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THE EFFECT OF ISOLATED CYANOBACTERIA ON RICE SEED GERMINATION AND GROWTH

Cyanobacteria make a major contribution to the fertility of soil. It has been suggested that cyanobacteria assist higher plant growth by supplying growth substances. There are numerous works about effect of cyanobacteria in paddy soils and rice growth in the world but very little research has been carried out in Afghanistan. In this research, cyanobacteria *Anabaena* sp were isolated from paddy soils of Baghlan Province of Afghanistan. The isolated cyanobacteria *Anabaena* sp was used as inoculums on three common varieties of Afghanistan's rice separately for two hours. After 10 days the result revealed that inoculation of rice seeds by cyanobacteria extracts can enhance seed germination, plant height and length of root in all treated seeds in comparing seeds control by water. In the next stage, seeds under experiment separately inoculated by cyanobacteria extracts and cultivated in nonorganic silt soil as well as treated by cyanobacteria extracts. After 12 days the morphological view showed differences in shoot height of treated plants in comparing to plant controlled by water. Therefore cyanobacteria *Anabaena* sp. recommended as an effective biofertilizer in paddy fields of Afghanistan.

Key words: Cyanobacteria, inoculation, biofertilizer, Baghlan, Shamarq, Chashmaishir.

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Күріштің өсуі мен тұқымның өнуіне бөлініп алынған цианобактериялардың әсері

Цианобактериялар топырақтың құнарлылығына үлкен үлесін қосады. Цианобактериялар өсімдіктердің жоғары деңгейде өсіп дамуына әсер ететіндігі жайлы мәліметтер бар. Әлем бойынша цианобактериялардың күріштің өсуіне және топыраққа әсері жайлы жұмыстар өте көп кездеседі, дегенмен Ауғанстанда бұл зерттеулер өте аз жүргізілген. Бұл зерттеуде *Anabaena* sp. цианобактериясы Ауғанстанның Баглан қаласы провинциясының топырағынан бөлініп алынды. Бөлініп алынған *Anabaena* sp. цианобактериясы құйылған ыдыста, Ауғанстанның үш түрлі күріштері екі сағат бойы ұсталынды. 10 тәуліктен соң, цианобактерия экстрактісінде ұсталынған күріш дәндерінің, өсімдіктің ұзындығы және тамыр ұзындығы, суда ұсталынған дәндермен салыстырғанда, артқаны байқалады. Тәжірибенің келесі кезеңінде күріш дәндері цианобактериямен байытылған топырақта және сумен байытылған топырақтарда өсірілді. 12 тәулік өткен соң цианобактериямен байытылған топырақта өскен өсімдіктің, сумен байытылған топырақта өскен өсімдіктен ұзындығымен, морфологиясы бойынша әртүрлілігін көрсетеді. Сондықтан, *Anabaena* sp цианобактериясы Ауғанстанның күріш алқаптарына тиімді биотыңайтқыш ретінде ұсыныла алады.

Түйін сөздер: цианобактерия, инокуляция, биотыңайтқыш, Баглан, Шамарк, Чашмайшир.

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Влияние выделенных цианобактерий на прорастание семян и рост риса

Цианобактерии вносят большой вклад в плодородие почвы. Показано, что цианобактерии синтезируют вещества, стимулирующие более высокий рост растений. Существует множество работ о влиянии цианобактерий на урожайность рисовых полей, но очень мало исследований проведено в Афганистане. В этом исследовании цианобактерия *Anabaena* sp. была выделена из рисовых полей провинции Баглан, Афганистан. Выделенную цианобактерию *Anabaena* sp. использовали в качестве инокулята на трех разновидностях риса Афганистана в течение двух часов. По истечению 10 суток было показано, что инокуляция семян риса цианобактерией улучшила всхожесть семян, рост растений и длину корня во всех обработанных семенах по сравнению с контролем. На следующем этапе эксперимента семена подвергали отдельной инокуляции экстрактами цианобактерии и культивировали в неорганической иловой почве. Через 12 дней результаты опыта показали различия в высоте побегов обработанных растений по сравнению с контролем. В связи с этим цианобактерия *Anabaena* sp. рекомендуется в качестве эффективного биоудобрения для рисовых полей Афганистана.

Ключевые слова: цианобактерия, инокуляция, биоудобрения, Баглан, Шамарк, Чашмайшир.

Introduction

Cyanobacteria are photosynthetic prokaryotes and colonizing microorganisms that are found throughout the world and they are exceptionally well adapted to a wide array of environmental conditions (Paerl et al., 2000:11-26, Karthikeyan et al., 2008:87-91 and Kirlwood et al., 2008:453-465). These organisms are one of the major components of the nitrogen fixing biomass in paddy fields. The agricultural importance of cyanobacteria in rice cultivation is directly related with their ability to fix nitrogen and other positive effects for plants and soil. After water, nitrogen is the second limiting factor for plant growth in many fields and deficiency of this element is met by fertilizers (Malik et al. 2001:1217-1220). The excessive use of chemical fertilizers has generated several environmental problems including the greenhouse effect, ozone layer depletion and acidification of water. These problems can be tackled by use of biofertilizers (Choudhury and Kennedy 2005:1625-1639, Rai 2006:1-28). Biofertilizers, more commonly known as microbial inoculants, include bacteria (*Azotobacter*), cyanobacteria and mycorrhizal fungi; they are natural, beneficial and ecological friendly, they also provide nutrients for the plants and maintain soil structure (Board 2004:9-49). Over the past six decades, reports have been published on the use of cyanobacterial inoculants (algalization) to enhance biological N₂ fixation in wetland rice fields. Cyanobacteria play an important role in maintenance and build-up of soil fertility,

consequently increasing rice growth and yield as a natural biofertilizer (Song et al. 2005:131-140). The acts of these algae include: (a) Increase in soil pores with having filamentous structure and production of adhesive substances. (b) Excretion of growth-promoting substances such as hormones (auxin, gibberellin), vitamins, amino acids (Roger and Reynaud 1982:147-168, Rodriguez et al. 2006:1-4). (c) Increase in water- holding capacity through their jelly structure (Roger and Reynaud 1982:147-168). (d) Increase in soil biomass after their death and decomposition. (e) Decrease in soil salinity. (f) Preventing weeds growth. (g) Increase in soil phosphate by excretion of organic acids (Wilson 2006:9-10). The indirect promotion of plant growth occurs when cyanobacteria prevent or counter deleterious effects of one or more phytopathogenic microorganisms (Moussa and Shanab, 2001:267-281, Rizk, 2006:212-215, Kim, 2006:138-142, Abo-Shady et al., 2007:3029-3038, Tassara et al., 2008:487-492, Kim and Kim, 2008:242-248). Several studies have reported that cyanobacteria can improve the plant growth by improving the soil structure as they have potential to secrete extracellular polysaccharides that help in soil aggregation and water retention (Hill et al., 1994:126-148, Mazor et al., 1996:121-130, Maqubela et al., 2009:79-92). In addition, the use of cyanobacteria can increase the carbon and nitrogen status of soil (De Caire et al., 2000:1985-1987, De Cano et al., 2002:2421-2431, Azia and Hashem, 2004:309-3011, Pandey et al., 2005:451-457, Malam Issa et al., 2007:209-219,

Obana et al., 2007:641-646, Nisha et al., 2007:49-56, Swarnalakshmi et al., 2007:307-313, Maqubela et al., 2009:79-92, Saadatnia and Riahi, 2009:207-212, Maqubela et al., 2009:79-92). Most paddy soils have a natural population of cyanobacteria which provides a potential source of nitrogen fixation at no cost (Mishra and Pabbi 2004:6-10). The paddy field ecosystem provides a favorable environment for the growth of cyanobacteria with respect to their requirements for light, water, high temperature and nutrient availability. This could be the reason for more abundant cyanobacteria growth in paddy soils than in upland soils (Roger and Reynaud 1982:147-168, Kondo and Yasuda 2003:105-111). In this research we studied the effect of isolated cyanobacteria on seed germination and plant growth on three common varieties of Afghanistan's rice (IR rice, Long red rice and Medium red rice)

Materials and methods

The aim of this research was effect of isolated cyanobacteria as a biofertilizer on three common varieties of rice plants in Afghanistan.

Soil samples were collected from the depth of 0–5 cm of soil surface in paddy fields of Shamarq and Chashmaishir villages of Baghlan province in the north east of Afghanistan in August, 2016. Soil sample transferred to the laboratory of photobiotechnology, Faculty of Biology and Biotechnology, al-Farabi KazNU. Standard microbiological and algological research methods carried out for isolation and purification of nitrogen fixing cyanobacteria used the bellow nutrient mediums.

Gromov's No6 nutrient medium consisted of (per liter of distilled water) NaNO_3 (1g), K_2HPO_4 (0.2g), $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ (0.2g), CaCl_2 (0.15g), NaHCO_3 (0.2g), EDTA (1ml), solution of microelement¹ (1ml) and solution of microelement² (1ml).

BG11 nitrogen free medium consisted (per liter of distilled water) K_2HPO_4 (40mg), $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ (75mg), $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ (36mg), Na_2CO_3 (20mg), Citrate Na(6mg), FeNH_4 Citrate(6mg), Solution of microelements¹, 1ml) and solution of microelement², (1ml). The collected soil was cultured in Petri dishes with a sterile Gromov's No6 liquid nutrient medium. After 14 days of cultivation, green algae grown on the surface of cultured medium in Petri dishes.

Green specimen of microalgae from the surface of cultured medium took by loop and transferred to the sterile liquid nutrient medium in the sterile flasks under the condition of lab box. The cultured

flasks were placed under the light (2000 lux) for 10 days. After 10 days the cultured growth and made the color of liquid nutrient green. For dilution and repeating the culture, 1ml of specimen from the liquid culture was took by pipette and transferred to sterile liquid nutrient medium in conical flasks, while the liquid was poured into the flasks in such a way that the volume occupied was not more than 1/3 – 1/4 of the volume of the flask. The culture was placed under the same intensity of light for 12 days. The specimen from the growth culture took by loop and transferred to the new sterile BG11 nitrogen free medium in the sterile flasks and placed under the same light condition 2000 lux. Algological pure culture was isolated by proper microscoping control (Gollerbah et al., 1951,644). The cultures in the liquid medium were hand shaken daily to prevent sticking of the cyanobacteria to the wall of the container.

To evaluate the activity of cyanobacteria, a suspension of cyanobacteria. A volume of 1 ml or more, depending on the purity of the storage culture, was transferred immediately to Petri dishes on frozen nutrient agar mixed with Gromov's medium and distributed on the surface of the agar with a sterile spatula.

For purification of cyanobacteria 0.1gr antibiotic (ampicillin) added to 1ml of water. 30 micro liters from the suspension antibiotic were distributed on the surface of each Petri dish with a sterile spatula.

The Petri dishes were placed in the light until the colonies formed. From the grown colony, specimen was transferred again to a liquid medium by loop. Pure culture of cyanobacteria *Anabaena* sp. was grown in conical flasks 250 – 1000 ml under the light (2000 lux) and temperature of 27 – 28 °C. Microscopy of isolated cultures of cyanobacteria is carried out with the help of MX 300T (Austria) and Axio Imager A1 («Carl Zeiss», Germany) microscopes (Gollerbah et al., 1951,644).

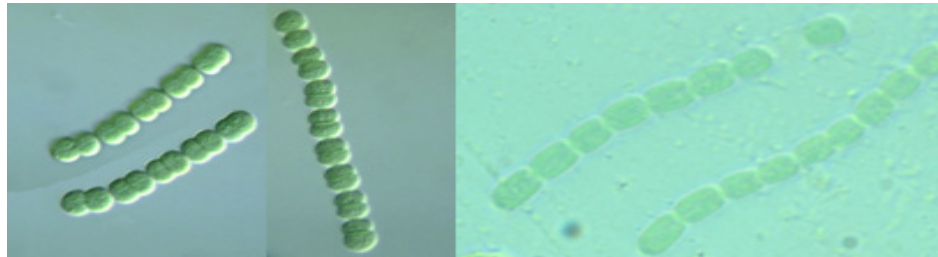
- Germination of seeds: Three variety of common rice (IR rice, medium red rice and long length red rice) collected from Baghlan province of Afghanistan and transferred to the laboratory of photobiotechnology, Faculty of Biology and Biotechnology, al-Farabi KazNU.

Petri dishes were prepared in the following way: sterile filter paper corresponding to the size of the cups bottom was placed at the bottom of sterile cups. Petri dishes were put in autoclave for one hour. Seeds of rice were soaked for two hours in suspensions of cyanobacteria extracts and in sterile water (control), and then seeds were placed in Petri dishes in the amount of 8 pieces in each

cup. Cultured seeds were placed under the light for 10 days and every 24 hours the seeds under experiment were treated with 1ml of sterile water. After 7 days germination of seeds, total length of shoots and roots measured by ruler in each Petri dish and counted the number of not germinated seeds for each cup.

- **Seed cultivation on soil:** Nonorganic silt soil were incubated and put in plastic cups not occupied

more than 3/4 parts of the cups. As previous new seeds of the test plants were soaked for one hour in suspensions of cyanobacteria and in sterile water to control. Then the amount of 10 seeds cultivated in soil of each cups. Cups were placed under the light for 12 days and every 24 hours the cultivated seeds in experiment were treated with 10 ml cyanobacteria extracts and sterile water to control for 12 days (Saadatnia and Riahi., 2009:207-212).



Culture of Cyanobacteria anabaena sp (Zoom. 1x 100)

Figure 1 – The morphology of isolated cyanobacteria, Anabaena sp.

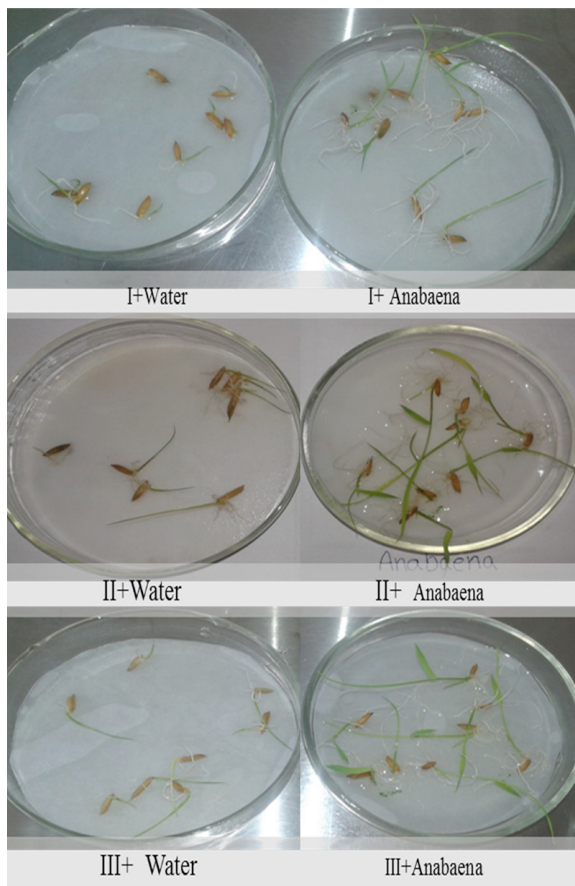


Figure 2 – Three varieties of rice (I-IR rice , II-Long red rice and III-Medium red rice) inoculated by cyanobacteria Anabaena sp. and soaked by water to control

Result and discussion

In the result of conducted research on soil of paddy fields in Afghanistan (Shamarq and Chashmaishir villages of Baghlan province). Pure culture of cyanobacteria of *Anabaena sp.* was isolated with the help of standard microbiological and algological research methods and morphologically identified with the help of MX 300T (Austria) and Axio Imager A1 («Carl Zeiss», Germany) microscopes (figure1).

According to the evaluation of conducted research, isolated cyanobacteria *Anabaena sp* had positive effect on three common varieties of Afghanistan's rice (IR rice, Long red rice and medium red rice). These varieties have good adaptation in Baghlan province. Germination of Inoculated seeds by caynobacteria extracts after 10 days compared to water control, the percentage of germinated seeds was 100 (figure 2). The result shows that caynobacteria *Anabaena sp.* had a positive effect on the growth of rice (germination, height of shoot and length of root system). The root length and plant height of one of the average plant from each experimented variety was measured by ruler, showed differences (figure3) as well as the graph of measuring showed differences (figure4).

In the next stage new seeds of the three common varieties Afghanistan's rice was separately cultivated in nonorganic silt soil and every 24 hours the cultivated seeds under the experiment

were treated by 10 ml cyanobacteria extracts and sterile water to control. After 12 days the result showed that there are morphological differences

in height of treated plant by cyanobacteria *Anabaena* sp. in comparing to plant treated by water (figure5).

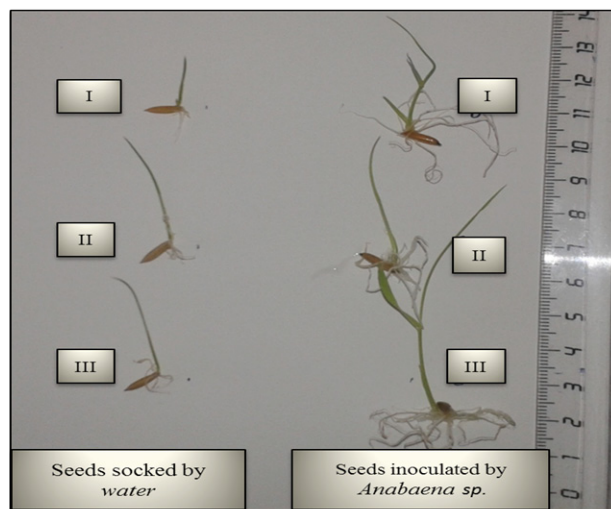


Figure 3 – The average effect of cyanobacteria *Anabaena* sp. on the root length and plant height of three varieties of rice (I-IR rice , II-Long red rice and III-Medium red rice) in comparing to water control

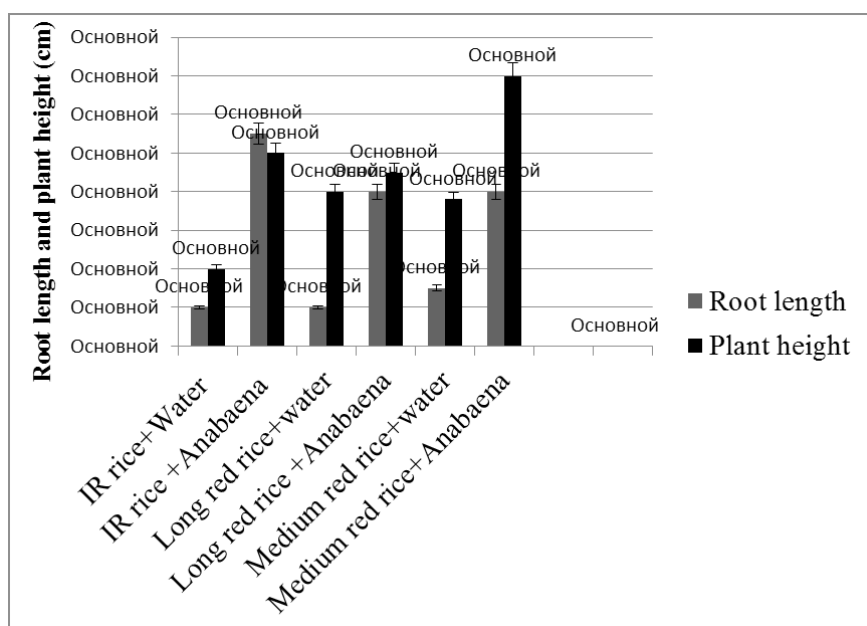


Figure 4 – The average effect of cyanobacteria *Anabaena* sp. on the root length and plant height of three varieties of rice (IR rice , Long red rice and Medium rice) in comparing to water control

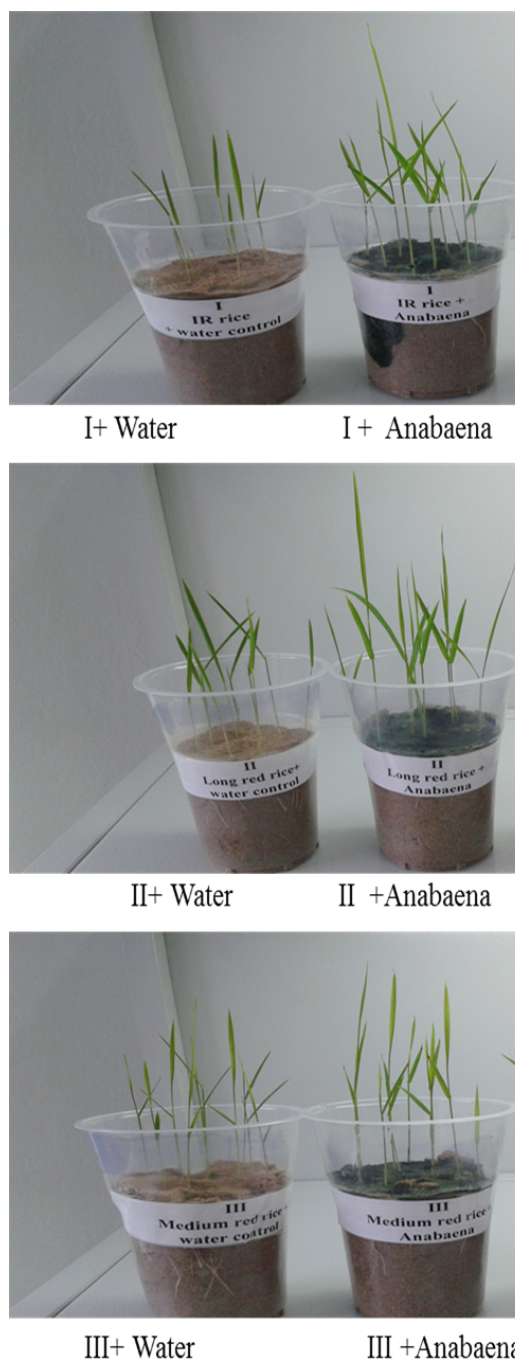


Figure 5 – Shows the morphological comparing of three varieties of rice (I-IR rice, II-Long red rice and III-medium red rice) treated by *Anabaena* sp. and compared to water treated seeds from the same variety in soil

Conclusion

Cyanobacteria are photosynthetic and prokaryotic microorganisms they are found in all over the world. They contribute the growth of plant by supplying growth substances. In this research,

cyanobacteria *Anabaena* sp was isolated from paddy soils of Baghlan Province in Afghanistan and used as inoculums on three common varieties of Afghanistan’s rice (IRrice, Long red rice and medium red rice). The result revealed that treatment of rice seeds by cyanobacteria extracts can enhance seed

germination and plant growth in all treated seeds as compared to control. In the next stage, cyanobacteria as inoculums experimented on cultivated rice in soil. The morphological view also showed differences

in shoots height of treated plants as compared to control by water. Therefore cyanobacteria *Anabaena* sp. is recommended as an effective biofertilizer in paddy fields.

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