

Full Length Research Paper

Association of N₂-fixing cyanobacteria with wheat (*Triticum vulgare* L.) roots

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Two locally prevailing heterocystous cyanobacterial isolates, collected from alluvial clay soil and identified as *Nostoc muscorum* and *Anabaena variabilis*, were tested (either singly or mixed) for their potency to colonize the roots of wheat seedlings. *N. muscorum* formed close association with root hairs, whereas *A. variabilis* showed loose binding. The mixture of both cyanobacterial strains formed weak association with the roots of wheat seedlings. The tight colonization of *N. muscorum* led to accumulation of Chl "a" in the roots, as well as showed a beneficial influence on stimulating nitrogenase activity. Such tight association by *N. muscorum* significantly increased the growth parameters of wheat seedlings. Dry weight of the inoculated plant seedlings roots augmented by more than 3-fold of the control, wherever the shoots were promoted by more than 36% as compared to the control has no inoculation. However, the loose attachment shown by *A. variabilis* contributed to the weak association of the mixture of both cyanobacterial agents.

Key words: *Nostoc muscorum*, *Anabaena variabilis*, *Triticum variabilis*, cyanobacterial isolates.

INTRODUCTION

It is known that the extensive application of mineral fertilizer is very costly and environmentally hazardous. So, application of biofertilizer is considered one of the most alternative solutions used to reduce soil pollution and agricultural expenses. In the recent years, several attentions have been drawn to use cyanobacterium that exists in the different soils as a biofertilizer for its capability for N₂-fixation and organic compounds assimilation (Prasanna et al., 2004). Efficiency of cyanobacteria to form such successful beneficial collaboration by invading the plant roots is of a considerable agronomic importance, particularly for cereal

production (Singh et al., 2011; Prasanna et al., 2013b). Likewise, some of cyanobacteria showed an effective role in stimulating soil fertility by producing plant growth enhancing substances, consequently increasing nutrients availability and plant uptake (Sergeeva et al., 2002; Prasanna and Kaushik (2006).

Aref and EL-Kassas (2006) pointed out that inoculation of the soil by genera *Nostoc* and/or *Anabaena* of cyanobacteria significantly augmented maize yield. A significant increase in chlorophyll content, dry weight, many enzymes activities as a result of cyanobacterial inoculation of wheat roots were reported by (Babu et al.,

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2015).

A significant promotion in dry weight, total nitrogen, chlorophyll a, chlorophyll b, carotenoids and pigments occurred in wheat plants treated with cyanobacterial biofertilizer (Dhar et al., 2015). Cyanobacteria also play an effective role in carbon and nitrogen cycling, wherever it enhance the production of ammonia and indolic compounds. They also reported that analysis of harvest stage of rice elucidated a remarkable augmentation in soil biomass carbon, nitrogen availability, grain yield and 1000 grain weight.

Smith and Crews (2014) showed that cyanobacteria may have a beneficial effect on inhibition plant diseases by reducing the herbivore or pathogen host population. Therefore, the present work aimed to estimate the potential of two locally isolated cyanobacterial strains in Egypt, namely *Nostoc muscorum* and *Anabaena variabilis* for developing association with wheat roots, as well as their effect on plant growth and nitrogenase activity for possibilities of utilizing it as a wide biofertilizer application.

MATERIALS AND METHODS

Isolation and cultivation of Cyanobacteria

Cyanobacterial isolates were collected from the surface layer (0 to 20 cm) of an alluvial clay soil (pH 7.5, EC 2.5 dSm⁻¹ and OM 1.07%). The isolated cyanobacteria were purified and identified to be *N. muscorum* and *A. variabilis*, following the technique described by Holt et al. (1994).

The axenic cultures were maintained in BG-11 medium (Allen and Stainer, 1968), and kept under constant conditions with continuous light (3000 lux) provided by four 18 W fluorescent tubes, and at a temperature of 26±2°C.

Root colonization assay of cyanobacterial isolates

Wheat seeds (*Triticum vulgare* L.) were germinated for three days and seedlings were placed in glass jars, each containing 50 ml of BG-11 medium containing 0.5% agar. To evaluate the root colonization ability of each cyanobacterial isolate in wheat seedlings, the prepared glass jars were divided into four groups as follows:

Applied treatments:

1. Control without inoculation "C".
2. Inoculation with *N. muscorum* "N".
3. Inoculation with *A. variabilis* "A".
4. Inoculation with a mixture of "N" + "A".

The experimental jars were inoculated with 4 ml of 15 day-old cyanobacterial culture of each strain. Each treatment was performed in three replicates and incubated in the presence of continuous light (at intensity of 3000 lux) and at temperature of 26±2°C. Medium in the jars was maintained at a constant volume via compensation wherever needed. Colonization was tested, after 15 days, by having transverse root sectors made by the microtome technique. The roots transverse sectors were examined under the light microscope by magnification power of 100x.

Nitrogenase activity and chlorophyll "a" content determination

Acetylene reduction technique was used to assay nitrogenase activity (Hardy et al., 1973) for cyanobacterial association with the roots of wheat seedlings. Whole plants (15 day-old) were placed, with their roots immersed in 5 ml medium, in sealed glass vessels each containing 17 ml of 10% (v/v) acetylene in air and incubated on an orbital shaker at a light intensity rate of 3000 lux for 60 min.

Wheat roots were extracted using methanol in the dark at 4°C, and content of chlorophyll "a" (Chl "a") was determined spectrophotometrically at 663 nm, according to MacKinney (1941). Moreover, growth of wheat seedlings were estimated in terms of shoot and root dry weights, which were then statistically analyzed, according to Gomez and Gomez (1984).

Statistical analysis

Complete randomize design was applied on the experiment, the LSD of 0.05% was calculated through analysis of variance (ANOVA) to make a comparison among the means of the obtained data of the tested treatments.

RESULTS AND DISCUSSION

Colonization of cyanobacteria with the roots of 15 days old wheat seedlings

Figures 1a, b, 2a and b show that, *N. muscorum* and *A. variabilis* had ability to colonize the roots of wheat seedlings. In the case of the mixture of both cyanobacterial strains, little colonization was observed, due certainly to contribution of *N. muscorum* strain. Such observations were detected in comparison with the control treatment without cyanobacterial inoculation, which showed no colonization occurred (Figure 1a). The attachment of cyanobacteria was characterized by long filaments loosely packed together, growing between root hairs. In the case of *Anabaena*, individual filaments were observed on the surface and around the roots in freshly cut transverse sections (Figure 2b) as they were not intimately associated with root epidermis. On the other hand, *Nostoc* formed tight associations, where packed filaments were observed on wheat roots. The cyanobacterial packages made an intimate contact with the root surface. In contrast, the strain forming a loose attachment (*Anabaena*) was not characterized by abundant sticky mucilage production.

Fadl-Allah et al. (2011) explained the process of colonization for the cyanobacteria forming a tight association with wheat roots, by the first stage of colonization of *N. muscorum* which might occur via migration of hormogonia. The hormogonia developed into long filamentous, and the long filaments were developed at the seriate stage, which consisted of filaments tightly packed in a mucilaginous sheath. They added that, in addition to forming a tight association, *N. muscorum* also appeared to penetrate some root cells as cyanobacterial mass.

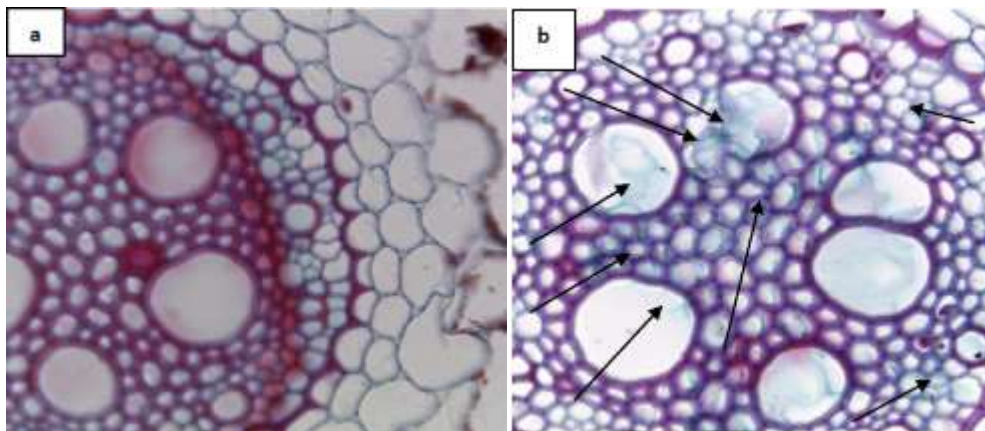


Figure 1. a) Control: wheat root without cyanobacterial inoculation; b) wheat root colonized with *N. muscorum* (as indicated by arrows).

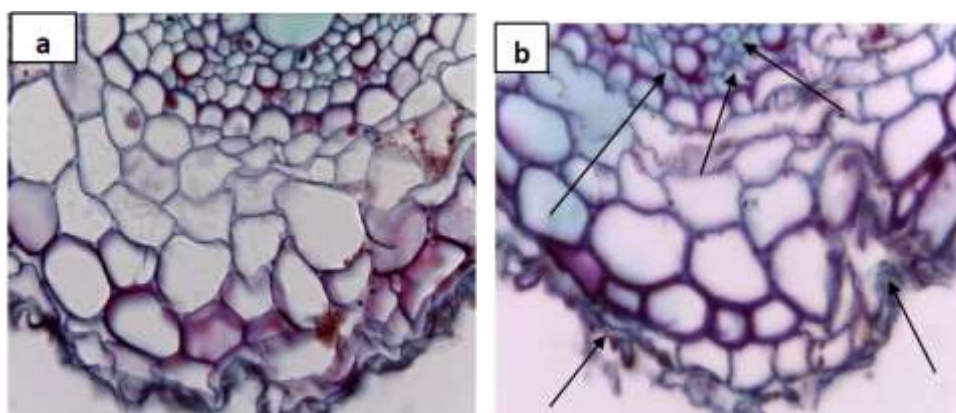


Figure 2. a) Root of wheat seedlings inoculated with *A. variabilis*; b) root of wheat seedlings inoculated with *N. muscorum* and *A. variabilis*. Little colonization is shown by *N. muscorum* (as indicated by arrows).

Table 1. Effect of cyanobacterial inoculation on some growth parameters of wheat seedlings (15 days old).

Treatment	Growth parameters of wheat seedlings							
	Roots					Shoots		
	Length (cm)	Fresh wt. (mg)	Dry wt. (mg)	Chl." a" ($\mu\text{g g}^{-1}$ dwt.)	*N-ase activity	Length (cm)	Fresh wt. (mg)	Dry wt. (mg)
Control	6.7	29.2	2.1	000	0.0	16.5	109.8	19.4
<i>N. muscorum</i> "N"	8.5	88.6	6.8	450	1.60	29.6	158.8	26.5
<i>A. variabilis</i> "A"	7.4	60.5	5.7	000	0.0	24.0	143.5	23.8
"A" + "N"	6.5	58.5	4.9	302	1.28	18.8	140.4	21.2
L. S. D. at 0.05%	1.15	27.0	2.2	—	—	1.7	32.1	1.4

*N-ase: Nitrogenase activity $\text{nmol C}_2\text{H}_4 \text{ h}^{-1}$.

Cyanobacterial inoculation affecting growth of wheat seedlings

Data listed in the Table 1 reveal that, inoculation with cyanobacterial isolates significantly increased the

measures of both roots and shoots of wheat seedlings, as compared to the un-inoculated seedlings. However, inoculation with *N. muscorum* was superior to *A. variabilis* and/or to their mixture in promoting the tested growth criteria of the plants, that is, length and fresh and dry

weights.

Results of the current study denoted that, inoculation of wheat plant roots with *N. muscorum* improved wheat seedlings growth, this agreed with previous findings reported by Gantar (2000) and Babu et al. (2015). In addition to its ability to fix N_2 and supply such essential nutrient to plants, cyanobacteria have been shown to produce compounds that stimulate the growth of plants.

In an earlier study by Prasanna et al. (2008, 2013a), it was proven that cyanobacteria had the ability to produce phytohormone "IAA". Nanjappan et al. (2007) evaluated the potential of plant growth promoting cyanobacteria as a biofertilizer for wheat. Gantar (2000) and Prasanna et al. (2012) showed that, the N_2 fixed by *Nostoc* sp. strain in association with wheat was taken up by the plants and supported their growth.

Effect of cyanobacterial inoculation on chlorophyll "a" content and nitrogeanase activity in roots of wheat seedlings

Chlorophyll "a" was not detected in roots grown either in absence of cyanobacteria, generally, or in the presence of *A. variabilis*, that was unable to form tight associations. The tight associations appeared by *N. muscorum* had a high Chl "a" concentration ($450 \mu\text{g Chl "a" g}^{-1}$ root dry wt.), as compared to *A. variabilis* which had a loose association with wheat plant roots.

Nitrogenase activity was not also detected in the roots grown either in the absence of cyanobacteria, or presence of *A. variabilis*. The tight associations of *N. muscorum* occurred a high nitrogenase activity ($1.6 \text{ nmol C}_2\text{H}_4 \text{ h}^{-1}$), as compared to *A. variabilis* which had a loose association with wheat roots.

Earlier studies have shown that the heterocystous cyanobacterium *Nostoc* sp. strain 2S9B was unusual among characterized cyanobacteria in its ability to form tight associations with wheat roots and to penetrate both root epidermis and cortical intracellular and intercellular spaces. *Nostoc* sp. strain 2S9B could easily co-exist within wheat tissue, making it a promising organism for achieving a biological association between a N_2 -fixing bacterium and wheat. Gantar and Elhai (1999) and Prasanna et al. (2013b) tested four heterocystous cyanobacteria, belonging to the genera, *Anabaena* and *Nostoc* for their ability to form associations with the roots of wheat seedlings under using hydroponics. The cyanobacterial strains formed close associations with wheat plants, and were able to enter through root hairs and penetrate the epidermal layer of wheat roots. Moreover, there was a significant higher indole acetic acid production and chlorophyll accumulation observed in the colonized roots.

Conclusion

The present study showed that the ability of cyanobacterium

N. muscorum strain to form tight association and colonization with wheat roots, enhanced nitrogenase activity consequently, plant growth through its potential role as a nitrogen fixer bacteria. So, it may be utilized as a biofertilizer and contribute in promoting the amount of nitrogen fertilizer needed by wheat crop. However, further studies must be undertaken to confirm the effect of this strain (*N. muscorum*) on other plants under Egyptian Conditions.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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