Evaluation of *Rhizobium trifolii* strains on nodulation of Trifolium sativus

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Abstract

- Eight strains of *Rhizobium trifolii* were evaluated for nodulating *Trifolium pratense* (red clover) and *Ttrifolium repens* (white clover).
- Plants were grown for seven weeks on sand-vermiculite (1:1) mixture under greenhouse condition. Shoot biomass yield and nodule dry weight were used to assess nodulation.
- There were distinct differences in shoot and root growth and development of both *T. pratense* and *T.repens* due to Rhizobia inoculation (Fig. 1-4)
- There was also a significant difference in both shoot dry matter yield and nodule weight of both types of clovers due to inoculation (Tables 1&2, Figures 5 & 6).
- The shoot biomass ranged from 7 to 274 mg/plant in *T. pratense, and 13 to 195 mg/plant in T. repens*.
- The degree of response in dry matter yield and nodulation in both types of clovers was different for different strains.
- Some of the strains gave several fold increase in dry matter yield and also showed specificity between the two types of clovers.

Introduction

- Biological nitrogen fixation (BNF) represents the major source of N-input in maintaining and improving the fertility of agricultural soils.
- The major one is the Rhizobium-legume symbiosis, which is known to be ideal for the supply of N to crops and improving the fertility of agricultural soils (Zahran 1999).
- The objective of this study was to identify strains, which have high potential in increasing the yield of *T. pratense* and *T. repens* through the supply of biologically fixed nitrogen.

Materials and Methods

- *T. pratense* and *T. repens* seeds were grown on sand and vermiculite mixture in Leonard Jars (Vincent, 1970) under greenhouse condition.
- Seeds were inoculated at planting with eight *Trifolium* strains of rhizobia, obtained from the USDA collections from Maryland, Alabama, and South Carolina areas
- Plants were supplied with N-free nutrient solution, and after seven weeks, shoot dry matter yield and nodule dry weight was assessed.
- Preparation of growth media as well as other isolation activities were carried out according to the procedure adopted by the Soybean Genomics and Improvement Laboratory of the Beltsville Agricultural Research Center of Maryland.

Results

- Results indicated that there were remarkable increases in shoot and root development of both *T. pratense* and *T. repens* in response to inoculation with some of the Rhizobia strains (Fig. 1-4)
- Significant increases were also observed in both shoot dry matter yield and nodule dry weight of both *T. pratense* and *T. repens*. The differences among strains were also significant (Tables 1 & 2, Fig. 5 & 6).
- In *T. pratense* the shoot biomass ranged from 7 mg/plant for *T. aureum* to 274 mg/plant for *T. nigrescens*.
- In *T. repens*, the range was from 13 mg/plant for *T. pratense* to 195 mg/plant for *T. incarnatum*. Such high response to inoculation is not uncommon (Beyene et.al. 2004;Young and Mytton, 1983; Brockwell, J. and Katznelson, J. 1975.)

Results

Nodule dry weight ranged from trace to about 10mg/plant in both *T. pratense* and *T. repens*.

In general, nodule mass had direct relationship with dry matter yield in both types of clovers

For T. pratense, the best performing strain in terms of dry matter yield was strain *T. nigrescens*, followed by *T. spp.* , *T. incarnatum* and *T. michelianum*.

- For *T. repens,* the best performing strain was *T. incarnatum*, followed by *T. michelianum, and T. nigrescens*.
- There was a general similarity in the response trend of *T. rapense* and *T. repens* to the Rhizobia inoculation.
- Strains *T. pratense* and *T. aureum* in particular, showed distinct specificity to *T. repens* (Vincent, 1954; Brockwell and J. Katznelson,1975) .
- Increase in yield of *T. pratense* by effective strains of *Rhizobia trifolli* has been also reported by other investigators (Materon and Hagedorn,1982).

Table 1. Effect of Rhizobia inoculation on shoot dry matter yield and nodule mass of *T. pratense* (Means with the same letter are not significantly different at 0.05 level)

Treatments	Dry matter yield (mg/plant)	Nodule mass (mg/plant)
Control	10 d	None (d)
T. *pratense* (mix)	57 c	2 c
T. *nigrescens*	274 a	10 a
T. *arvense*	15 c	None (d)
T. *lapaceum*	13 c	None (d)
T. *michelinum*	73 c	5 b
T. *spp*	205 b	7 b
T. *pratense*	13 c	None (d)
T. *aureum*	7 d	None (d)
T. *incarnatum*	203 b	8 ab

Table 2. Effect of Rhizobia inoculation on shoot dry matter yield and nodule mass of *T. repens* (Means with the same letter are not significantly different at 0.05)

Treatment	Dry matter yield (mg/plant)	Nodule mass (mg/plant)
Control	11 c	None (c)
T. repens (mix)	69 bc	3 bc
T. igrescens	112 ab	4 b
T. *arvense*	19 c	None (c)
T. *lapaceum*	16 c	None (c)
T. *michelinum*	131 ab	6 b
T. *spp*	58 bc	6 b
T. *pratense*	13 c	None (c)
T. *aureum*	14 c	None (c)
T. *incarnatum*	195 a	9 a

Figure 1. Effect of selected strains of rhizobia inoculation on growth of *T. pratense*, as compared to negative and positive controls

Figure 2. Effect of selected strains of rhizobia inoculation on growth of *T. repens*, as compared to positive and negative controls

Figure 3. Effect of selected strains of rhizobia inoculation on shoot and root development of *T. pratense*, as compared to positive and negative controls

Figure 4. Effect of selected strains of rhizobia inoculation on shoot and root development of *T. repens*, as compared to positive and negative control.

1 = Control, 2 = T. pratense (positive control), 3 = T. nigrscens, 4 = T. arvense, 5 = T. lapaceum, 6 = T. michelinum, 7 = T. spp., 8 = T. pratense, 9 = T. aureum, 10 = T. incarnatum

1 = Control, 2 = *T. pratense* (positive control), 3 = *T. nigrescens*, 4= *T. michelinum*, 5 = *T. spp.*, 6 = *T. incarnatum*

Figure 5. Effect of Rhizobia inoculation on dry matter yield of *T. pratense* and *T. repens*

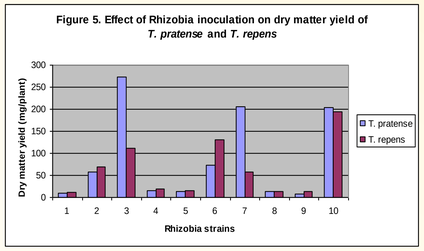
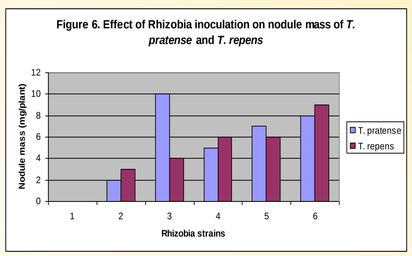


Figure 6. Effect of Rhizobia inoculation on nodule mass of *T. pratense* and *T. repens*



Conclusion

- Both *T. pratense* and *T. repens* showed significant response in plant growth, dry amtter yield and nodulation to selected rhizobia strains.
- There were significant differences among the strains tested. *T. nigrescens* gave the highest dry matter yield and nodule mass for *T. pratense*, while *T. incarnatum* was the highest in dry matter yield and nodule weight for *T. repens*.
- The research results warrant further inoculation studies on *Trifolium pratense* and *Ttrifolium* repens, in order to identify the most effective N-fixing rhizobium strains under field conditions.

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