Selection of Effective Microorganisms

for Bio-fertilizers in China

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Part I Recent Rhizobium selection

Since 1960s a lot of scientists have been engaged in isolation and evaluation of rhizobium from legume crops such as peanut and soybean, a wide range of bio-fertilizers have been developed just as mentioned at the report of last FNCA. Before isolation and selection of rhizobium, we always introduced ^{15}N natural Abundance method into evaluation of nitrogen fixation evaluation by legume. That is based on that the nitrogen fixing activity of legume was closed related to its $\delta^{15}N$ value. But, according to our experience, two points of view should be considered: (1) Reasonable evaluation only could obtained from the $\delta^{15}N$ value of shoot, not that of roots or nodule (Table 1) and (2) $\delta\ ^{15}N$ Value of some none-nodulated crops(without any nitrogen fixation ability) is very low.

Table 1 $\delta^{15}N$ value and %Ndfa of different parts of Clark bean (Sand culture experiment)

	^{15}N natural Abundance method		15N direct method
Parts of plants	$\delta\ ^{15}N$	%Ndfa	%Ndfa
Whole plant	-0.69%	83.8%	56.78%
Shoot	-2.25%	53.2-55.7%	
Roots	3.07%	-386.6%	
nodule	10.54%	-31.8%	

For evaluation of nitrogen fixation of inoculated rhizobium, we used ^{15}N direct method and ^{15}N dilution method (A_N value methods). For this method, selection of reference none-legume crop is of significant importance.

Recently such works turned their focus on

(1). Screening of rhizobia from native crops grown at West China such as Sichuan, Qinghai, Tibet and herbage or forage grass;
(2). Effect of rhizobia applied with seed of none-legume crops on yield and nitrogen fixation
(3). Interaction between rhizobia and crops
  (a. Interaction between rhizobia and native host crops;
  (b. Interaction between rhizobia and other legume crops;
  (c. Interaction between rhizobia and none-legume crops(Rice, Wheat, maize and potato);
(4). Competition between native rhizobia and inoculated rhizobia;

Part II Selection of associated nitrogen fixing bacteria

Since 1977 when two strains of associated nitrogen fixing bacteria were isolated from rhizosphere of rice roots, A-15(Alcaligenes faecalis) and E-26(Enterobacter), a large quantity of associated nitrogen fixing bacteria have been isolated, even some strain were successfully isolated first In China. A lot of related studies on associated nitrogen fixing bacteria and its effect has been conducted for these two dozens of years, but mainly focus on strain A-15 and E-26. It showed that A-15(Alcaligenes faecalis)

(1) can not only be adsorbed on root surface, but also enter into rice roots and live there;
(2) Can exude ammonium which could utilized by rice, 1/3 nitrogen fixed utilization by rice during a course of 69 h;
(3) exude plant regulator, such as IAA and GA3, to promote the growth of rice;
(4) Contains nitrate reductase and increase the uptake of nitrogen by rice roots;
(5) Increase the uptake of nutrients by rice roots

Part III Results of experiment in 2002

Determination of Nitrogen fixation of microorganisms and their contribution to wheat

At Shijiazhuang city, an field trial was conducted to measure the nitrogen fixation(Difference method and ^{15}N dilution method) and study their effect on yield of wheat. Two microorganisms were used, A-15 and BS (An gene modified Alcaligenes faecalis), seed was dressed with bacteria solution at dosage of 300 ml/ha. In Fig 1-4, N represents 150 kgN/ha applied.

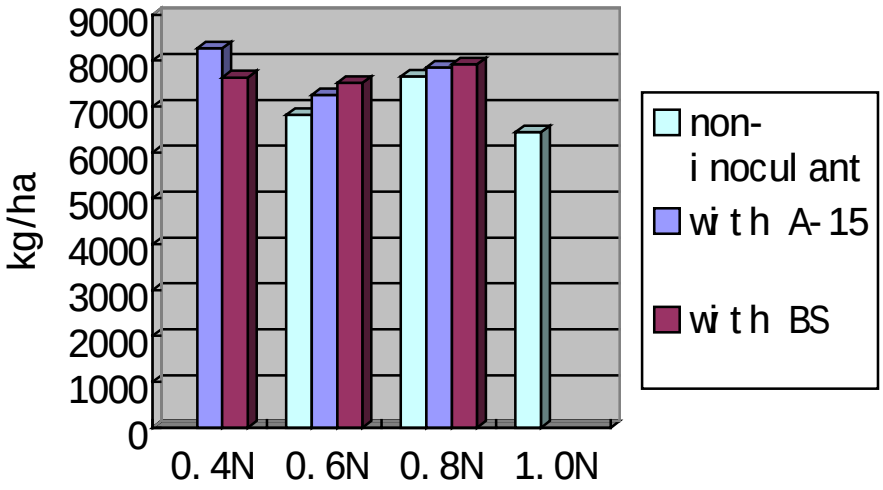


Fig 1 Biomass of wheat with relation to different incorporation of fertilizer and microorganisms

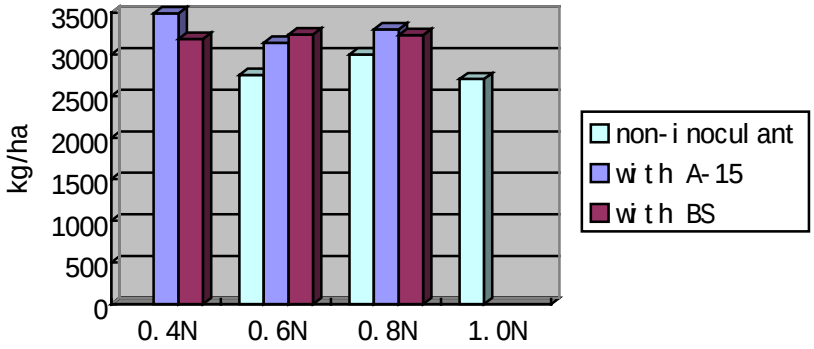


Fig 2 Yield of wheat with relation to different incorporation of fertilizer and microorganisms

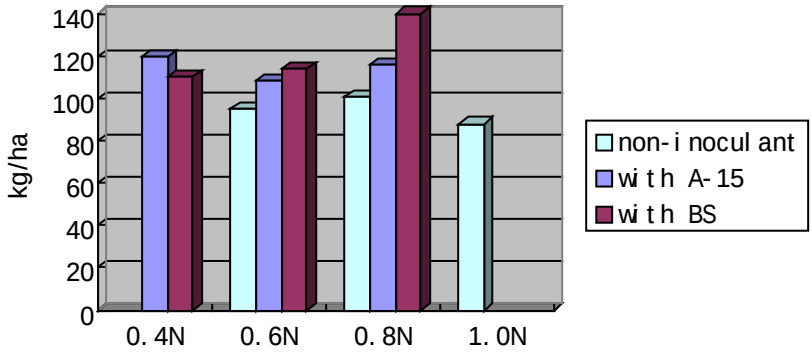
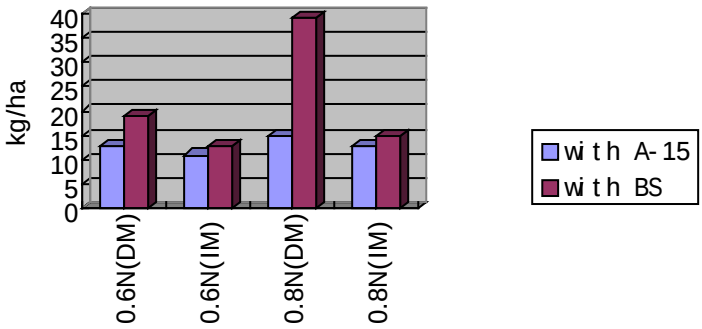


Fig 3 Nitriogen uptake by wheat with relation to different incorporation of fertilizer and microorganisms



DM-difference method, IM-isotopic method

Fig 4 Nitrogen fixation by different incorporation of fertilizer and microorganisms

The results shown at Fig 1-4 indicated these two strain of associated nitrogen fixing bacteria could increase biomass, yield and nitrogen uptake of wheat regardless of the nitrogen fertilizer

application level. But for nitrogen fixation, BS, an gene modified bacteria, seemed to be better than the A-15, an bacteria isolated directly from rice roots, 19~39kgN/ha and 13~15 kgN/ha respectively, by difference method and 13~15gN/ha and 11~13 kgN/ha by isotopic method..

Part IV Plan of FNCA bio-fertilizer project for 2003

1. Selection of suitable carriers for A-15 and BS bio-fertilizers (vermiculite, active carbon, animal manure and so on);
2. Set-up of the techniques for A-15 or BS direct Incorporation with macronutrient fertilizers(compound fertilizers);
3. Identification of suitable irradiation dosage for carrier sterilization;
4. Establishment of Quality control standard for associated nitroen fixing bacteria fertilizers (both the number of living microorganism and effectiveness);
5. Demonstration of bio-fertilizers with A-15 and BS
   The crops to be inoculated: rice and winter wheat
   Location: Sichuan and Hebei province, one hectare for each
   Carrier sterilization method: Irradiation by ^{60}Co