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Enhancement of Nodulation Efficiency of Mungbean *Rhizobia*

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A B S T R A C T

Rhizobium-mungbean symbiosis was influenced by the rhizospheric application of naringenin which increase nodule numbers. There was significant improvement in the number of nodules in 15 µM concentration of naringenin over control, 5 µM and 10 µM concentration of naringenin. Among the four different isolates of Rhizobia used, maximum nodulation was observed in the isolate NAULN-1 in all the treatment including control. Treatment with the isolate NAULN-1 and 5 µM, 10 µM and 15µM concentration of naringenin concentration showed 29, 37 and 40 numbers of nodules over the 29 nodules in control treatment. Maximum no. of nodules found in 15 µM concentration of naringen in all the isolates.

Introduction

The symbiosis between rhizobia and legumes is the result of millions of years of co-evolution. Among the 19,000 species of legumes described so far, only a small proportion has been studied for their nodulation ability (Balachander *et al.*, 2007).

For the establishment of an effective symbiosis two main classes of bacterial symbiosis genes are needed: nodulation and nitrogen fixation genes. Nodulation genes (e.g. *nod ABC*) encode enzymes responsible for the biosynthesis and secretion of Nod factors, which are host determinant lipochito-oligosaccharides (LCOs) that interact with the plant flavonoids (Downie, 1998).

Nitrogen fixation genes (*nif* and *fix*) include the structural genes for the nitrogenase (*nifHDK*), the enzyme responsible for atmospheric nitrogen fixation (Kaminski *et al.*, 1998).

The initial interaction between the host plant and free-living rhizobia is the release of a variety of chemicals by the root cells into the soil. Some of these encourage the growth of the bacterial population in the area around the roots (the rhizosphere). Nodule formation is controlled by extracellular bacterial signal molecules, called nod factors, which are recognized by the host plant (Lerouge *et al.*, 1990; Schultze and Kondorosi, 1998).

Flavonoids secreted by the root cells activate the *nod* genes in the bacteria which then induce nodule formation. The whole nodulation process is regulated by highly complex chemical communications between the plant and the bacteria.

Synthetic falvonoids are used as one of the soil amendment to improve the microclimate around the root zone. It can enhance the activities of the bacteria around it and result in the higher nodulation efficiency.

The natural flavonoids released from seeds and roots of plant cultivars limited nodulation. Adding flavonoids to the rhizosphere enhance nodulation and N_2 fixation. Atmospheric biological nitrogen fixation by the *rhizobium* is directly proportionate to the number of effective nodule developed on root. Under the circumstances if the number of nodules is increased on the legume roots, they will certainly result in to the higher biological nitrogen fixation in a particular ecosystem.

Materials and Methods

Collection of nodule samples

Collection of nodule samples was collected from different mungbean growing villages of Navsari district. The plants were uprooted and fully developed pink colored nodules of desired size were selected carefully to collect the nodules. All the samples were collected at the early flowering stage i.e. from around 50-60 days old crop.

Isolation of *Rhizobium* Bacteria

Intact nodules were surface sterilized, crushed in sterile distilled water and streaked on YEMA medium containing Congo red for isolation of *Rhizobium* bacteria. One colony from each location was selected as an isolate and tentatively named as NAULN-1, NAULM-2, NAULA-4 and NAULP-6 collected from NAU Farm, Maroli, Abrama and Pethan village of Navsari district respectively.

Naringenin treatment

Seeds of mungbean cultivar (Variety-GM-4) obtained from the Pulses Research Station, Navsari Agricultural University were treated with different isolates of *rhizobium* before sowing. The experiment was conducted based on Complete Randomized Design for layout. Naringenin (sigma) was applied to the soil at the time of sowing. Naringenin solution 5µM, 10 µM and 15 µM prepared and applied to the different pot and one pot was kept without treatment as control. Plants from each different treated pot were uprooted on 45 days after sowing for observation of no of nodules.

Results and Discussion

Efficiency of *Rhizobium*-mungbean symbiosis was enhanced by the application of naringenin. To study the enhancement of nitrogen fixation ability through chemical agent, all the four bacterial isolates were used. Nodulation efficiency was tested by the pot method and applying 5 µM, 10 µM and 15µM concentration of naringenin.

Study of the data presented in table 1 and figure 1 indicated that among the four different isolates of Rhizobia used, maximum nodulation was observed in the isolate 1 in all the treatment including control. Total numbers of nodules were 29, 20, 18, and 24 respectively in the control in the isolates NAULN-1, NAULM-2, NAULA-4 and NAULP-6 respectively.

There was no significant difference in the control and 5 µM concentration of naringenin in any of the treatment. There was significant improvements in the number of nodules in 10 µM concentration of naringenin over control and 5 µM concentration of naringenin.

Similarly there was significant improvement in the number of nodules in 15µM concentration of naringenin concentration over 10 µM concentration of naringenin.

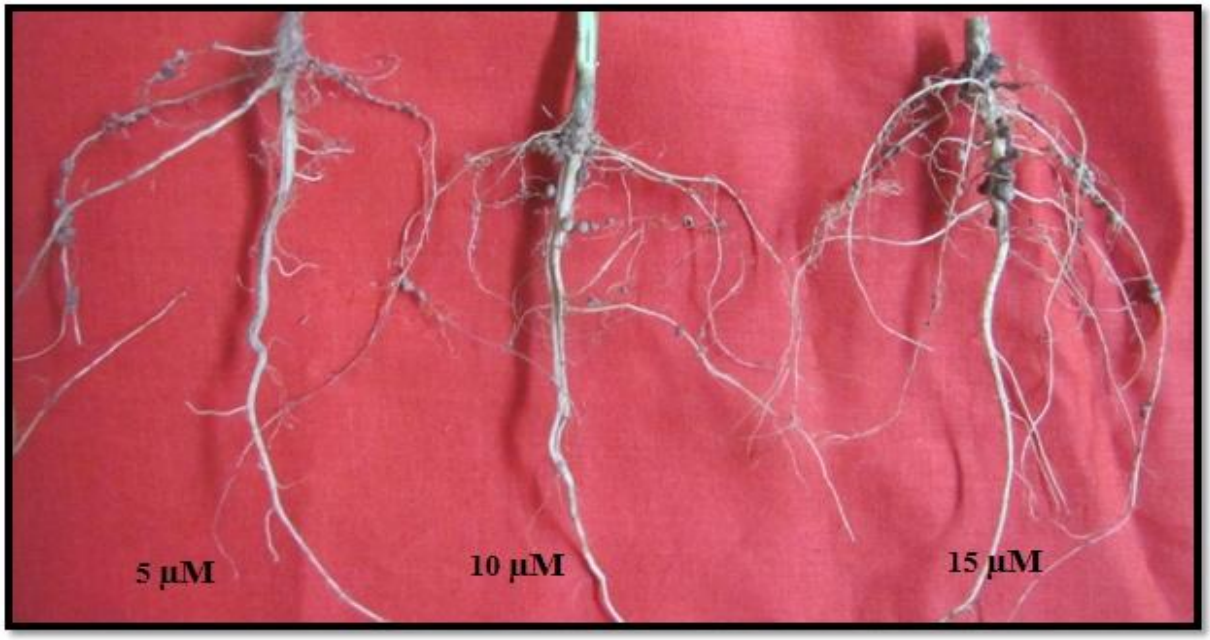
Treatment with the isolate NAULN-1 and 5 µM, 10 µM and 15µM concentration of naringenin concentration showed 29, 37 and 40 numbers of nodules respectively.

Treatment with the isolate NAULM-2 and 5 µM, 10 µM and 15µM concentration of naringenin concentration showed 22, 29 and 33 numbers of nodules respectively.

Fig.1 Pot Experiment (Naringenin treatment)

Mungbean plant in pot

Plant uprooted at 45



Nodulation observed in plant treated with NAULN-1 and 5 µM, 10 µM and 15 µM Naringenin concentration

Table.1 Effect of rhizosphere application of *nod* regulators (naringenin) on Enhancement of nodulation

Sr. No.	Naringenin concentration	No. of Nodules formed by NAULN-1	No. of Nodules formed by NAULM-2	No. of Nodules formed by NAULA-4	No. of Nodules formed by NAULP-6
1	**5 µM**	29	22	20	22
2	**10 µM**	37	29	27	30
3	**15 µM**	40	33	29	34
4	**Control (No Nar)**	29	20	18	24
5	**CD**	1.988	1.540	1.406	1.540
6	**CV**	3.825	3.846	3.884	3.636
7	**SEm**	0.645	0.500	0.456	0.500

Treatment with the isolate NAULA-4 and 5 µM, 10 µM and 15µM concentration of naringenin concentration showed 20, 27 and 29 numbers of nodules respectively. Enhancement of nodulation per treatment with the isolate NAULP-6 and 5 µM, 10 µM and 15µM concentration of naringenin concentration showed 22, 30, 34 numbers of nodules respectively.

Results were in accordance with addition of 10 µM luteolin (Kapulnik *et al.,* 1987) and naringenin (Jain *et al.,* 1990) to the rhizosphere of alfalfa seedlings increased nodulation. Naringenin which is known to be an inducer of nod genes of *Rhizobium leguminosarum* (Zaat *et al.,* 1987). Flavonoids present in root exudates of legumes are known to induce the expression of nod genes (Peters *et al.,* 1986). Host controlled flavone limitations to root nodulation is known in alfalfa (Kapulnik *et al.,* 1987). The increase in symbiotic efficiency observed in the present investigation was perhaps due to the effect of naringenin on nod genes of *Rhizobium*.

From this investigation it is fact that nodulation has been enhanced by the application of naringenin opens up another technology for maximizing symbiotic nitrogen fixation.

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