Therefore, during the process of seed formation, nitrogen is needed by plant, due to nitrogen is a key element of protein. Protein is the largest component of the cell, where more than 50% of the seeds dry weight contain protein component (Istanti et al., 1999).

The adequacy of nitrogen availability will be able to increase the number of seeds per plant, which will further increase the dry weight of seeds per plant. This is consistent with the result, the increasing doses of *Azotobacter* or urea, they will increase the number of seeds per plant and dry weight of seeds per plant. The yield of maize per area is influenced by the yield per individual plant. The higher the yield per individual plant, it will increase the yield per area (Rizky et al, 2015). This is consistent with the result, in which the higher the dry weight of seeds per plant, it will be followed by an increase in dry weight of seed per plot and per hectare

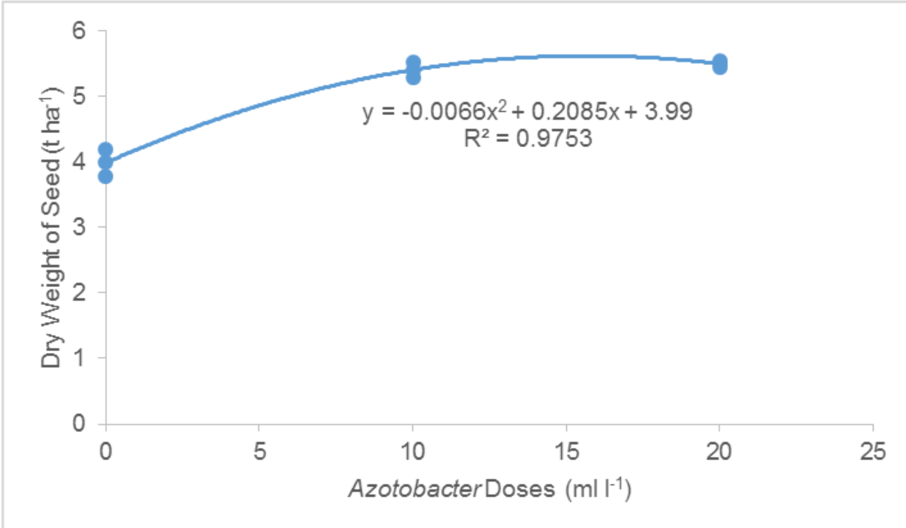

Figure 1. Regression between doses of *Azotobacter* with seeds dry weight

Improvement in crop production due to Azotobacter inoculation has been reported in several crops. Azotobacter inoculation increased the yield 10-23% in wheat Triticum aestivum and 13-19% in oat Avena sativa more than the control (Damir et al. 2011).

Based on Figure 2, it shows that the dry weight of seeds produced by maize plant was influenced by the level of urea. The level of urea gave effect on the dry weight of seeds at 97.60% (R^2 = 0.9760). Based on the equation of the regression analysis, it is known that maize yield would reach the optimum value when the dose of urea supplied was 153.50 kg ha^{-1}. Therefore, if the urea dose given to the plant more than 153.50 kg ha^{-1}, the maize yield will be not different statistically with it

CONCLUSION

There were no significant interaction between the dose of *Azotobacter* and urea, either on the growth and yield parameters, or on the plant nitrogen uptake. The inoculation of *Azotobacter* was not give any significant effect on the plant nitrogen uptake. Nitrogen uptake of maize reached the optimum value when the dose of urea supplied was 263.50 kg ha^{-1}. The yield of maize was increased due to *Azotobacter* inoculation. However, increasing the dose of *Azotobacter* more than 10 mL L^{-1} did not increase the maize yield. Maize yield reached the optimum value when the dose of urea applied 153.50 kg ha^{-1}

.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

ACKNOWLEGEMENT

Thanks are extented to Akbar Saitama and Akbar Hidayatullah Zaini for his assistance and direct involvement of this study.

AUTHOR CONTRIBUTIONS

DH and MR developed the concepts and also designed the experiments. NWR as researchers.

REFERENCES

Arun, K.S., 2007. Bio-fertilizers for sustainable agriculture, sixth edition, Agribios Publishers, Jodhpur, India. pp 76-77

Bachtiar, R.R., Chang, W.I., Anindita R. and Mustadjab, M., 2014. Research article supply response and maize price volatility in Indonesia. Greener J. Buss & Manag Stud. 4: 58–69

Baral, B.R. and Adhikari, P., 2013. Effect of *Azotobacter* on growth and yield of maize. J.

Agric. 11: 141–147.
Damir, O., Mladen, P. I., Božidar, S. and Sran, N., 2011. Cultivation of the bacterium *Azotobacter chroococcum* for preparation of biofertilizers. Afr. J. Biotechnol. 10: 3104-3111
Danapriatna, N., 2010. Biochemical nitrogen fixation by non symbiotic bacteria. J. CEFARS, 1: 1–10
Espinoza, L. and Ross, J., 2013. Maize production handbook, Cooperative Extension Service University of Arkansas, Arkansas, USA. pp 23–27
Gardner, F.P., Pearce R.B. and Mitchell, R.L., 2003. Physiology of crop plants, Iowa State University Press, Iowa, USA. pp 12.
Gordon, W.B., 2014. Management of urea-containing fertilizers for no-tillage corn using nitrogen stabilizers and coated-granule technology. J. Plant Nutr., 37: 87-94
Hindersah, R., Sulaksana, D.A. and Herdiyantoro, D., 2014. Changes in level of N available and population *Azotobacter sp.* in the rhizosphere of sorghum (*Sorghum bicolor* L.) planted in two soil ordo by inoculating *Azotobacter sp.* J. Agrologia 3: 10–17.
Huber, D.M., Tsai C.Y. and Stromberger, J.A., 1994. Interaction of K with N and their influence on growth and yield potential of maize. In: Annual corn and sorghum research conference, 48. Proceedings, American Seed Company Association, USA. pp 165-176.
Istanti, A., Prasetyo, T.I. and Listyorini, D., 1999. Cell biology, Science Faculty of Malang State University, Malang, Indonesia. pp 83.
Jamilah and Safridar, N., 2012. Effect of urea, active charcoal and zeolite on growth and yield of rice (*Oryza sativa* L.). J. Agrista 16: 153–162
Muyasir, 2013. Responses of Corn Doubles Cobs (*Zea mays* L.) on Urea and Compost Fertilization. J. Land Resorces Manag. 2: 250-254
Rao, N.S.S, 2010. Soil microorganisms and plant growth, UI-Press, Jakarta, Indonesia. p. 100–124.
Rizky, D., Irmansyah, T. and Ginting, J., 2015. Biological Fertilizer Aplication in Some Plant Spacing Response on Growth and Yield of *Brassica oleraceae* var. *acephala.* J. Online Agrotech. 3: 483-488
Rosmarkam, A. and Yuwono, N.W., 2002. Soil fertility studies, Kanisius Publisher, Yogyakarta, Indonesia. pp 95–186
Salhia, B.M., 2010. The effect of *Azotobacter chroccocum* as nitrogen biofertilizer on the growth and yield of *Cucumis sativus*, The Islamic University. Gaza, Gaza, Palestine. pp 23 -25
Saribay, G.F., 2003. Growth and nitrogen sixation dynamics of *Azotobacter chroccocum* in nitrogen-free and OMW containing medium. Middle East Technical University, Aralik, Turkey. pp 4 - 5
Sitompul, S.M. and Gurito, B., 1995. Plant growth analysis, Gadjah Mada University Press, Yogyakarta, Indonesia. pp 152–217
Syam'un, E., Kaimuddin and Dachlan, A., 2012. Vegetative growth and N uptake rice plant of application fertilizing N inorganic and microbial fixation non-symbiotic N. J. Agrivigo 11: 251–261
Usharani, G., Sujitha D. and Sivasakthi, S., 2014. Effect of *Azotobacter sp.* for the improvemezt of growth and yield of pearl millet (Cumbu) (*Pennisetum Glaucum* L.). Int. J. Adv. Multi. Res., 1: 102–106
Widiyawati, I., Sugiyanta, Junaedi, A. and Widyastuti, R., 2014. The role of nitrogen-fixing bacteria to reduce the rate of inorganic nitrogen fertilizer on lowland rice. J. Agron. Indonesia, 42: 96–102