BioBoost: a new sulfur-oxidizing bacterial inoculant for canola

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Abstract

To hasten the process of sulfur (S) oxidation in soil, a S-oxidizing bacterial inoculant BioBoost was used as canola seed treatment to meet the plant S requirement and to increase canola production in Western Canada. BioBoost is a contaminant free peat based inoculant, having a shelf life over five months with the adequate level of viable bacterial cells. The active ingredient of the inoculant BioBoost is a selected strain of *Delftia acidovorans* isolated from Canadian soil, which is also a canola plant growth promoting rhizobacteria (PGPR). Our multi year multi sites field studies with BioBoost showed that the bacterial inoculant significantly enhanced canola performance and yield. Being PGPR, BioBoost inoculant promoted canola production irrespective of the soil S status of the fields. Seed analysis showed that BioBoost inoculant helped in canola S-uptake but did not change the seed quality traits like oil, protein, oleic acid, linolenic acid and glucosinolate content of canola seed. Thus, this research has developed a bacterial inoculant that improves canola production without having any effect on the seed quality aspect of canola. In fact, this research has developed a new microbial inoculant BioBoost which is the first S-inoculant for canola to the best of our knowledge.

Media summary

A new microbial inoculant to enhance canola production has been developed that would shortly be available as a commercial product in Canada and United States.

Key Words

PGPR, Sulfur oxidation, Canola growth, Inoculant.

Introduction

Use of plant growth promoting rhizobacteria (PGPR) as microbial inoculant to increase agricultural production, biocontrol of plant pathogens and aid in bioremediation is getting world wide attention. Potential positive impacts of PGPR have been demonstrated in crops like radish, potato, sugarbeet, bean, barley, vegetables, canola, pea, peanut and many other crops. Although PGPR may reveal huge potential for crop production, for a microbial inoculant to be commercially feasible, it must be economically mass-produced and formulated into a cost-effective, uniform and readily applicable form (Walter and Paau 1997). Success of microbial inoculant for enhanced crop production is also greatly influenced by the number of viable cells introduced into soil (Duquenne et al. 1999) as biological activity may decline rapidly with handling and storage procedure. Thus, it is critical that the PGPR inoculant product be in such a formulation, which would not only deliver adequate bacterial population but also have enough product shelf life.

Presumably the most successful and recognizable PGPR to be used as microbial inoculant for agricultural crops is that based on *Rhizobium spp.* through symbiotic nitrogen fixation (Chanway et al. 1989). Several other mechanisms like phytohormone production (Brown 1974), extracellular siderophore production (Kloepper et al. 1980), effects on ion uptake by roots (Lifshitz et al. 1987) and induced systemic resistance (Wei et al. 1996) have also been credited for their growth promoting activity. Although the mechanism of enhanced sulfur oxidation by PGPR resulting in increased crop performance have been established (Grayston and Germida 1991; Banerjee 1995) but little information is available on successful application of sulfur-oxidizing PGPR as inoculant for agricultural crops.

Canola (*Brassica napus* L. cv), like other oil seed crop has high sulfur (S) demand. Crop growth and production are declined when canola is grown in S deficient soils in Europe and elsewhere (Scherer 2001). Fertilizer industry is promoting elemental S fertilizer in recuperating S deficiency because of its inherent attractiveness of being concentrated form, slow release characteristics and an industrial by-product. However, elemental S must be oxidized to sulfate form to become plant available. This conversion is generally carried out by S-oxidizing soil microorganisms that need 18-24 months. To accelerate this process of S oxidation, BioBoost, a S-oxidizing PGPR (Banerjee and Yesmin 2002) inoculant was utilized as canola seed treatment to meet the plant S requirement and to increase canola production. Others may have isolated and identified canola PGPR for canola growth promotion (Kloepper et al. 1988; Bertrand et al. 2001), but our research most probably the first instance that used S-oxidizing PGPR to produce the first commercial inoculant for canola. But much works are needed urgently to demonstrate the mass production of this S-oxidizing inoculant to be technologically and commercially viable.

Methods

BioBoost inoculant

The active ingredient of the product BioBoost is the S-oxidizing rhizobacteria identified as *Delftia acidovorans* RAY209 using 16S rDNA method. The strain RAY209 is inoculated to the gamma irradiated sterile Canadian sedge peat powder of 300 mesh. The inoculated packets are incubated at 28^{o}C for 7 days for microbial proliferation and are ready to use for seed inoculation.

Field trials

To test the efficacy of the product BioBoost in the Canadian prairies, four field trials in year 2002 (Dauphin, Miami, La Salle and MCDC) and seven field trails in year 2003 (Dauphin, Neepawa, La Salle, Elm Creek, Gladstone, MacGregor and Millet) were carried out. The trials were laid as Randomized Complete Block Design (RCBD) with six to eight replications in each site. Each replication was considered as a block and the treatments were randomized within each block. Sulfur fertilizer was added either as elemental sulfur (ES) or as sulfate sulfur (SO_4) at seeding. Elemental S was applied with seed, and, sulfate S was broadcasted and recked into the soil. Fungicide (Helix) treated, herbicide-tolerant canola, cultivar Libred 799 RR was used in these trials. The seeding rate of the canola used was 6 lbs/ac (6.72 kg/ha). The ratio of canola seed: sticker: peat inoculant was 1000 g : 40 ml : 60 g. Coating of seeds with peat inoculant was done on the field site just before seeding. Seeding was done using plot seeder. During the experiments, all the plots were maintained following the standard maintenance for canola production in the region. All the plots were harvested singly with plot combine. For each plot, harvested seed samples were bagged separately and weigh them to get yield per plot basis. Seed moisture was measured to get moisture corrected yield result (8.5% seed moisture basis).

Nutrient uptake and Seed quality

From each plot small sub samples were prepared for seed nutrient and quality analyses. Seed nutrient analyses were done using acid digestion and Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) method, and, other seed quality analyses were done using Near Infra Red Spectroscopy (NIRS) method.

Results

Table 1. 2002 Field trials: canola yield (kg/ha).

Treatment	Location

	Dauphin	Miami	La Salle	MCDC
Control (C)	988.14	1348.53	2336.41	2145.45
C+ES	970.34	1322.75	2367.25	2154.65
C+SO4	1115.00	1344.18	2398.62	2160.76
C+BioBoost	1096.63	1358.65	2367.92	2165.10
C+ES+BioBoost	1120.13	1497.61	2411.15	2222.69
C+SO4+BioBoost	1132.59	1378.67	2524.65	2209.72
LSD (5%)	*71.63*	*33.55*	*58.66*	*NS*

BioBoost is a contaminant free peat inoculant for canola containing 100 million ($1X10^8$) viable cells of a selected strain of *Delftia acidovorans* per gram of peat and optimized to survive to the desired level in peat bag over five months from the date of production. Table 1 showed that the BioBoost inoculant increased canola yield in different field trials in the year 2002. The bacteria alone or in combination with other S fertilizers has increased canola yield by 109-145 kg/ha, 10-149 kg/ha, 32-188 kg/ha and 20-77 kg/ha in Dauphin, Miami, La Salle and MCDC, respectively. Although not all the increases were significant but positive trends in canola production were observed in all the sites upon bacterial inoculation. When inoculant was used in combination with elemental S or sulfate S yield increase was more evident over control. Inoculant in combination with ES showed significant ($p<0.05$) yield increase above control in Dauphin, Miami and La Salle.

Table 2. 2003 Field trials: canola yield (kg/ha).

Treatment	Location						
	Dauphin	Neepawa	La Salle	Elm Creek	Gladstone	MacGregor	Millet
Control (C)	1492.55	796.60	2161.01	2649.93	1342.17	2336.81	1489.39
C+ES	1467.60	1083.41	2163.70	2650.13	1599.46	2464.88	1560.86
C+SO4	1603.88	1095.67	2270.58	2700.36	1788.35	2507.55	1639.01
C+BioBoost	1574.24	1238.22	2248.75	2700.44	1705.04	2439.46	1733.14
C+ES+BioBoost	1625.25	1295.72	2280.19	2811.76	1722.05	2583.83	1771.21

C+SO4+BioBoost	1618.14	1301.12	2265.34	2648.27	1903.51	2541.23	1682.47
LSD (5%)	*70.56*	*123.58*	*89.18*	*81.81*	*287.50*	*NS*	*NS*

Table 2 also showed that the BioBoost inoculant increased canola yield in different field trials in the year 2003. Although not all the increases were significant but positive trends in canola production were observed in all seven sites upon bacterial inoculation. Significant ($p<0.05$) increases in canola yield were obtained due the bacterial inoculation in Dauphin, Neepawa, La Salle, Elm Creek and Gladstone. When inoculant was used in combination with ES or sulfate S yield increase over control was also quite evident. Inoculant in combination with ES showed significant ($p<0.05$) yield increase above control in Dauphin, Neepawa, La Salle, Elm Creek and Gladstone.

Table 3. Seed sulfur uptake of canola inoculated with BioBoost in 2002 Dauphin field trial site.

Treatment	S concentration (%)	S uptake (kg/ha)	% Change in S uptake from control
Control (C)	0.43	4.25	0
C+ES	0.43	4.13	-2.8
C+SO_4	0.46	5.10	20.0
C+BioBoost	0.44	4.83	13.7
C+ES+BioBoost	0.43	4.84	13.9
C+SO_4+BioBoost	0.46	5.18	21.9
LSD (5%)	*NS*	*0.42*	-

Table 4. Seed quality of canola inoculated with BioBoost in 2002 Dauphin field trial site.

Treatment	Oil Content (%)	Protein Content (%)	Oleic acid (%)	Linolenic acid (%)	Glucosinolate content (µmol/g)
Control (C)	49.13	22.10	58.35	7.77	14.45
C+ES	48.43	23.08	57.65	7.93	14.52
C+SO_4	48.22	23.45	58.08	8.27	15.02

C+BioBoost	48.57	22.57	58.23	8.25	15.45
C+ES+BioBoost	48.93	22.25	57.83	8.02	14.97
C+SO_4+BioBoost	48.45	23.08	58.72	8.85	15.48
LSD (5%)	*NS*	*NS*	*NS*	*NS*	*NS*

Results of the field seed samples analyzed for S concentration and seed quality showed that with yield increase, use of inoculant increased S uptake (Table 3) and retained all quality aspects of canola seed (Table 4). Thus, BioBoost inoculant with ES combination not only increase canola yield significantly compared to control but also compete with sulfate S treatment.

Conclusion

The overall field efficacy results with BioBoost evidently showed that this inoculant can work as canola PGPR to enhance canola production in Western Canada. This naturally occurring S-oxidizing rhizobacterial inoculant retained all the seed quality aspects of canola. The BioBoost peat inoculant can also provide farmers with agronomic benefits of reduced input cost and better crop yield in an environment friendly manner. Thus, this research has developed a new microbial inoculant that is the first S-inoculant for canola to the best of our knowledge.

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