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Different Response of Hydroponically Grown Vegetables to the Addition of *Nostoc* sp. GIA13a as an alternative nitrogen source

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Abstract. Vegetable growth needs nitrogen (N) supply in the form of nitrate and or ammonium. Alternative environmental-friendly nitrogen can be provided by using N₂-fixing cyanobacteria, such as *Nostoc*. This study investigated the effect of *Nostoc* sp. GIA13a to spinach (*Amaranthus* sp.) and water spinach (*Ipomoea aquatica*) grown in modified Nutrient Film Technique (NFT) system provided with Hoagland Solution. The treatments of nitrogen in Hoagland Solution were as follows: nitrate+GIA13a (NGia), nitrate without GIA13a (N), ammonium+nitrate (AN), ammonium+GIA13a (AGia) and ammonium without GIA13a(A). Inoculant of 2 g was added at the 1st and 14th day after planting (dap). Experiment was carried out for 21 days. The result showed that spinach and water spinach had different physiological responses to the occurrence of *Nostoc* sp. Gia13a in nutrient solution. Total chlorophyll of spinach was increased in treatment with GIA13a inoculation, while it was decreased in water spinach. Ammonium consumption of water spinach was higher than spinach as shown by ammonium concentration on the nutrient solution after 14 days experiment. Inoculation of *Nostoc* sp. Gia13a also triggered the growth of lateral shoot of water spinach. During experiment, chlorosis symptom was observed in young leaves of both plants. Chlorosis symptom in water spinach occurred in all treatment except NGia, while in spinach the symptom was found only in AGia and A.

1. Introduction

Hydroponic farming has grown in urban communities. Under hydroponic system, plants are grown in a soil less medium on containers, which can be arranged vertically in order to save the space. Due to their space efficiency and its cleanliness, hydroponic farming systems have become increasingly popular in urban communities.

Among the many nutrients contained in a hydroponic medium, nitrogen is the highest in concentration. Plants require nitrogen to build many cell components, including the photosynthetic enzyme of RuBisCo in their leaves. Therefore, nitrogen deficiency, in plants, rapidly leads to chlorosis, which later inhibits plant growth. Nitrogen is usually provided in the form of ammonium (NH₄⁺) or nitrate (NO₃⁻). The long-term industrial production of ammonium and nitrate creates



environmental burdens, because the processes use a great deal of fossil-fuel energy. In order to reduce the consumption of fabricated ammonium and nitrate, environmentally friendly nitrogen suppliers, such as N_2 -fixation cyanobacteria, can be used as an alternative source of nitrogen.

Nitrogen-fixing cyanobacteria are commonly found in soil [1-3]. The vegetative cells of N_2 -fixation cyanobacteria are organized in a filamentous form. Vegetative cells are able to change into specialized cells, called heterocyst cells, which contain nitrogenase enzymes. Inside the heterocyst cells, catalyzed by the nitrogenase, dinitrogen is reduced into ammonium, which can later be released into the environment. Thus, the ability of Cyanobacteria to fix N_2 provides ammonium in the soil [4,5]. Moreover, the biomass of cyanobacteria also provides nitrogen when the population of cells decays.

The application of N_2 -fixing cyanobacteria as a nitrogen supplier has been studied mostly in paddy plants. An inoculant of a mixture of cyanobacteria was able to increase the amount of rice grown, in certain studies [5,6]. Paudel et al. (2012) reported a 20.9% increase of rice grain yield (as compared to control) when using a mixture of *Nostoc*, *Anabaena*, *Westiellopsis*, *Aulosira* and *Scytonema* [5]. This result was higher than previous amount of rice grain yield (19%) reported by Mishra & Pabbi in 2004 [7] when using a mixture inoculant of *Anabaena*, *Nostoc*, *Aulosira*, and *Tolypothryx*. Application of N_2 -fixing cyanobacteria could bring an improvement in the yield of rice (ranging from 5-25%) as well as direct and indirect influences on the quality of physico-chemical properties of soil [6].

Nitrogen fixing cyanobacteria can potentially be applied as a source of nitrogen for other plants under hydroponic systems, such as vegetables. A variety of vegetable plants, such as lettuce (*Lactuca sativa* L.), spinach (*Amaranthus* spp.), and water spinach (*Ipomoea aquatica* L.), have been cultivated in hydroponic systems. Spinach and water spinach are popular hydroponic vegetable in Indonesia. The young terminal shoots and leaves are used as leafy vegetables and in salads. Both vegetables have high potential for commercial cultivation due to its high nutrient value (especially its iron content), agreeable taste, and fast harvest.

The two primary forms of inorganic nitrogen taken up by plants are nitrate and ammonium. In a hydroponic system, ammonium can immediately be oxidized into ammonia, which is toxic for plants. Nitrogen, in the form of nitrate, is thus a preferable nitrogen source. Nitrogen-fixing cyanobacteria, in contrast, exhibit a preference for ammonium, which their cells readily uptake. This assimilation is sustained by a regulatory phenomenon termed nitrogen control (N control) that ensures that permeases and enzymes of the assimilatory pathways for alternative nitrogen sources are not expressed when the cells are exposed to a non-limiting concentration of ammonium [8]. The opposing nitrogen uptake between plants and N_2 -fixing cyanobacteria may be an advantage for application of cyanobacteria in hydroponic systems. The aim of this study was to examine the application of *Nostoc* sp. GIA13a as a nitrogen source in hydroponic systems using Hoagland mediums for spinach and water spinach growth.

2. Material and Methods

2.1. Design experiment

The study used a Nutrient Film Technique (NFT) hydroponic system. Hoagland nutrient solution was used as basic medium. Total volume of nutrient solution was 6 L. Nutrient solution's replacement was carried out on day 14th of the 21-day experiment. Plant biomass, chlorophyll content and ammonium-nitrate concentration were measured at the end of experiment.

The treatments were as follows: nitrate+GIA13a (NGia), nitrate without GIA13a (N), ammonium+nitrate (AN), ammonium+GIA13a (AGia) and ammonium without GIA13a(A). Inoculant of 2 g was obtained by running the 21-day-old culture through centrifugation. Inoculant was added at the 1st and 14th day after planting (dap). Experiment was carried out for 21 days.

The complete Hoagland medium recipe is as follows: 795 μM KNO_3 , 603 μM $\text{Ca}(\text{NO}_3)_2$, 270 μM MgSO_4 and 109 μM KH_2PO_4 ; micronutrients: 40.5 μM $\text{Fe}(\text{III})\text{-EDTA}$, 20 μM H_3BO_4 , 2 μM MnSO_4 , 0.085 μM ZnSO_4 , 0.15 μM CuSO_4 and 0.25 μM Na_2MoO_4 . Acidity of the solution is adjusted to 6.1 pH [9].

2.2. Co-cultivation of spinach and water spinach in the hydroponic system

The plant grains used for the experiment were selected by soaking the potential grains in water. Floating grains were eliminated, and the submerged grains were preserved. The selected grains were then placed on rockwool to let them germinate. Three grains were put onto each section of rockwool. The seedling growth took place for 5 days at a room temperature of 30°C. On the sixth day, the rockwools with growing seedlings were put inside the net pots and co-cultivated at the hydroponic system. Each treatment was provided with 8 net pots (24 individual plants). The system was placed at cultivation house outdoor. Vegetables were harvested at day 21st after co-cultivation.

2.3. Measurement of chlorophyll

Chlorophyll content was measured following Prsa et al. 2007 [10]. One gram of leaves was grinded on a mortar. After all biomass was extracted, 10 mL 96% ethanol was added. The solution was centrifuged at 1500 rpm for 10 minutes. Supernatant was transferred to glass measurement and the volume was adjusted to 100 mL with the same concentration of ethanol. The absorbance of solution was then measured with spectrophotometer (Genesys 10S UV- Vis) at 649 and 665 nm. The results were counted with formula below:

$$\text{Total chlorophyll (mg/L)} = (20 \times \text{OD}_{649}) + (6.1 \times \text{OD}_{665})$$

$$\text{Chlorophyll a} = (13.7 \times \text{OD}_{649}) + (5.76 \times \text{OD}_{665})$$

$$\text{Chlorophyll b} = (25.8 \times \text{OD}_{649}) + (7.7 \times \text{OD}_{665})$$

3. Result and Discussion

The fresh and dry weight of spinach were higher in Hoagland Solution with nitrate as single nitrogen source (Treatment N and NGia). Compare with these two treatments, treatments with ammonium or combined ammonium-nitrate as nitrogen source had lower biomass (Table 1). Different with spinach, water spinach showed that combined ammonium-nitrate had the highest biomass amongst all treatments. Ammonium as the only nitrogen source could not support the growth of water spinach, showed by low biomass dry weight (1.80±0.5 on Treatment AGia and A) (Table 1).

Table 1. Biomass yield of spinach and water spinach at the end of experiment (21 days)

Treatment	spinach			water spinach		
	fresh weight (g)	dry weight (g)	water content (%)	fresh weight (g)	dry weight (g)	water content (%)
NGia	66.54±11.2	4.24±0.8	93.63	42.21±9.8	2.60±0.6	93.84
N	88.18±24.9	5.18±1.7	94.13	31.49±9.4	2.00±0.6	93.65
AN	30.14±8.7	2.1±0.6	93.03	47.83±12.8	2.70±0.7	94.36
AGia	16.66±2.7	1.02±0.3	93.88	23.89±3.5	1.80±0.5	92.47
A	22.72±6.5	1.72±0.6	92.43	27.23±7.1	1.80±0.5	93.39

Value after chlorophyll content was SD value

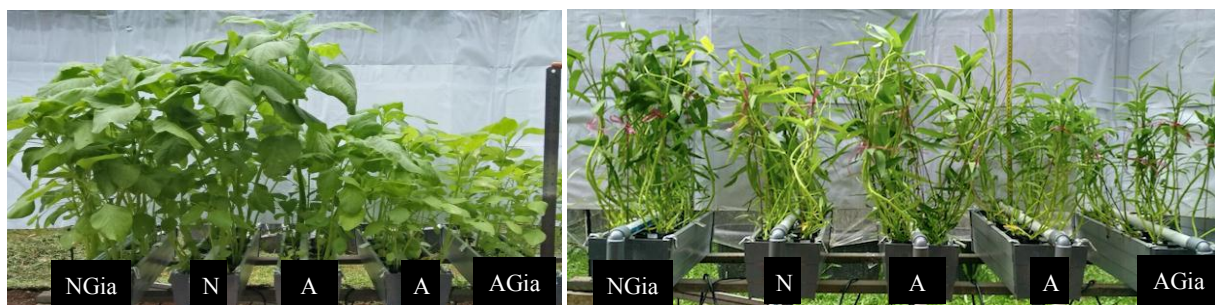


Figure 1. Growth of spinach and water spinach at 21 dap

Plants have their specific preferences and dependencies in response to different nutrients. For example, aspen plants were reported to have greater efficiency in nitrate acquisition than conifers [11]. The fern *Nephrolepis exaltata* and the black cottonwood *Populus trichocarpa* are low-nitrate need plants, so low-nitrate environments have no effect on their growth [12]. Some leafy-vegetables, like lettuce and rocket (*Eruca sativa*) plants, tend to accumulate nitrate in their leaves in order to maintain high turgor pressure [13]. This study showed that spinach and water-spinach had different response on the presence of nitrate and ammonium. While spinach preferred nitrate to support its growth, water spinach used combined ammonium nitrate. At the end of experiment, the content of nitrate in water spinach medium solution remained high (430 ppm) while it was only 10 ppm in spinach's.

It was expected that GIA13a inoculation could substitute or add certain percent of nitrogen on nutrient solution. However, the inoculant so far did not have significant effect on the growth enhancement of plants, both in the presence of nitrate and ammonium. *Nostoc* sp. GIA13a has been applied in paddy [14]. Compare to Control, paddy planted in soil garden inoculated with strain GIA13a obtained the highest number of dry weight biomass and filled grains. However, application of strain GIA13a in hydroponic system was different to previous application in soil. The growth of inoculant biomass, presence of competitor, and response of plants are several factors that influence the potential of strain GIA13a. The amount of biomass inoculant is important. Along the experiment, biomass population may decrease so that adequate population has to be maintained on the system. In this study, 2 g biomass inoculant (fresh weight) was not enough even with addition of another 2 g at 14 dap.

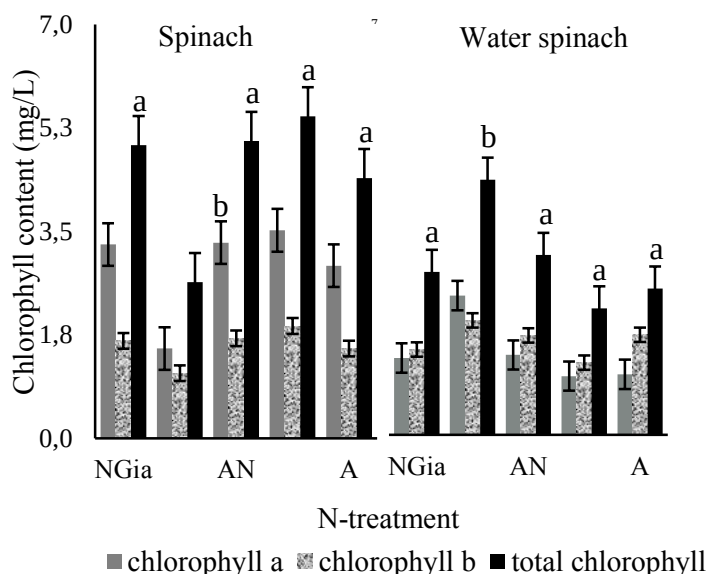


Figure 2. Chlorophyll content of spinach and water spinach at the end of

Another explanation is there may be a competition between strain GIA13a and plants for ammonium. The presence of nitrogen on the environment gives signal to cell to stop heterocyst production so that the activity of nitrogenase is suppressed. There is evidence that the preferential order of nitrogenous

compounds for cyanobacteria is ammonia, followed by nitrate, then urea [15]. Ammonium plays an important role on the GS-GOGAT cycle, as a metabolic pathway of nitrogen assimilation in N₂-fixing cyanobacteria [16]. Ammonium is incorporated into glutamine to synthesize glutamate, one of the amino acids essential for cell metabolism. So, instead of supporting plant growth, addition of strain GIA13a had opposite effect on plant.

Although strain GIA13a did not have effect on the growth of spinach and water spinach, it affected the content of chlorophyll. Inoculation of *Nostoc* GIA13a resulted high performance of total chlorophyll in spinach, but not water spinach (Figure 1). In spinach, statistically, the four Treatments (NGia, AN, AGia, and A) did not have different total chlorophyll content. Significant difference was only showed in Treatment N, when nitrate was provided solely as nitrogen source (2.64 mg/L). Regardless statistical analyses, inoculant treatments (NGia and AGia) had higher results than non-inoculant treatments (AN and A). Total chlorophyll of Treatment AGia was higher (5.45 mg/L) than Treatment NGia (4.96 mg/L). Chlorophyll a spinach of Treatment AGia also showed higher value (3.52 mg/L) than NGia (3.28 mg/L). Combined ammonium-nitrate (Treatment AN) or ammonium as sole nitrogen source (Treatment A) did not affect much on total chlorophyll of spinach.

In water-spinach, the effect of treatments was more vary. Statistically, combined ammonium-nitrate treatment (AN) showed no different value with all treatments. Treatment with nitrate as nitrogen-source (Treatment N) was different significantly with inoculant treatments (AGia and NGia) and ammonium (Treatment A). Total chlorophyll of Treatment N was the highest amongst all treatments. Differences of Treatment N not only showed in total chlorophyll content but also on the content of chlorophyll a. In water spinach, application of *Nostoc* GIA13a did not have effect to the synthesis of chlorophyll.

4. Conclusion

Nitrogen, in the form of nitrate (NO₃⁻), is more suitable for spinach, while water spinach prefer combined nitrogen of ammonium and nitrate. *Nostoc* sp. GIA13a has potential use for “nitrogen supplement” for hydroponic spinach. Addition of *Nostoc* GIA13a can promote the production of spinach chlorophyll in the presence of ammonium.

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