Colonization of the alfalfa rhizosphere by plant growth promoting rhizobacteria.

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Abstract

Plant growth promoting rhizobacteria (PGPR) were evaluated for their ability to colonize the alfalfa rhizosphere and increase dry matter yield of alfalfa. Field trials at Beaverlodge, AB., Sun Prairie, WI. and Outlook, SK. demonstrated that PGPR's Pseudomonas 31-12 (Ps.) and Serratia 2-68 co-inoculated with Rhizobium meliloti increased the dry matter yield by 11, 9 and 7%, respectively. In the first year of a three year field study planted in May 1993 at Outlook, the effect of formulation carriers on colonization of the alfalfa rhizosphere by spontaneous rifampicin resistant mutants (rif^+) of PGPR's Ps. 31-12 and Serratia 2-68 was measured. No rif^+ microorganisms were detected in the rhizosphere of uninoculated alfalfa plants. Ps. 31-12 was the better colonizer of the rhizobacteria examined with populations exceeding 10^6 cells per root 169 days after planting. The population of Ps. 31-12 was 10 times greater 35 and 169 days after planting in the peat formulation than in the clay formulation. Similarly, the population of Serratia 2-68 in the alfalfa rhizosphere was 1000 times greater in the peat formation than in the clay formulation. The populations of these microorganisms will be measured over the next two growing seasons.

Introduction

For over a century we have managed the rhizosphere of leguminous crops by applying Rhizobium to legume seed. This practice ensures the development of N_2-fixing root nodules enabling up to 70% of the nitrogen requirements of some legumes' to be met. Interest in the rhizobacteria has greatly increased over the last decade because of reports of plant growth promotion following seed inoculation. The PGPR (plant growth promoting rhizobacteria, Kloepper et al., 1988) are a specialized group of bacteria that actively colonize and persist in and on the roots of plants. These microorganisms have been reported to improve growth of cereals, oil seed crops, vegetables and annual and perennial legumes (Kloepper et al., 1988). The term nodulation promoting rhizobacteria (NPR) was suggested following studies showing an increase nodule number and mass for soybeans co-inoculated with PGPR and Bradyrhizobium.

Both direct and indirect growth promotion mechanisms were identified to be active in these bacteria. Direct effects occur from bacteria-produced compounds (Yoshikawa et al. 1993) with phytohormonal properties or from products that facilitate uptake of nutrients such as nitrogen, phosphorus and iron (Kloepper et al. 1991, Grimes and Mount 1987). Indirect effects occur when the activities of the rhizobacteria results in reduced populations of deleterious microbes, particularly those of plant pathogens (Bull et al. 1991; Dahiya et al. 1988; Handelsman et al. 1990; Liao 1989; Loper and Buyer 1991; Reddy et al. 1994, Voisard et al. 1989; Weller 1988).

Further effects observed in plants following co-inoculation of rhizobacteria and Rhizobium are improved root and shoot weight, plant vigour, nodulation, N_2 fixation (C_2H_2 reduction) and yield by various legumes such as bean (Grimes and Mount, 1983), chickpea (Del Gallo and Fabbri, 1991), soybean (Halverson and Handelsman, 1991, Singh and Subba Rao, 1979, Polonenko et al., 1987, Li and Alexander, 1988), alfalfa, clover (Handelsman et al., 1990, Harris, 1953, Yahalom et al., 1987), lentil and pea (Chanway, Hynes and Nelson, 1987).

We have observed in previous field studies carried out by Esso and Agriculture Canada scientists that PGPR can increase the dry matter yield of alfalfa by as much as 12%. Field trials were conducted to gain more understanding of the colonization characteristics by these rhizobacteria on alfalfa. We measured colonization of the alfalfa roots using rifampicin resistant strains of PGPR formulated in the peat and clay powder. The goal of this project was to: 1. Monitor the population levels of the introduced PGPR in the rhizosphere of alfalfa over a 3 year period. 2. Determine which formulation is best for the PGPR.

Materials and Methods

Treatments

The bacterial strains, PGPR's 31-12, 2-68 and Rhizobium meloliti were formulated in clay at Lipha Tech, Milwaukee, WI. and peat at Esso Ag Biologicals, Saskatoon. Alfalfa cv Beaver was inoculated with the clay and peat plus sticking agent (Nitra Coat) using reccommendated rates and seeded within 48 hours. Treatments including untreated seed, Rhizobium meloliti alone and Rhizobium meloliti plus PGPR were planted into 1.5 by 2.4 m plots in a randomized complete block deseign.

Colonization study

Spontaneous rifampicin-resistant PGPR's 31-12 and 2-68 mutants were selected on PAF supplemented with 100 ppm rifampicin (R) (Sigma Chemicals, St Louis, Mo). For monitoring the population of rifampicin-resistant PGPR's on alfalfa roots, 50 ppm benomyl (B) (Wilson's, Dundas, Ont.) was also added to PAF+R medium. Three plants per plot were carefully up-rooted and excess soil removed from the roots. The roots were suspended in sterile water and agitated at high speed for two minutes. Aliquots from serial dilutions were plated onto PAF+R+B medium. Colony counts were made from the appropriate dilutions after 48 hours of incubation at 24^0 C and the numbers averaged.

Acetylene reduction assay

Seven plants per plot were carefully up-rooted and excess soil removed from the roots. Roots were separated from shoots and placed in a jar with screw cap and 5% of the jars' gas phase was replaced with acetylene. After 30 minutes 5 mL of gas was removed from the jars and placed into vacu-tainers. Gas samples were analyzed later (within 30 days) for C_2H_2 and C_2H_4 using a Poropak R column fitted into a Shimadzu gas chromatograph GS8A equipped with an flame ionization detector.

Harvest

Plots were cut using a plot combine equipped with a scale to measure plot fresh weight yield. Grab samples were taken to obtain moisture content of the alfalfa and a measure of the dry matter yield.

Results and Discussion

Colonization Study

Plants were removed from the plots and the PGPR from the roots were enumerated following serial dilution and plating onto selective medium. Table 1 shows that the population of PGPR 31-12 on alfalfa roots was larger than plants similarly treated with PGPR 2-68 and that larger populations developed from alfalfa seed inoculated with the microorganisms formulated in peat than in the clay powder. No, PGPR were detected on the uninoculated plants (Table 1).

After the first year of a three year study we report that PGPR 31-12 and 2-68 formulated in peat and clay can establish and maintain themselves in the rhizosphere of

alfalfa. Enumeration of these PGPR will continue next year to determine their ability to survive in the rhizosphere of alfalfa over winter.

Table 1. Colonization of the rhizosphere of alfalfa by PGPR

Treatments	Microorganisms/root	
	35 DAP	169 DAP
Rhiz.+31-12 C	2.40E+07	1.40E+06
Rhiz.+2-68 C	2.60E+07	2.00E+03
Rhiz. C	ND	ND
Rhiz.+31-12 P	1.30E+08	2.00E+07
Rhiz.+2-68 P	7.50E+08	1.50E+06
Rhiz. P	ND	ND
Uninoculated	0	0

Note DAP= days after planting. ND= not done

Acetylene Reduction Study

Nitrogen fixation was acessed by measuring the acetylene reduction activity of the nodulated roots. Uninoculated plants had developed root nodules indicating a indigenous population of R. meloliti was present at this site. However, co-inoculation of alfalfa with PGPR particularly in the clay formulation improved acetylene reduction activity over the Rhizobium alone treatment (Table 2).

Table 2. Effect of PGPR on nitrogen fixation (acetylene reduction) by alfalfa

Treatments	umol C2H4/g dry root
Rhiz.+31-12 C	0.569
Rhiz.+2-68 C	0.694
Rhiz. C	0.347
Rhiz.+31-12 P	0.474
Rhiz.+2-68 P	0.624
Rhiz. P	0.607
Uninoculated	0.413

Note: Acetylene reduction assay done 42 DAP. C and P refer to clay and peat based formulations.

Effect of PGPR on Dry Matter Yield

The 5th and 6th cuts were taken from the alfalfa trial started in 1991 concluding this three year study. The effect of four PGPR co-inoculated with R. meloliti on dry matter yield is shown in Table 3. PGPR 31-12 increased the dry matter yield of alfalfa between 5 and 12% in 5 of 6 cuts over the Rhizobium alone treatment (Table 3). These results confirm earlier Beaverlodge and Wisconsin studies demonstrating the benefits of co-inoculating alfalfa with PGPR 31-12 and Rhizobium.

Table 3. Effect of PGPR on the Dry Matter yield of Alfalfa at Outlook, SK.

Treatments	1991		1992		1993		Cumulative
	Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	Cut 6	
31-12 + Rhizobium	5.17	4.03	8.53	6.20	9.23	5.39	38.55
.2-68 + Rhizobium	4.65	4.10	8.88	5.94	8.45	5.23	37.26
61-9A + Rhizobium	4.78	3.78	8.25	5.86	8.63	5.21	36.51
G2-26 + Rhizobium	4.73	4.07	7.88	5.57	8.50	5.44	36.19
Rhizobium	4.85	3.79	7.65	5.92	8.63	5.54	36.38
Untreated	4.82	3.83	8.72	5.82	8.95	5.27	37.40

Conclusions

- PGPR 31-12 increased the dry matter yield of alfalfa 6%, averaged from 6 cuts over 3 years.
- PGPR 31-12 was the better colonizer of the rhizobacteria examined with populations exceeding 10^6 cells per root 169 days after planting.

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