Improved nitrogen fixation of soybean by novel seed treatments

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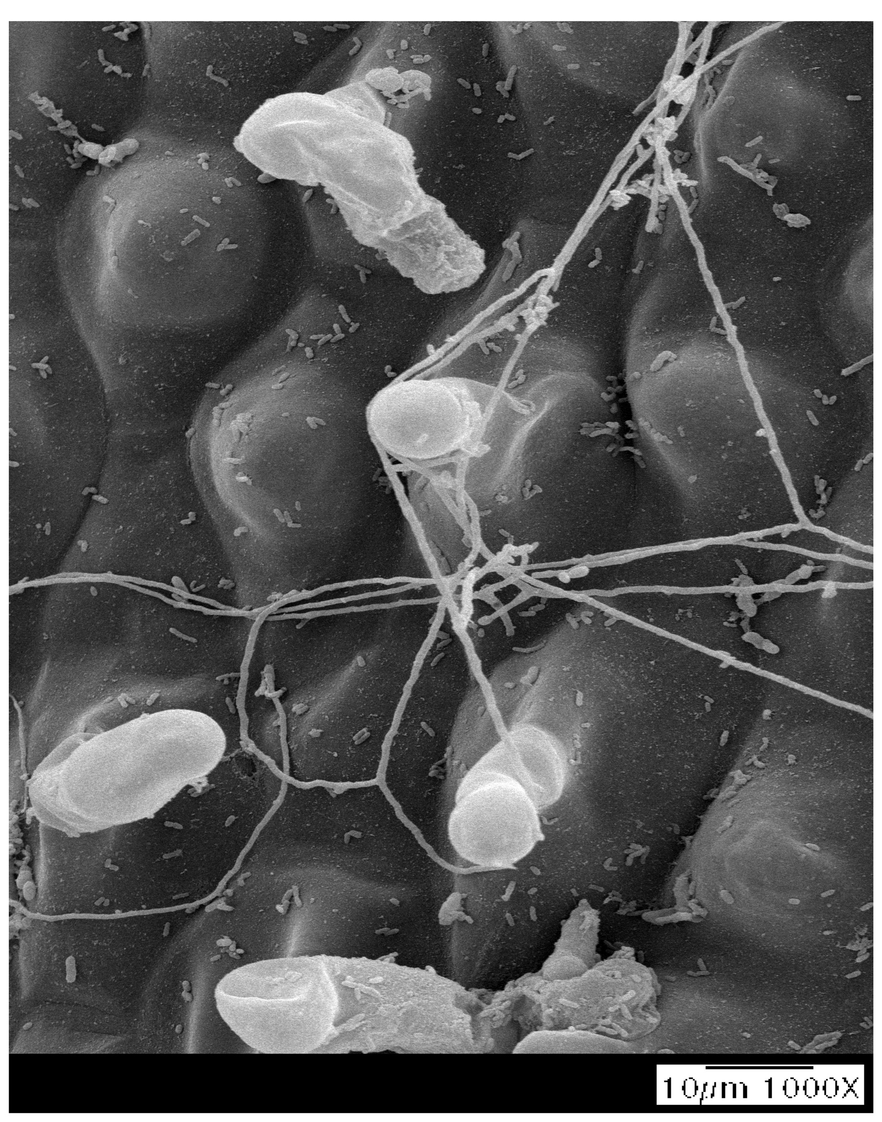
Introduction

- The continuing interest in improving inoculation technology is driven by the recognition that the native soil bradyrhizobia dominant in soybean nodulation are competitive and may be ineffective in nitrogen fixation. Common seed inoculation techniques to date have limited success to boost the nodule occupancy by inoculation to a level significant enough to show an inoculation response.
- The order of susceptibility by the native bradyrhizobia in the soils of southern Illinois is kanamycin > tetracycline > oxytetracycline> rifampicin> neomycin. Additionally, it was estimated that less than 20% of native bradyrhizobia in these soils are resistant to kanamycin.
- Previous studies from our laboratory had shown that co-inoculation of kanamycin/neomycin-resistant *Bradyrhizobium japonicum (B. japonicum)* strains derived from USDA 110 and *Streptomyces kanamyceticus (S.kanamyceticus)* ATCC #12853 on soybean seeds (cultivar LS90-1920) led to greater nodule occupancy of KNI strains and shoot nitrogen content of host plant grown in Southern Illinois soil (Gregor et al., 2003). Production of kanamycin by inoculated *S.kanamyceticus* was postulated to be responsible for these results.
- Nodulation can also be improved by co-inoculation of soybean with *Bradyrhizobium japonicum* and other soil bacteria that enhance plant vigor or relieve the abiotic constraint on plant growth.
- This study demonstrated that our novel seed treatment of the soybean cultivar LS90-1920 had improved nitrogen fixation and nodule occupancy over the treatments without the bacterial co-inoculants.



Evidences of soybean root colonization by *S.kanamyceticus*

Objective and Hypotheses

- Novel seed treatments intended to improve the nodule competency by a selected inoculant against native bradyrhizobia were evaluated with the soybean cultivar LS90-1920 grown in southern Illinois soils.
- We hypothesized that the nodule occupancy by a bradyrhizobial inoculant may be improved by the ecological selection of seed treatment. Balanced amounts of nutrients and protectants should enhance the survival of all bacterial inoculants in the seed environment. Colonization of *S.kanamyceticus* on soybean rhizoplane should impede the competition of native soil bradyrhizobia on root infection. Early seed germination mediated by emergence-promoting rhizobacteria (*Pseudomonas putida* strain G11-32 or 17-29) should maximize root exposure to viable inoculants.

Materials and Methods

- The seed treatment composition used in this study included bacterial nutrient solution consisted of sucrose, sodium glutamate and yeast extract; bacterial inoculants (liquid medium); gum acacia; and seed coating materials. They were applied by the order described above to the soybean seeds.
- The following bacteria were used in this study. One derivative of *Bradyrhizobium japonicum* strain USDA 110 (KNI-3), which is kanamycin/neomycin-resistant, was chosen from a collection of antibiotic-resistant isolates from our laboratory. *Streptomyces kanamyceticus* was purchased from the American Type Culture Collection (ATCC) listed as ATCC # 12853. Two strains of *Pseudomonas putida* (G11-32 and 17-29) were obtained from Allelix Inc.
- Soil of (six inch depth) was collected from the Agronomy Research Center of Southern Illinois University in Belleville and classified as a Bethalto silty clay loam (Fine, silty, mixed, superactive, mesic Udollic Endoaqualf). This soil was mixed with sand in a 1:2 ratio to grow soybean in a randomized complete block split design . Four levels of seed coating materials (activated charcoal and graphite, both with or without vermicompost) were the main plot treatments and six levels of inoculation (1: non-inoculated, 2: non-inoculated but grown with N fertilizer, 3: KNI-3 alone, 4: KNI-3 and *S.kanamyceticus*, 5: KNI-3, *S.kanamyceticus* and *P. putida* 17-29, 6: KNI-3, *S.kanamyceticus* and *P. putida* G11-32) were the subplot treatment with four replications. The experiment was repeated three times under greenhouse condition.
- The data were subjected to analysis of variance (ANOVA) and least Squares Means contrast of the treatment effects. Mean separation (P < 0.05) was achieved by the Tukey's multiple range test or Student's *t*-test with JMP Statistical Discovery Software.

Results

Table 1. Effect of Seed Coating Material on Nodule Occupancy of Soybean Cultivar LS90-1920 by *B. japonicum* Strain KNI-3

Seed Coating Material[a]	Nodule Occupancy by KNI-3 (% plant^{-1})
AC	15 b[c]
ACV	14 b
G	18 b
GV	25 a

[a] AC: activated charcoal, ACV: activated charcoal and vermicompost, G: graphite, GV: graphite and vermicompost.

[b] Means in the same column followed by the same letter are not significantly different (P≤0.05) according to the Student *t* test

Table 2. Inoculation Responses of Soybean Cultivar LS90-1920 Grown in a Bethalto Silty Clay Loam

Seed Treatment[a]	Nitrogenase Activity	Nitrogenase Specific Activity	Nodule Biomass	Shoot Biomass	Nodule Number	Nodule Occupancy by KNI-3
	(μmole C_2H_2 hour^{-1} plant^{-1})	(μmole C_2H_2 hur^{-1} · Plant^{-1}.g^{-1} nodule	(mg plant^{-1})	(mg plant^{-1})	(plant^{-1})	(% plant^{-1})
Inoculum						
NI[b]	22.58 a[c]	307.12 a	58.3 a	552.4 b	25 a	0 b
NI+MN	2.25 b	117.80 b	8.1 b	978.8 a	3 b	0 b
KNI3	23.39 a	310.45 a	56.6 a	550.9 b	30 a	22 a
KNI3-SK	25.37 a	313.71 a	57.9 a	563.4 b	26 a	25 a
KNI3-SK-S2	28.6 a	389.55 a	57.8 a	542.3 b	26 a	27 a
KNI3-SK-S4	29.25 a	413.86 a	57.1 a	577.8 b	28 a	25 a

[a] Seed coating material: equal portion of graphite and vermicompost.

[b] NI: Not inoculated with bacterium, NI: Not inoculated with bacterium and grown in mineral nitrogen (NH_4NO_3), KNI3: Inoculated with *B. japonicum* strain KNI-3, KNI3-SK: Inoculated with KNI-3 and *S. kanamyceticus*. KNI3-SK-S2: Inoculated with KNI-3, *S. kanamyceticus* and *Pseudomonas putida* strain 17-29, KNI3-SK-S4: Inoculated with KNI-3, *S. kanamyceticus* and *Pseudomonas putida* strain G11-32.

[c] Means in the same column followed by the same letter are not significantly different (P≤0.05) according to the Tukey's multiple range Test.

Results

Table 3. Least-Square Means Contrast of Selected Inoculum Treatments on Soybean Cultivar LS90-1920

Measurement	P-value				
	Contrast 1[a]	Contrast 2[b]	Contrast 3[c]	Contrast 4[d]	Contrast 5[e]
nitrogenase activity	**0.02***	**0.041***	0.184	**0.013***	**0.016***
nitrogenase specific activity	**0.005****	**0.007****	**0.009****	0.082	0.196

Inoculum treatments: (1) NI: Non-inoculated, (2) KNI3: Inoculated with *B. japonicum* strain KNI-3, (3) KNI3-SK: Inoculated with KNI3 and S. *kanamyceticus,* (4) KNI3-SK-S2: Inoculated with KNI3, S. *kanamyceticus* and *Pseudomonas putida* strain 17-29,(5) KNI3-SK-S4: Inoculated with KNI3, S. *kanamyceticus* and *Pseudomonas putida* strain G11-32.

Seed coating materials (SCM): (1) AC: activated charcoal, (2) ACV: activated charcoal and vermicompost, (3) G: graphite, (4) GV: graphite and vermicompost.

[a] $\mu_{NI} = (\mu_{KNI3\text{-}SK\text{-}S2}+\mu_{KNI3\text{-}SK\text{-}S4})/2$, SCM: GV.

[b] $\mu_{KNI3} = (\mu_{KNI3\text{-}SK\text{-}S2}+\mu_{KNI3\text{-}SK\text{-}S4})/2$, SCM: GV.

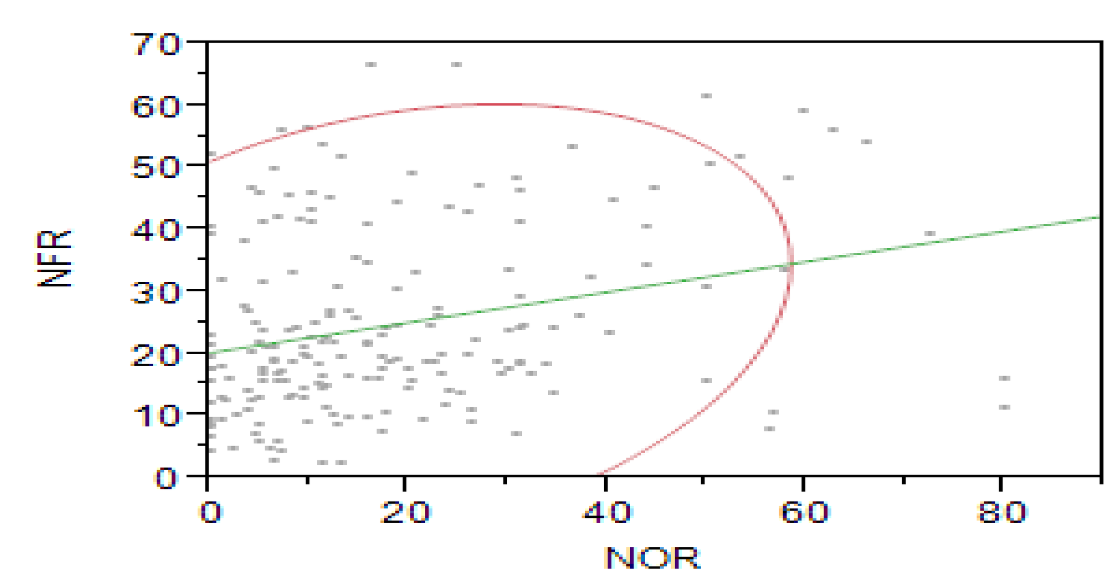
[c] $\mu_{KNI3\text{-}SK} = (\mu_{KNI3\text{-}SK\text{-}S2}+\mu_{KNI3\text{-}SK\text{-}S4})/2$, SCM: GV.

[d] $(\mu_{NI(AC)}+ \mu_{NI(ACV)} + \mu_{NI(G)} +\mu_{NI(GV)})/4 = (\mu_{KNI3\text{-}SK\text{-}S2\ (GV)}+ \mu_{KNI3\text{-}SK\text{-}S4\ (GV)})/2$

[e] $(\mu_{KNI3(AC)}+ \mu_{KNI3(ACV)} + \mu_{KNI3(G)} +\mu_{KNI3(GV)})/4 = (\mu_{KNI3\text{-}SK\text{-}S2\ (GV)}+ \mu_{KNI3\text{-}SK\text{-}S4\ (GV)})/2$

Significance level: *0.01< P ≤ 0.05, ** P ≤ 0.01.

Figure 1. Linear Regression for Data on Nitrogenase Activity of Soybean and Nodule Occupancy by KNI-3



NFR: Nitrogenase Activity (μmole C_2H_2 hour^{-1}plant^{-1})

NOR: Nodule Occupancy by KNI-3 (% plant^{-1})

Discussion

These findings suggest that the improved rate and efficiency of nitrogen fixation by soybean is related to greater nodule competency by the soybean inoculant following seed treatment with *Streptomyces kanamyceticus* and selected *Pseudomonas putida* strains. However, the presence of *S.kanamyceticus* in this novel seed treatment does not always guarantee greater nodule occupancy of *B. japonicum* inoculant as predicted by our working hypothesis. Optimization of the dosage and function of each bacterial inoculant by seed treatment will be further investigated to improve nodule competency and seed quality.

Summary

- Seeds coated by graphite and vermicompost gave rise to higher nodule occupancy of KNI-3 than by the other coating materials (Table 1).
- no significant differences in terms of nodule biomass, shoot biomass , nodule number and nodule occupancy were determined between all inoculum treatments (Table 2).
- the linear regression of nitrogen fixation rates and nodule occupancy of the *B. japonicum* inoculant (KNI-3) from soybeans receiving the inoculum treatments with KNI-3 were highly significant (P<0.001)(Fig 1).
- When graphite and vermicompost were used as seed coating material, the combined mean nitrogen fixation rate and combined mean nitrogenase specific activity of soybean receiving *S.kanamyceticus* and *Pseudomonas putida* strain G11-32 or 17-29 as co-inoculants with KNI-3 was significantly higher than those soybeans with other inoculum treatments (Table 3).
- The nitrogen fixation rate was still significantly higher when the combined mean of dual co-inoculant treatments were compared to the combined treatment mean of KNI-3 alone or without inoculation regardless of seed coating material (Table 3).