Formation of lateral roots and root hairs of Ciherang rice as a result of *Nostoc* colonization

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Abstract. We performed an interaction study between the cyanobacteria *Nostoc* sp. CPG24 and GIA13a and the roots of Ciherang rice (*Oryza sativa* var. Ciherang) in a hydroponic system. For this purpose, 14-day-old rice seedlings were cultivated in 10× BG11 N-free, aerated liquid medium. The plants were incubated at room temperature and grown in a 12:12 light–dark cycle. To better understand the role of *Nostoc* colonization on root growth, we exposed the rice samples to four different treatments: P1 (0.4 g *Nostoc* CPG24 strain inoculum), P2 (0.4 g *Nostoc* GIA13a strain inoculum), K1 (no inoculation of *Nostoc* and IAA), and K2 (addition of 15 ppm IAA). Regardless of the strain, we found that *Nostoc* filaments were attached to the root surface, particularly in the regions of formation and elongation of lateral roots and root hairs. The root elongation and number of lateral roots were high under the K2 and P2 treatments, whereas they were substantially reduced under the P1 treatment. We demonstrate that the *Nostoc* strain GIA13a induces the formation of new lateral roots, whereas the *Nostoc* strain CPG24 stimulates the formation of root hairs.

Keywords: Ciherang rice, interaction, *Nostoc*, root

1. Introduction

*Nostoc*, nitrogen-fixing cyanobacteria, is commonly found in rice fields [1-3]. Reportedly, the presence of *Nostoc* in rice paddies influences the growth of rice. As nitrogen-fixing bacteria, *Nostoc* fixes atmospheric nitrogen to add ionic ammonium to the soil of rice fields [4-6]. In addition to ammonium, *Nostoc* also produces the plant hormone auxin, triggering a growth response in rice and increasing root colonization by *Nostoc* filaments [7, 8]. Hendrayanti et al. [9] reported that five different Indonesian *Nostoc* strains (CPG8, CPG24, TAB7d, GIA13a, and BAD5) influenced root elongation in the rice variety Ciherang. In addition, of these five strains, a single inoculation of *Nostoc* strains CPG24 and GIA13a influenced plant height and reduced the number of empty grains in harvest [9]. Considering their potential impact on plant growth, further work has been conducted to better understand the interaction between plant roots and the *Nostoc* strains CPG24 and GIA13a.

The interaction between *Nostoc* sp. and rice roots may occur in three distinct forms: attachment, penetration, or symbiotic association [5]. Among these, attachment is the most common, and it occurs when free-living cyanobacteria attach to the surface of the root [10]. *Nostoc* sp. can also inhabit rice root tissue as an endosymbiont. Fitrianti et al. studied the interaction between two *Nostoc* strains (CPG24
and GIA13a) and the roots of Ciharang rice using in vitro culture [11]. In their study, rice seedlings were co-inoculated with both Nostoc strains in a 20-cm test tube containing blue–green 11 liquid medium without a nitrogen source (BG11 N-free). Although Fitrianti et al. showed that the attachment of Nostoc filaments occurred in the epidermis of the root surface, the effect of this attachment on the structure and development of rice root was not identified. Further, the in vitro culture used in the study by Fitrianti et al. had nutrient deficiency and oxygen depletion as its limitations, while also being unable to mimic the growing conditions of a rice paddy. To build on these previous works, we used a hydroponic system that better reflects natural conditions and provides an alternative method to study the interaction of different Nostoc strains with rice roots. Using a hydroponic system, we inoculated rice plants with four different treatments. Herein, we reveal how each treatment affected the overall growth of the root.

2. Experimental

2.1. Nostoc culture

The Nostoc strains CPG24 and GIA13a were isolated from the soil within a rice field. The Nostoc strain CPG24 was isolated from Ciptagelar village, within the National Park of Halimun–Salak, West Java, whereas the Nostoc strain GIA13a was isolated from Gianyar, Bali. The cultures were maintained on BG11 N-free medium, provided with a light intensity of 3000 lux (L:D = 14:10) at room temperature (23–25 °C).

2.2. Selection of rice plants

The Ciharang rice seedlings used for this experiment were selected by soaking the grain in water. Floating grains were discarded, whereas tan grains with brown patches were selected for further use. Then, the selected grains were placed into a container for 14 days at room temperature (30 °C) to allow for germination. Germinated plants were washed thrice using sterile distilled water. We recorded the height of rice seedlings and selected 36 13.03–14.58 cm long plants for cultivation in a 110-mL test tube (d = 5 cm).

2.3. Installation of rice plants in a water culture system

Three individual rice seedlings were wrapped with gauze approximately 1 cm above the cotyledon. Then, group-of-three rice seedlings were inserted into the hole of a styrofoam test tube cap to ensure that the cotyledon of each plant adhered to the bottom side of the styrofoam cap. The tube was filled with 95 mL 10× concentrate of the BG11 N-free medium, and the cap containing the three rice plants was mounted on the mouth of the tube. Each tube was adjusted such as to maintain an approximately 2 cm distance between the root tip and medium. Next, an aerator hose was inserted into the tube, and the level of aeration was set so as avoid the removal of the medium from the tube. The tubes were placed in the room under the same conditions for rice seeding, but only this time, they were provided with light illumination and L:D cycle of 12:12 [12].

Further, we exposed each group of the three rice seedlings to the following treatments: K1 (rice seedlings without inoculation of Nostoc and IAA), K2 (rice seedlings + 15 ppm IAA), P1 (rice seedlings + 0.4 g Nostoc strain CPG24 inoculum), and P2 (rice seedlings + 0.4 g Nostoc strain GIA13a inoculum). The K1 and K2 treatments served as our negative and positive controls, respectively. Each treatment was performed in triplicates. We obtained 0.4 g of fresh weight Nostoc for each treatment by centrifuging 21-day-old culture. We performed a chlorophyll analysis for three samples of 0.4 g of each Nostoc strain. The results for the triplicates of the Nostoc strain CPG24 were 15.813, 15.918, 17.126 mg/L, whereas those for the Nostoc strain GIA13a were 8.69, 8.26, and 7.22 mg/L. Each rice plant was grown in triplicates, totaling 36 individual plants for the experiment. After the inoculation, the tubes containing the plants were placed in a growth chamber (L:D 12:12, 30 °C) for 14 days.
2.4. Data analysis

We obtained four different measures of vegetative growth: root length, number of lateral roots, whole plant fresh weight, and whole plant dry weight. To obtain the number of lateral roots and root hairs, we observed a 1 cm long root section from each plant under a stereo microscope. The presence of IAA in the media was measured using spectrophotometry, and the roots were imaged using Olympus SZX16 stereo and Hirox KH-8700 digital microscopes. For SEM analyses, the root samples were outsourced to the Laboratories of Zoology, Indonesian Institute of Science (LIPI), Cibinong.

3. Results and discussion

At the completion of the experiment, the rice plants reached minimal and maximal heights of 15.45 and 16.57 cm, respectively, whereas the root lengths ranged from 8.25 to 9.08 cm. During the incubation, the plants showed symptoms of chlorosis, and some leaves possessed necrotic spots. The growth of Nostoc filaments was found only at the top of the tube approaching the root (figure 1).

Colonization of the Nostoc filaments was first observed on the 3rd day after the inoculation. As new roots developed on the 7th day, the filaments soon attached to the surface of the root elongation zone. Further observation using SEM demonstrated that the attachment of the Nostoc strain CPG24 was concentrated at the epidermis of the root, particularly at the root division and maturation zones, whereas the Nostoc strain GIA13a was concentrated only at the maturation zone (figure 2). We could experimentally confirm earlier reports by Fitrianti et al. [11] that only the Nostoc strain CPG24 colonized the rice root tip by observing the roots using an SEM and stereo microscope.

The Nostoc strains CPG24 and GIA13a are free living. As the filaments grew and colonized the root, they also adhered to the aerator hose and tube wall of our hydroponic system. The behavior of these two strains of attaching to the substrate within the hydroponic system reflects their benthic lifestyle, which is an adaptation to living in the soil of a rice field. Although these strains were cultured in liquid medium during the experiment, the filament growth patterns did not change to the planktonic type.

The colonization of the root filaments is in response to the presence of chemical signals released by rice root, including mannose, galactose, glucuronic acid, amino acids, and growth hormones [13]. Nostoc is able to biosynthesize its carbon sources, a result of the photosynthetic abilities of cyanobacteria. However, Nostoc obtains and utilizes amino acids and hormones from the environment, a process that is more efficient for Nostoc than independent biosynthesis. Conversely, the root obtains benefits from the Nostoc filament colonization of the root tip (known as division zone),

![Figure 1](image1.jpg) (a) (b) (c)

**Figure 1.** Rice roots after 14 day co-cultivation. The Ciherang rice root (a, b) with or (c) without Nostoc attachment. (a) Colonization of the Nostoc strains CPG24, (b) GIA13a, and (c) filaments on the roots. The colonization by filaments is marked with a black arrow.
Figure 2. Visualization of roots using (a, b, and d) scanning electron, and (c) digital 3D microscopes. (a) Filaments of the *Nostoc* strain GIA13a attached on the surface of a P2 treatment root; (b) the *Nostoc* strain CPG24 filaments on the root during the P1 treatment; (c) colonization of the *Nostoc* strain CPG24 filaments at the meristematic and elongation zones of the root tip under the P1 treatment (120× magnification); and (d) the root tip of P2 (inoculation with GIA13a) showing no colonization by the filaments. *Nostoc* filaments are indicated with an arrow.

Table 1. Effect of various treatments on lateral roots and root hairs and the concentration of IAA.

| Treatments | Mean of root elongation (mm) | Lateral root Number (per cm) | Lateral root Length (mm) | Hair root presence* | Concentration of IAA (ppm) |
|------------|-------------------------------|-----------------------------|--------------------------|---------------------|---------------------------|
| K1         | 124                           | 22                          | 0.11–1.80                | ++                  | 2.58                      |
| K2         | 140                           | 29                          | 0.21–7.35                | ++                  | 3.04                      |
| P1 (CPG24) | 114                           | 16                          | 0.22–2.67                | +++                 | 3.20                      |
| P2 (GIA13a) | 148                          | 26                          | 0.27–1.65                | +                   | 2.00                      |

* +++: abundance; +: rare

wherein *Nostoc* secretes a mucilaginous sheath to the outer layer of its cell wall made of polysaccharides [14], protecting the sensitive root tip and meristematic cells from damage.

The root measurements from all treatments revealed that inoculating with the GIA13a strain (P2) resulted in the highest root elongation (table 1).

Further, the treatment with IAA alone (K2) had greater root elongation than that with K1 and P1. Table 1 also shows the performance of lateral and hair roots. All treatments with the addition of *Nostoc* or IAA had longer lateral roots when compared with the negative control (K1). Inoculation with the strain GIA13a increased the number of lateral roots (26 roots/cm) but did not greatly affect the amount of root hairs. In contrast, the treatment with the strain CPG24 had no significant effect on the number of lateral roots (16 roots/cm), but it increased the presence of root hairs. Therefore, the two strains have
Table 2. Average fresh and dry weights of the rice roots after 14-day co-cultivation.

| Number of repetitions | Average fresh weight (g) | Average dry weight (g) |
|-----------------------|--------------------------|------------------------|
|                       | K1          | K2          | P1          | P2          | K1          | K2          | P1          | P2          |
| 1                     | 0.198      | 0.179      | 0.288      | 0.170      | 0.019      | 0.018      | 0.030      | 0.012      |
| 2                     | 0.211      | 0.195      | 0.275      | 0.150      | 0.022      | 0.019      | 0.027      | 0.010      |
| 3                     | 0.185      | 0.170      | 0.226      | 0.170      | 0.019      | 0.016      | 0.023      | 0.010      |
| 4                     | 0.168      | 0.171      | 0.235      | 0.170      | 0.019      | 0.024      | 0.030      | 0.011      |
| 5                     | 0.178      | 0.210      | 0.240      | 0.150      | 0.019      | 0.022      | 0.030      | 0.012      |
| 6                     | 0.168      | 0.176      | 0.201      | 0.160      | 0.021      | 0.022      | 0.026      | 0.013      |
| Average               | 0.185      | 0.184      | 0.244      | 0.162      | 0.019      | 0.020      | 0.028      | 0.011      |

different effects on the growth of rice roots. Interestingly, the combination of *Nostoc* (the strain GIA13a or CPG24) and IAA results in a negative effect on root growth, with roots becoming shorter and having a reduced number of lateral roots. Sergeeva et al. previously reported that some free-living and symbiotic *Nostoc* strains have the ability to produce substances similar to IAA, an auxin hormone [15]. IAA plays a role in inducing the formation of lateral roots [16] and the formation and elongation of root hairs [17]. The present study demonstrates that inoculation with *Nostoc* influences the formation or elongation of lateral roots or root hairs, depending on the strain.

Six individual plants for each treatment were selected for weighing (table 2). Notably, there were no significant differences in terms of fresh or dry weight of roots in all treatments. The incubation time (14 days) was possibly too short to notice significant changes in root weight. In general, the average heights of the rice plants observed under all treatments (15.45–16.57 cm) were normal based on the study by Moldenbauer et al. for 14 day old rice crops (10–20 cm high) [18].

4. Conclusion
Colonization of the *Nostoc* strains CPG24 and GIA13a on the Ciherang rice roots influences the formation or elongation of lateral roots and root hairs. The *Nostoc* strain GIA13a induces the formation of new lateral roots, whereas the *Nostoc* strain CPG24 stimulates the formation of root hairs.

References
[1] Sinha R P and Hader D P 1996 *Photochem. Photobiol.* 64 887-96
[2] Prasanna R and Nayak S 2007 *Wetlands Ecol. Manag.* 15 127-34
[3] Choudhary K K 2011 *Bangladesh J. Plant Taxon.* 18 73-6
[4] Vaishampayan Aet al. 2001 *Bot. Rev.* 67 453-516
[5] Nilsson M, Bhattacharya J, Rai A N and Bergman B 2002 *New Phytol.* 156 517-25
[6] Mishra U and Pabbi S 2004 *Resonance* 9 6-10
[7] Hashtroudi M S, Ghassempour A, Riahi H, Shariatmadari Z and Khanjir M 2013 *J. Appl. Phycol.* 25 379-86
[8] Hussain A, Shah S T, Rahman H, Irshad M and Iqbal A 2015 *Front. Plant Sci.* 6 46
[9] Hendrayanti D, Kusmadji L R, Yuliana P, Amanina M A and Septiani A 2012 *Makara J. Sci.* 16 203-8
[10] Ahmed M, Lucas J S and Hasnain S 2010 *Plant Soil* 336 363-75
[11] Fitrianti A, Hendrayanti D and Kusmadji L R 2012 *Proc. Int. Conf. on Life Science & Biological Engineering* (Amsterdam: North-Holland/American Elsevier) p 517
[12] Podar D 2013 *Plant growth and cultivation Plant Mineral Nutrients: Methods and Protocols* ed Maathuis F J M (New York: Springer Science+Business Media)
[13] Bacilio-jiménez M, Aguilar-Flores S, Ventura-zapata, Perez-campos E, Bouquelet S and Zenteno E 2003 Plant Soil 249 271-7
[14] Kumar K, Mella-Herrera R A and Golden J W 2010 Cyanobacterial heterocysts Cell Biology of Bacteria (New York: Cold Spring Harbor Laboratory Press)
[15] Sergeeva E, Liaimer A and Bergman B 2002 Planta 215 229-38
[16] McSteen P 2010 Cold Spring Harb. Perspect. Biol. 2 a001479
[17] Chen Y H, Chen Y Y, Shu Y Y, Hong C Y and Kao C H 2012 Plant. Cell Rep. 31 1085-91
[18] Moldenbauer K, Counce P and Hardke J 2013 Rice Growth and Development Arkansas Rice Production Handbook ed Hardke J T (Arkansas: University of Arkansas Division of Agriculture) chapter 2 pp 9-20