J. Indian bot. Soc. ISSN - 0019 - 4468 Vol. 93 (3 & 4) 2014 : 254-258

COMPETITIVE EFFICIENCY OF INDIGENOUS RHIZOBIA FOR NODULATION, NITROGEN FIXATION AND YIELD OF *VIGNA RADIATA* UNDER SALINITY

MALVIKA SRIVASTAVA AND SHILPI SRIVASTAVA

Plant Physiology And Biochemistry Laboratory
Department of Botany, D.D.U. Gorakhpur University, Gorakhpur-273009
drmalvika.srivastava@gmail.com | shilpi.srivastava212@gmail.com

Surface sterilized seeds of *Vigna radiata* were inoculated with 72h grown culture of rhizobia isolated separately from *Melilotus indica, Lathyrus sativus, Vicifaba, Medicago denticulate, Sesbania sesban* as monoculture or as mixed inocula. An uninoculated sample served as control. For mixed inoculum, appropriate inocula were mixed in a proportion of constant optical density (i.e. 0.05) at 610 nm. Seeds were allowed to germinate in earthenware pots filled with soil containing native soil rhizobia. The pots were watered with saline water containing 100mM NaCl. Hoagland's nutrient solution was also supplied every 4th day in each pot. Various parameters such as number of nodules, nitrogenase and nitrate reductase activity, total nitrogen and protein content, and yield were studied. Results confirmed the effect of indigenous rhizobia against native soil rhizobia in terms of nodulation, nitrogen fixation and yield of *Vigna radiata* grown under salinity stress.

Keywords : Efficiency, *Rhizobium,* salt stress, *Vigna*, indigenous rhizobia.

Legumes are pillars in the development of sustainable agriculture, because they are responsible for a substantial part of global flux of nitrogen from atmospheric nitrogen to fixed forms such as ammonia, nitrate and organic nitrogen. Wild legumes have greater sensitivity and adaptability in particular agro-climatic conditions than the cultivated legumes. In the near future, there is an option where cultivated legumes can be replaced by wild legumes for food, fuel, fodder, medicine and nitrogen fixation. Legumes are capable of fixing atmospheric nitrogen through symbiotic association with many species of *Rhizobium* and other genera.

Saline conditions may affect the legume-*Rhizobium* symbiosis by reducing the survival of rhizobia via inhibiting the infection process, affecting nodule development and function, or reducing plant growth (Singleton and Bohlool 1984). Competitiveness of native rhizobia also pose barrier to the benefits of inoculation (Shamreldin and Werner 2004). Rhizobia have been utilized in agriculture to increase the yield of leguminous plants (Wadhwa *et al.* 2010) through their use as inoculants to seed or less often, soil. Nodulation of leguminous crops by *Rhizobium* largely depends on the presence of a specific and compatible strain in soil for a particular legume.

The sensitivity of different rhizobial species and strains to abiotic stress varies considerably (Vriezen *et al.* 2007). Mishra and Srivastava (2003) studied the response of five rhizobial isolates/strains obtained from healthy crown nodules of wild legumes viz. *Melilotus indica, Lathyrus sativus, Vicia faba, Medicago denticulata, Sesbania sesban* growing in salt affected area of Gorakhpur district to study their effect on nodulation, nitrogen fixation and yield of *Vigna radiata* under salinity and they reported that these rhizobial strains isolated from saline soil under a particular agro-climatic condition were more efficient in increasing the nitrogen fixation efficiency and yield of crop plants.

Therefore, in view of its efficiency under saline conditions, further experiments were conducted to study the competitive efficiency of selected strains with native soil rhizobia in imparting tolerance against salt stress.

MATERIALS AND METHODS

Surface sterilized seeds of *Vigna radiata* were inoculated with 72h grown culture of rhizobia isolated separately from *Melilotus indica,*

Received on. May 9, 2014 Accepted on August 11, 2014

Lathyrus sativus, Vicia faba, Medicago denticulata and *Sesbania sesban* as monoculture or as mixed inoculum. An uninoculated sample was kept as control. For mixed inoculums, appropriate inocula were mixed in a proportion of constant optical density (i.e. 0.05) at 610 nm. Seeds were allowed to germinate in earthenware pots filled with soil containing native soil rhizobia. The pots were watered with saline water containing 100m M NaCl. Hoagland's nutrient solution was also supplied every 4th day in each pot. Nitrogenase activity in detached root nodules was determined by the method of Srivastava *et al.* (1980) and nitrate reductase activity in primary leaves by following the method of Streeter and Bosler (1972). Total free amino acids were estimated by the method of Yemm and Cocking (1955) and total nitrogen was estimated by Donnen's (1932) micro-kjeldahl method. For the estimation of protein content, the amount of insoluble nitrogen fraction as obtained by micro-kjeldahls digestion method was multiplied by factor 6.25. Yield parameter was studied after 60th day of sowing.

RESULT

Nodule number : A significant difference in nodule number per plant formed in the test plant with different isolates/strains and with mixed inocula (Fig. 1) was observed. The maximum nodule number was formed at 36th day. The response of various isolates/strains was in the order :

Isolates/StrainNo.1>2>3>mixed>4>5>control.

Enzymes of Nitrogen Metabolism

Nitrate Reductase Activity : Significant difference in the nitrate reductase activity in the fresh leaves of plants raised from the seeds inoculated with different isolates/strains, mixed culture and uninoculated control was observed. (Fig. 1). The maximum NR activity was measured at 30th day of plant growth. The response of various isolates/strains was in the order :

Isolates/Strain No.1>2>3>mixed>4>5>control.

Nitrogenase Activity : A significant variation in nitrogenase activity in the fresh nodule of plants raised from seeds inoculated with different isolates/strains, mixed culture and uninoculated control was observed (Fig. 1). The maximum nitrogenase activity was observed at 36 days after germination. The response of various isolates/strains was in the order :

Isolates/Strain No.1>2>3>mixed>4>5>control.

Biochemical Estimations

Total Nitrogen Content : Total nitrogen content in dry leaves varied considerably among the plants raised from seeds inoculated with different isolates/strains, mixed culture and uninoculated control (Fig. 2). The response of various isolates/strains was in the order :

Isolates/Strain No. 1>2>3>mixed>4>5>control.

Total Protein Content : Total nitrogen content in dry leaves varied considerably among the plants raised from seeds inoculated with different isolates/strains, mixed culture and uninoculated control (Fig. 2). The response of various isolates/strains was in the order :

Isolates/Strain No. 1>2>3>mixed>4>5>control.

Yield : Yield is measured in g/100 seeds. Yield varied considerably among the plants raised from the seeds inoculated with different isolates/strains, mixed culture and uninoculated control (Fig. 2). The response of various isolates/strains was in the order :

Isolates/Strain No. 1>2>3>mixed>4>5>control

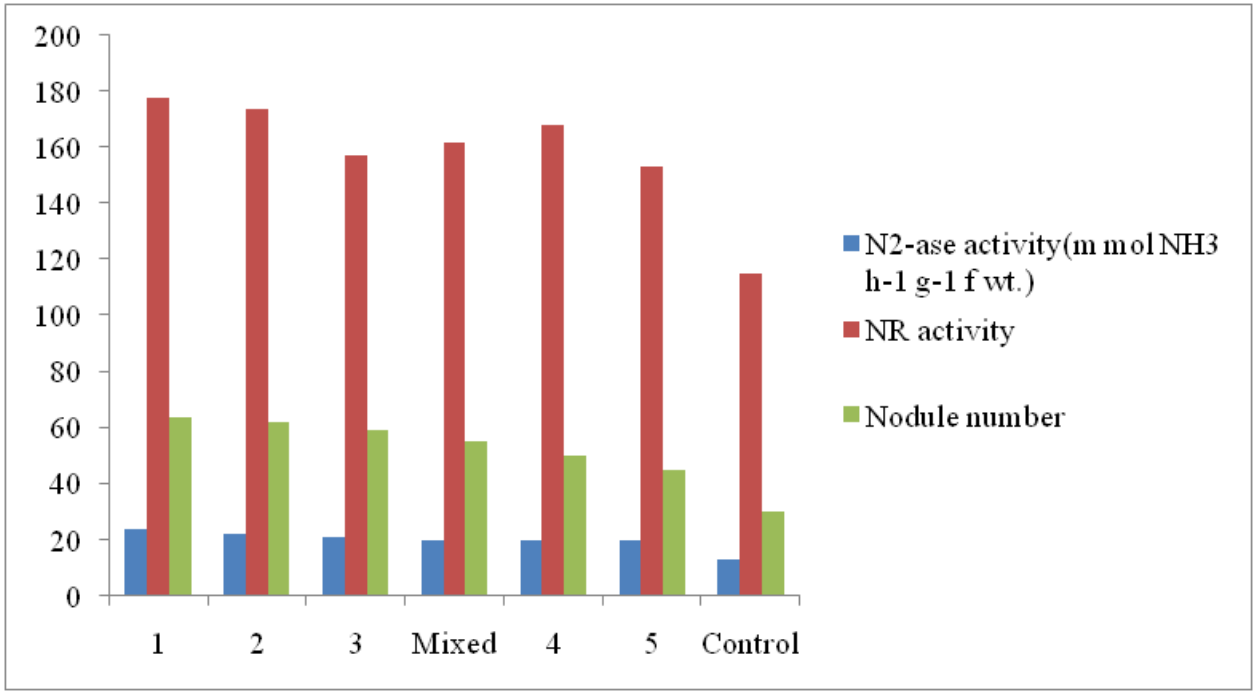

Figure 1. *Vigna radiata :* N2-ase activity and NR activity at the day of optimum activity and nodule number at the day of optimum nodule formation in plants raised from seeds inoculated with different isolates/strains/mixed inoculum of the *Rhizobium.*

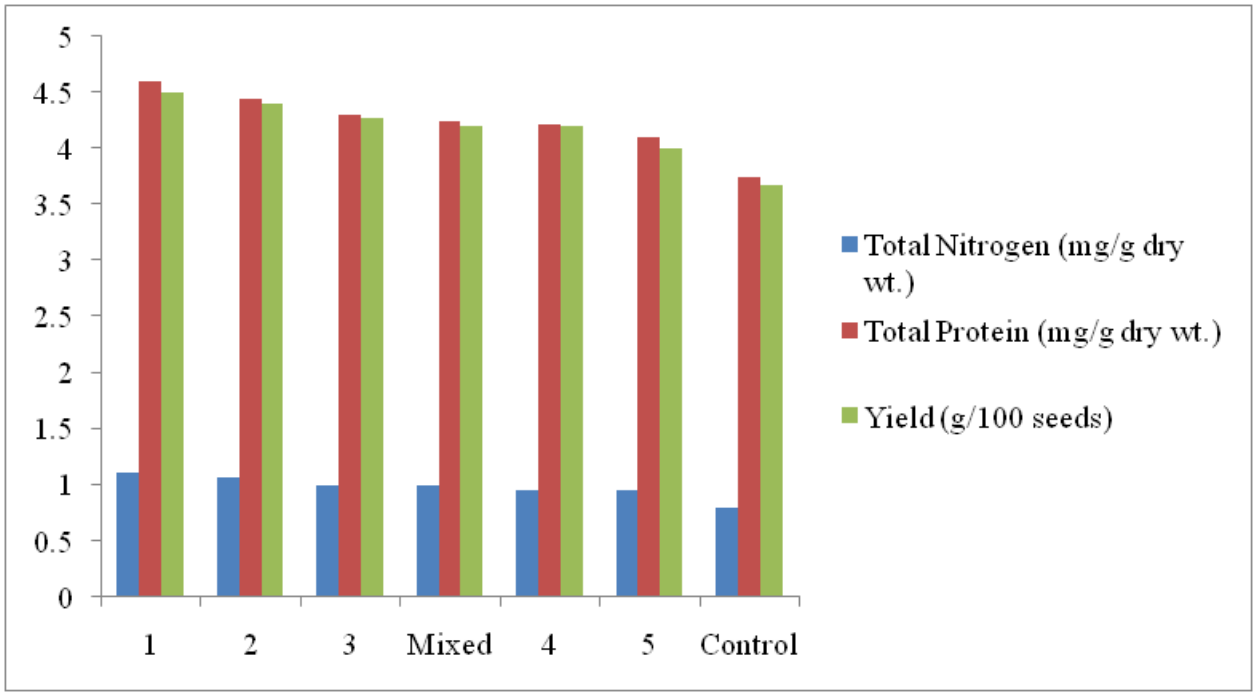

Figure 2. *Vigna radiata :* Total nitrogen content, total protein content and yield at the day of optimum activity in plants raised from seeds inoculated with different isolates/strains/mixed inoculum of *Rhizobium*.

DISCUSSION

Number of nodules per plant increased in response to various strains in the test plant. The presence of well developed large and healthy nodules is the characteristics of the effectiveness of rhizobial strains. The strains could tolerate salinity and are able to grow well in saline soils. Masshady *et al.* (1998) reported effective symbiosis of *Rhizobium meliloti* with *Medicago sativa* under saline condition (100 mM NaCl). Kruppasamy *et al.* (2011) reported an increase in nodule number in *Samanea saman* by stress tolerant *Rhizobium* inoculation.

Nitrogenase is the most important enzyme for determining the nitrogen fixation by root nodules. Nitrogenase activity increased with the inoculation of effective rhizobial strains to the test plant (Naeem *et al.* 2004). The maximum nitrogenase activity was measured at 36 days after germination. More resistant and more efficient rhizobial strains exhibited more nitrogenase activity in salt stressed condition. Nitrate reductase activity increased in stressed condition as compared to control. NR activity was found to be strain dependant. Sharma *et al.* (2011) observed that *Rhizobium* inoculation enhanced the NR activity. NR activity correlates well with more nitrogen fixation and increased protein synthesis.

Total nitrogen and protein content in dried leaves were similar to nitrogenase activity. Athar and Johnson (1996) reported that some strains of rhizobia significantly increased shoot nitrogen content in stress condition. Yield increased in the test plant inoculated with different rhizobial strains. Increase in seed yield due to *Rhizobium* inoculation has been reported by Ashraf *et al.* (2002) and Abbasi *et al.* (2008).

Hence, the ability of certain strains of *Rhizobium* isolates to dominate nodulation in a multi-strain environment is termed as competitiveness. Enhanced competitive ability in an inoculant strain is a key requirement for successful nodule formation, and subsequent N_2-fixation. Stress factors such as salinity, drought, heat, etc suppress the growth and symbiotic characteristics of most rhizobia; however, several strains, distributed among various species of rhizobia are tolerant to stress effects and form effective symbiosis with their host legumes. Thus it may be concluded that *Rhizobium*-legume symbiosis is superior to other nitrogen fixing system with respect to nitrogen fixing potential and adaptation to severe conditions. The isolation of effective and persistent rhizobial strains used to inoculate the other legume crop is a strategy to improve the efficiency of the *Rhizobium*-legume symbiosis. The rhizobia from wild legumes have better traits than the homologous/native soil rhizobia. The effective rhizobial strains have ensured their improved productivity and profitability ultimately increasing the yield of pulse crop under saline conditions.

REFERENCES

Abbasi MK, Majeed A, Sadiq A. and Khan SR 2008 Application of *Bradyrhizobium japonicum* and phosphorus fertilization improved growth, yield and nodulation of soybean in the sub-humid hilly region of Azad Jammu and Kashmir, Pakistan. *Plant Prod. Sci*. **58** 368-376.

Ashraf M, Ali S and Hassan I 2002 Interaction of *Rhizobium japonicum* strains and soybean genotypes. *Pak J Soil Sci* **21** 49-54.

Athar Mohammad and Douglas A Johnson 1996 Influence of drought on competition between selected *Rhizobium meliloti* strains and naturalized soil rhizobia in alfalfa. *Plant and soil* **184** 231-241.

Doneen LD 1932 A micromethod for nitrogen estimation in plant materials. *Plant Physiol* **7** 717-720.

Karuppasamy K Nagaraj S and Kathiresan K 2011 Stress tolerant *Rhizobium* enhances the growth of *Samanea saman* (JECQ) Merr. *African Journal of Basic & Applied Sciences* **3** (6) 278-284.

Mashhady A S, Salem S H, Barakh F N, and Heggo A M 1998 Effect of salinity on survival and symbiotic performance between *Rhizobium meliloti* and *Medicago sativa* in Saudi Arabian soils. *Arid Soil Res. Rehabil*. **12** 3–14.

Mishra T and Srivastava M 2003 Nitrogen metabolism and yield of *Vigna radiata* under saline condition in response to salt tolerant strains of *Rhizobium*. *Indian J Plant Physiol*, (special issue) vol. **8** 354-357.

Naeem F I, Muhammad Ashraf M, Malik K A and Fauzia Y Hafeez 2004 Competitiveness of introduced *Rhizobium* strains for nodulation in fodder legumes. *Pak J bot*, **36** (1) 159-166.

Shamseldin A and Werner D 2004 Selection of competitive strains of *Rhizobium* nodulating *Phaseolus vulgaris* and adapted to environmental conditions in Egypt, using the gus-reporter gene technique. *World Journal of Microbiology & Biotechnology* **20** 377–382.

Sharma P, Sardana Virender and Kandola S S 2011 Response of Groundnut *(Arachis hypogaea L.)* to *Rhizobium* Inoculation. *Libyan Agriculture Research Center Journal International* **2** (3) 101-104.

Singleton P W, Bohlool B 1984 Effect of salinity on the nodule formation by soybean. *Plant Physiol* **74** 72–76.

Srivastava R C, Mukherji D and Mathur S N 1980 A freeze/thaw technique for estimation of nitrogenase activity in detached root nodules of *Vigna mungo*. *Ann Appl Biol* **96** 235-241.

Streeter J G and Bosler M E 1972 Comparison of *in-vivo* and *in-vitro* assays for nitrate reductase in soybean leaves. *Plant Physiol* (Lancaster) **49** 448-450.

Vriezen J A C, De Bruijn F J and Nusslein K 2007. Responses of Rhizobia to dessication in relation to osmotic stress, oxygen and temperature. Appl Environ Microbiol **73** 3451–3459.

Wadhwa K, Dudeja S S and Yadav R K 2010 Molecular diversity of native Rhizobia trapped by five field pea genotypes in Indian soils. *Journal of Basic Microbiology* vol. **50**1–9.

Yemm E and Cocking E C 1955 The determination of amino acids with ninhydrin *Analyst* **80** 209-213.