Growth promoting effects of the diazotroph *Azorhizobium caulinodans* on Canadian wheat cultivars

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Abstract

Production of wheat typically requires intensive use of chemical fertilizers. Reliance on fertilizers may be decreased by exploitation of plant growth promoting organisms. This work examines the responsiveness of Canadian cultivars of Hard Red Spring wheat to inoculation with *Azorhizobium caulinodans*, a diazotroph isolated from the legume *Sesbania rostrata*. This bacterium has been shown to colonize wheat roots through crack entry of the lateral roots. Inoculation of wheat cultivar CDC Teal grown in field soil caused increases in grain yield and total biomass of 34 and 49%, respectively. Inoculated plants produced more tillers and had larger leaf area than un-inoculated plants. In contrast, wheat cultivar AC Taber did not benefit from inoculation. A screening bio-assay was developed to test whether the effects of inoculation are common within Canadian wheat cultivars. Low levels of nitrogenase activity were detected in preliminary studies. It is yet to be verified whether the nitrogen being fixed is sufficient to be of significant benefit. We have hypothesized that the observed effects may be caused by other growth promoting mechanisms. For example, we have observed changes in root morphology that may increase nutrient and water use efficiencies and drought tolerance.

Media summary

Prospects of microbial plant growth promoting inoculants to decrease nitrogen use and environmental sustainability of agriculture.

Key Words

PGPR, Nitrogen fixation, Endophytic, Leaf area, Nutrient use efficiency.

Introduction

Nitrogen is a very important plant nutrient whose limitation can affect yield considerably. Production of chemical nitrogen fertilizers besides being costly, depletes non-renewable resources and poses human and environmental hazards. To complement and eventually substitute mineral fertilizers with biologically fixed nitrogen would represent an economically beneficial and ecologically sound alternative (Glick et al. 1999). Bacteria are abundantly present in the rhizosphere and in close vicinity of the root. It has long been recognized that several genera of these rhizobacteria have the ability to promote plant growth and these have been termed plant growth promoting rhizobacteria or PGPR. Some of these rhizobacteria interact with the plant in different mutually beneficial ways and may thereby promote plant growth or yield by direct or indirect mechanisms. Direct growth promotion may be through biofertilization through synthesis of elements or compounds utilizable by the plant or by aiding the plant in uptake of nutrients and/or water. Indirect growth promotion on the other hand may entail biocontrol of infection by phyto-pathogenic organisms.

The bacteria that provide some benefit to plants are of two general types: those that form a symbiotic relationship, which involves formation of specialized structures or nodules on host plant roots, and those that are free living in the soil; the latter are often found near, on or even within the roots of plants (Kloepper et al. 1988). Large-scale application of PGPRs to crops as inoculants would be very attractive as it would substantially reduce the use of chemical fertilizers and pesticides, which often pollute the environment. *Azorhizobium caulinodans* is a stem and root nodulating nitrogen fixing bacterium that was isolated from the stem nodules of *Sesbania rostrata* (Dreyfus et al. 1988). Studies have shown endophytic colonization of roots of non-legumes e.g. wheat, where it stimulates root development accompanied by increased yield and N content (Sabry et al. 1997). Quispel (1991) has suggested that only in endophytic systems are the prerequisites for effective nitrogen fixation likely to be fulfilled in non-legume rhizobial interactions. Considerable levels of nitrogenase activity have been detected in wheat plants inoculated with *A. caulinodans* (Sabry?et?al. 1997). However, the level to which the fixed nitrogen benefits the plant remains to be verified.

With more than 61.5 million acres of grains and oilseeds grown annually in Canada, the use of diazotrophic PGPR in non-legumes represents an enormous opportunity for agriculture. Our objective therefore is to evaluate the potential of improving wheat production in Canada through inoculation with *A. caulinodans* and to develop commercial inoculants that can be made available to producers.

Methods

Greenhouse experiments

The experiments were performed in a greenhouse with photoperiod of 16 hours using natural light supplemented with sodium halide light bulbs. Day and night temperature in the greenhouse ranged from 20 to 32?C and 14 to 20?C, respectively while relative humidity was from 10 to 70% throughout the experiments. Plants were grown in soil beds (4.2 x 2.5 x 0.6 m) filled with topsoil diluted with peatmoss. The dilution was carried out to achieve a starting nitrogen level of approximately 20 kg/ha and the final ratio of soil to peat was approximately 1:3. In the trial conducted in 2000, seeds of wheat cultivars CDC Teal and AC Taber were surface sterilized in 20% domestic bleach and rinsed 3 times with distilled water. *A. caulinodans* inoculum was produced as described in Sabry et al. (1997). The seeds were then soaked in inoculum for 20 minutes before sowing into the soil beds. A group of seeds were soaked in re-autoclaved inoculum and used as controls. At maturity, plants were harvested, and plant biomass, grain yield and yield components were determined.

In 2002, experiments were conducted to verify the results obtained in the 2000 trials. Wheat cultivar CDC Teal was tested under similar conditions and settings described above. During this trial, we measured plant performance by taking plant height, number of tillers, leaf area, and canopy cover at 5 and 7 weeks after seeding as well as the grain yield at maturity.

Screening assay in growth chamber

Following confirmation of the results obtained in the first experiment and our discovery that the two cultivars did not respond equally to inoculation with *A. caulinodans*, we decided to develop a quick screening assay to test the response of several elite cultivars of wheat to *A. caulinodans*. In the assay, plants were grown in small cups filled with autoclaved nutrient free sand. We adopted the inoculation protocol of Sabry et al. 1997. Control wheat plants received re-autoclaved inoculum. The assay was developed using cultivar CDC Teal. Plants were grown for 5 to 6 weeks and harvested for root and shoot weight determination. This assay has now been used to screen 4 more cultivars for responsiveness to inoculation.

Results

In a preliminary trial in 2000, inoculated plants of CDC Teal produced larger leave laminas and were somewhat delayed in their phenology. At maturity, inoculation lead to a 49% increase in above-ground biomass and consequently, grain yield increased by 34%. In contrast, cultivar AC Taber did not benefit from inoculation (data not shown). Results of the 2002 experiment are summarized in Tables 1 and 2. Visual observations can be made from Figure 1.

Figure 1. Wheat cv. CDC Teal grown in the presence (right) and absence (left) of *A. caulinodans*.

Table 1. Means and standard errors of growth parameters at 7 weeks of inoculated and un-inoculated plants of wheat cv CDC-Teal grown in soil beds in a greenhouse

Treatment	Height (cm)	L-Area (cm^2)	LAI[1]	Tillers	Biomass ($g^{-5plant}$)
Control	37.72?0.72	52.12?5.01	0.35?0.03	0.83?0.19	4.89?0.50
Inoculated	38.09?0.50	62.11?5.32	0.50?0.04	1.17?0.10	5.63?0.36
Difference. (%)	1	19	41	40	15

[1]LAI means leaf area index

Table 2: Means and standard errors of yield and yield parameters of inoculated and un-inoculated plants of wheat cv CDC-Teal grown in soil beds in a greenhouse

Treatment	Biomass (g)	Seed yield (g)	Seed number
Control	1.87?0.12	0.96?0.08	31.38?1.64
Inoculated	2.70?0.12	1.35?0.06	43.79?1.51
Difference. (%)	43	40	38

The results indicate that at 7 weeks after seeding, inoculated plants performed considerably better than their un-inoculated controls. Consequently, plant biomass and grain yield were superior in the inoculated plants, which confirms results obtained for the cultivar in the preliminary experiment. Nitrogen content was higher in the inoculated plants (data not shown). The cultivar screening bio-assay also separated clearly inoculated from un-inoculated plants of CDC Teal, which was consistent with results obtained in experiments where plants were grown to maturity (Table 3). We have now screened several elite cultivars of Canadian Red Hard Spring wheat to gain information into the genetic mechanisms of strain/cultivar specificity.

Table 3: Means and standard errors of growth parameters at 4 weeks of inoculated and un-inoculated plants of wheat cv CDC-Teal grown in pots filled with sand in a growth chamber

Treatment	Height (cm)	L-Area (cm^2)	Tillers	Biomass (g $plant^{-1}$)
Control	9.98?0.53	26.65?1.32	0.00?0.00	0.19?0.01
Inoculated	11.75?0.33	41.73?4.86	1.50?0.29	0.28?0.03
Difference. (%)	18	57	100	47

Conclusion

The results of these studies show considerable growth promotion by *A. caulinodans* on wheat cultivar CDC Teal. AC Taber on the other hand did not benefit from inoculation, which suggests that growth promotion may be cultivar specific. Mechanisms of growth promotion in wheat by this bacterium are yet to be fully elucidated. Although we found some level of nitrogenase activity, which conforms to the studies of Sabry et al. (1997), there is a need to verify that the nitrogen being fixed is beneficial to the plants. We are currently looking at nitrogen and water use efficiencies as other possible explanations for growth promotion by the bacterium in wheat.

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