ENHANCEMENT OF SOYBEAN (GLYCINE MAX L.) GROWTH BY BIO-FERTILIZERS OF NOSTOC MUSCORUM AND NOSTOC RIVULARE

ESSAM NAGEH SHOLKAMY*, HESHAM M. EL-KOMY2 AND HAYSSAM M. ALI1

1Department of Botany and Microbiology, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia
2Department of Botany and Microbiology, Faculty of Science, Minia University, Minia, Egypt
3Department of Timber Trees Research, Sabahia Horticulture Research Station, Horticulture Research Institute, Agriculture Research Center, Alexandria, Egypt
*Corresponding author’s e-mail essam_92003@yahoo.com

Abstract

In the present study the nitrogenase activity of Nostoc muscorum and Nostoc rivulare was evaluated in vitro; the test showed that Nostoc muscorum and Nostoc rivulare have the ability to fix nitrogen. In a pot experiment under field conditions, the results of the present study showed that inoculation of the soybean plant with Nostoc muscorum and Nostoc rivulare, either alone or in combination with N-fertilizer at 50 and 100 kg N/ha, caused a significant increase in the growth of these plants, as reflected in plant height, leaf area, weight of plant as well as the legume weight of soybeans. The combination of biofertilization and N-fertilization, especially at 100 kg N/ha, had more effect on both the growth of soybeans and nitrogenase activity compared to biofertilization alone. Nostoc muscorum and Nostoc rivulare are a promising biofertilizers for achieving an efficient association between N2 fixing cyanobacteria and soybeans; and thus enhancement of the growth.

Key words: Cyanobacteria, Soybean, Biofertilization, Ammonium sulphate.

Introduction

The sandy soils in Saudi Arabia have 2 major problems in respect to agriculture: low fertility and inadequate water retention. Nitrogen is a vital element for animal and plant growth, since it is an essential part of many of the chemical compounds, such as proteins and nucleic acids, which are the basis of all life forms. It spite of its abundance (it constitutes 78% of the earth’s atmosphere) it is one of the most limiting factors for crop growth. The reserves of nitrogen present in the soil and available for use by plants are continually depleted by such processes as microbial de-nitrification, soil erosion, leaching, chemical volatilization and, perhaps most important, the removal of nitrogen containing crop residues from land. The nitrogen reserve of agricultural soils must therefore be replenished periodically in order to maintain an adequate level for crop production. This replacement of soil nitrogen is generally accomplished by the addition of chemically fixed nitrogen in the form of commercial inorganic fertilizers or by the activity of biological nitrogen fixation systems (Schoot, 1990). Cyanobacteria are friendly biofertilizers for rice based collecting systems, being the major components of wetland rice ecosystems. They are, in addition, easily available and serve as inexpensive sources of natural biofertilizers (Omar, 2000; Ladha & Reddy, 2003; Vaishampayan et al., 2001; Ahamd et al., 2014). The association between cyanobacteria and plants can profit crop plants by improving the physical and chemical properties of the soil because cyanobacteria have ability to produce the biologically fixed nitrogen, growth promoting substances and different types of secondary metabolites and significant amounts of organic matter which have been solubilized otherwise insoluble phosphates (Goyal, 1993). These processes are called nitrogen fixation, and may be either chemical or biological. The increasing international emphasis on environmentally sustainable development through the use of renewable resources is likely to focus attention on the potential role of biological nitrogen fixation in supplying nitrogen for agriculture (Dixon & Wheeler, 1986; Peoples, et al., 1995). This expanded interest in ecology has drawn attention to the fact that BNF is ecologically safe and that its greater exploitation could reduce the use of fossil fuels and be useful in reforestation and the restoration of misused lands to productivity (Sprent & Sprent, 1990; Burris, 1994).

Several plants form a special symbiosis with the photosynthesizing cyanobacteria, such as the herb Gunnera with Nostoc (Bergman et al., 1992) and the tiny water fern, Azolla, with the filamentous Anabaena (Braun-Howland & Nierzwicki-Bauer, 1990). Cyanobacteria are normally located in the intercellular spaces of the tissues except in the case of Gunnera, where bacteria are present inside the cells of the shoot apex (Bergman et al., 1992). Sholkamy et al. (2012) reported a potential role of Nostoc muscorum and Nostoc rivulare as biofertilizers for the enhancement of maize growth under different doses of n-fertilizer; this present study extends this work by aiming to isolate Nostoc spp. and to evaluate their role in the enhancement of soybean growth in sandy soil.

Material and Methods

Isolation, purification and identification of Nostoc: One ml of dilute soil suspension was transferred to each sterile Petri dish. Melted but cooled nitrogen free BG11 medium was then poured into the Petri dish (Rippka & Herdman, 1992) and this was supplemented with agar for solidification (1%), at a kept at a light intensity of 3000 lux.
Three plates were prepared for each sample; the cultures from each plate were incubated at 24°C ±1 for 15 days according to the method described by Abdel-Hafez et al. (2000). Developing cultures were identified according to Desikachary (1959). Pure isolates were maintained on N2 containing BG11 medium for further studies. After 15 days, cyanobacterial cultures were harvested by centrifugation (8000 rpm for 10 min), suspended in sterile H2O (4g/ 250 ml), and used as an inoculum at an amount of 10⁶ heterocysts per seed of wheat plant (El-Shahed, 2005).

**In vitro evaluation of nitrogenase activity of two Nostoc isolates:** The nitrogenase activity of two of the cyanobacterial isolates was evaluated in the 15 days’ old culture using the acetylene reduction technique, which was described by Hardy et al. (1973). For this evaluation, a Gas Chromatograph, ATIUNICAM 610-GLC (UK) equipped with a glass column filled with activated alumina, was used.

**Pot experiments of soybean:** Surface sterilized soybean seeds (Glycine max L.) were left to germinate on wet sterile filter paper in Petri dishes for three days in the dark. Six grains were transplanted into a pot containing 2 kg of a mixture of sand and clay soil in a ratio of 2:1 respectively. The pots were then inoculated with N. muscorum or N. rivulare (at 10⁶ heterocysts per seedling). One week later, a dose of Ammonium sulphate (at either 100 or 50 kg N/ ha) was added on three periods (i.e. the 100 kg N/ha dose was added in three periods, each of 33.3 kg N/ha). The pots were divided into nine groups with three replicates for each treatment, as illustrated in Figs. (8a, b, c: 1). Seedlings of the first group were not inoculated with N. rivulare or N. muscorum, and received no additional dose of ammonium sulphate. 2) Seedlings of the second group were also not inoculated with N. rivulare or N. muscorum, but were treated with half a dose of ammonium sulphate (50 kg N / ha). 3) Seedlings of the third group were not inoculated with N. rivulare or N. muscorum, but were treated with a full dose of ammonium sulphate (100 kg N/ha). 4) Seedlings of the fourth group were inoculated with N. rivulare, but received no ammonium sulphate. 5) Seedlings of the fifth group were inoculated with N. rivulare, and were also treated with a half dose of ammonium sulphate. 6) Seedlings of the sixth group were inoculated with N. rivulare and a full dose of ammonium sulphate. 7) Seedlings of the seventh group were inoculated with N. muscorum, but no ammonium sulphate. 8) Seedlings of the eighth group were inoculated with N. muscorum and a half dose of ammonium sulphate. 9) Seedlings of the ninth group were inoculated with N. muscorum and a full dose of ammonium sulphate. At the beginning of the second week after sowing, plants were thinned down to two plants per pot. The experiment was performed in a wire proof greenhouse maintained at 26 ± 5°C under natural daylight. Pots were irrigated with water as needed according to field capacity. A month after cultivation, soybean plants were harvested and growth parameters and nitrogenase activity were determined.

**Determination of plant growth:** The length of the tested plants were measured (cm) and they were then weighed in order to find their fresh weight (mg) and then dried in an oven at 105°C to a constant mass for further analysis. Leaf area was determined according to Norman & Campbell (1994). Chlorophyll a, Chlorophyll b and carotenoids were determined using the spectrophotometric method recommended by Metzner et al. (1965). The versene (disodium dihydrogen ethylenediamine tetraacetie acid) titration method (Schwarzenbeck & Biederman, 1948) was employed for the determination of Ca²⁺ and Mg²⁺ concentrations. Sodium and potassium were determined in the samples photometrically by flame photometry according to the method of Golterman et al. (1978).

**Statistical analysis:** The triplicate sets of data for the various parameters evaluated were subjected to ANOVA (Analysis of variance) in accordance with the experimental design (i.e. a completely randomized design) using the SPSS11 statistical package in order to quantify and evaluate L.S.D values, which were calculated at a P level ≤ 0.05 (Steel & Torrie, 1960).

**Results**

The results showed that both of the cyanobacterial isolates tested have the ability to fix nitrogen. As shown in Figs. (1, 2, 3, 4, 5, 6 and 8) shoot length, root length, leaf area, plant weight and the number of legumes increased significantly with the addition of combined nitrogen compared to plants not fertilised with nitrogen. The results also indicated that both half and full doses of N-fertilizer, together with N. muscorum and N. rivulare inoculation, significantly increased soybean growth as well as legume number and legume weight compared to inoculation with N. muscorum or N. rivulare alone. N. muscorum was more effective in enhancing the growth of the soybean plants compared to inoculation with N. rivulare. The results in Fig. 7 show that the addition of a half or full dose of combined nitrogen caused an increase in pigement content, with biofertilization also improving from pigement content. The results also indicated that the growth of soybean plants inoculated with N. muscorum together with a half dose or full dose of combined nitrogen significantly increased compared with the growth of soybean plants inoculated with N. rivulare at half dose or full dose of combined nitrogen. Overall, therefore, it was evident that N. muscorum and N. rivulare demonstrated a nitrogen fixing capacity on soybean roots. Furthermore, in comparison to non-fertilised plants, this nitrogen fixing activity was enhanced by the addition of N-fertilizer. Pot experiments, in which large pots containing non-sterilized sandy soil were used to test the ability of cyanobacteria to improve the growth of tested plants under field conditions. Results of the present study showed that inoculation of soybean plant with N rivulare and N. muscorum caused a significantly increase in growth of this plant, represented in plant height, leaf area, weight of plant as well as legume weight of soybean, pigment and minerals content.
Fig. 1. Effect of *N. rivulare* and *N. muscorum* inoculation on the shoot length of the soybean plant with N-fertilization at half and full dose. L.S.D (5%) = 2.86

Fig. 2. Effect of *N. rivulare* and *N. muscorum* inoculation on fresh weight of the soybean plant with N-fertilization at half and full dose. L.S.D (5%) = 0.39

Fig. 3. Effect of *N. rivulare* and *N. muscorum* inoculation on the root length of the soybean plant with N-fertilization at half and full dose. L.S.D (5%) = 2.13.

Fig. 4. Effect of *N. rivulare* and *N. muscorum* inoculation on the dry weight of the soybean plant with N-fertilization at half and full dose. L.S.D (5%) = 0.033.

Fig. 5. Effect of *N. rivulare* and *N. muscorum* inoculation on the leaf area of the soybean plant with N-fertilization at half and full dose. L.S.D (5%) = 40.

Fig. 6. Effect of *N. rivulare* and *N. muscorum* inoculation on the fresh weight of soybean legume with N-fertilization at half and full dose. L.S.D (5%) = 0.04
Results of the present study showed that shoot length, root length, leaf area, plant weight and the number of legumes increased significantly with the addition of combined nitrogen compared to plants not fertilised with nitrogen. These results are in accordance with the recent findings of Nanjappan et al. (2007), Sholkamy et al. (2012) and Al-Noaim & Hamad (2004) who established that cyanobacteria improved the growth, respectively, of wheat, maize and rice grown in pot experiments. Additionally, the stimulatory effect of tested cyanobacteria on the growth of the tested plants agrees with the earlier findings of Obreht et al. (1993) and Rai et al. (2000) who established that strains of Nostoc and Anabaena positively affect plant growth by making a high contribution to N2 fixing activity in liquid and soil cultures. Growth stimulation by cyanobacteria isolates could be attributed to production of auxins / bioactive molecules (Sergeeva et al., 2002), production of secondary metabolites linked to the bio-control of bacterial and fungal diseases (Biondi et al., 2004) or improvements to soil structure through secretion of mucilage and polysaccharides assisting soil aggregation (Gantar et al., 1993; Aziz & Hesham, 2003 and 2004; Nisha et al., 2007). These results also showed that inoculation of soybean plants with N. muscorum isolate had a stronger positive effect on the growth of these plants than inoculation with N. rivulare. The second main finding of our study was that the combination of biofertilization (N. rivulare and N. muscorum) and N-fertilization (at half and full doses) significantly increased the growth of soybean plants, more than those of control non-inoculated and fertilized plants. These results are in accordance with previous observations by Al-Noim & Hamad (2004); Rekhi et al. (2000) who established that the combination of biofertilization and N-fertilization at 180 Kg/ha significantly increased the growth of rice as measured by leaf area, plant height and grain yield. The results also substantiate the findings of Nayak et al. (1986); Wang (1986), and accord with the observations of Sholkamy et al. (2012) who established that biofertilization by Nostoc muscorum or Nostoc rivulare significantly increased shoot length, leaf area of maize, either alone or in combination with N-fertilizer at 50 and 100 kg N/ha. The combination of biofertilization and N-fertilization, especially at 100 kg N/ha had more effect on the growth of maize and on nitrogenase activity compared to biofertilization alone. Similarly, Hussein & Radwan (2001); Bassal et al. (1996); Kreem (1993) established that increasing nitrogen application rates increased the number of tillers/ hill, number of filled grains/ spike, 1000 grain weight and grain yield of wheat plants. Similar conclusions were reported by Singh et al. (1992); Wang (1986) who observed that Azolla biomass in the soil increases rice stem height and the number of spikes/ hill over the control. The stimulatory effect of the addition of N-fertilizer on nitrogen fixing activity is due to its stimulation of root growth, as well as the stimulation of the growth of the cyanobacterial population in soil cultivated with soybean. Similar conclusions were reported by Jha et al. (2001); Jha & Prasad (2007) who established that chemical fertilizers at recommended doses caused
increases in cyanobacterial population and nitrogenase activity in paddy soil. The results of the current study showed that N-fertilization at half and full doses increased cyanobacterial abundance (MPN/ g soil) in soil cultivated with soybean plants. Similar conclusions had been reported by Nanjappan et al. (2007) showing that biofertilization by cyanobacteria promoted wheat plant growth, and also that the application of N-fertilizer at the recommended level stimulated the growth of the diazotrophic cyanobacterial population in tested soil. Similar conclusions were reported by Roger & Reynaud (1977) who observed higher N2 fixing cyanobacteria biomass in fertilized pots than unfertilized pots.

Conclusion

It can be concluded that Nostoc muscorum and Nostoc rivulare could be used as renewable biofertilizers for improvement of soybean growth and decreasing use of N-fertilizers

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References


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