Rhizobacterial influence on healthy stand establishment of canola grown in *Rhizoctonia solani* infested fields of Saskatchewan

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Abstract

Pre-emergence seedling damping-off, seedling blight, and brown girdling root rot caused by *R. solani* are important diseases of canola/rape seed in western Canada. Annual yield losses in excess of 20-30% have been reported in several infected fields. Cultural control methods or resistant cultivars are currently unavailable for these diseases. Chemical fungicides have been developed for use to control the disease, but the success rate has been varied. However, the use of chemicals is becoming less acceptable from an environmental point of view. Several studies indicated that biological control using plant growth-promoting rhizobacteria may also be effective in controlling *R. solani*. Field plots were established in Saskatoon, Regina and Melfort, SK, in 1990, 1991 and 1992 to evaluate the potential of rhizobacterial strains as seed treatments to increase the healthy stand of canola CV. Westar grown in *R. solani* infested field. The bacteria were formulated either in sterile peat or in a liquid carrier and applied to seed just before planting. Bacterized seed were mechanically planted in replicated field plots artificially infested with *R. solani*. Final healthy stand was measured 30 days after planting. Grain yields were determined by harvesting the plots. Seed bacterization significantly increased the final healthy stand compared to non-bacterized controls. Strains which increased stand showed *in vitro* antagonistic activity to not only *R. solani* but also other pathogens such as *Pythium ultimum, Fusarium solani* and *F. oxysporum*. Some of these strains induced root elongation of canola under laboratory conditions. Rhizosphere colonization, chemical compatibility and shelf-life of the important bacteria will be discussed.

Introduction

Pre- and postemergence seedling damping-off or brown girdling root rot of canola/rape seed crops caused by *R. solani* are important diseases in western Canada (Berkenkamp and Vaartnou, 1972; Kaminski and Verma, 1985). The root rot severity in canola grown fields of Saskatchewan and Manitoba is generally low (Petrie, 1973). Canola grown fields in Alberta, particularly in the Peace River region have been severely affected, at times as high as 80-100% with annual yield losses in excess of 20-30 % as previously reported (Sippell *et al.*, 1985). The root rot is characterized by a brown girdling of the root and basal stem which causes poor pod formation, pod sterility, loss of seed weight,

lodging and uneven ripening. Cultural control or resistant cultivars are currently unavailable for this disease. Though chemicals have potential in suppressing the disease, the use is becoming less acceptable from an environmental point of view. The development of resistance to many fungicides by major pathogens and public concern over chemical fungicides in foods and the environment have created interest in alternative methods of disease control. Biological control of soil-borne diseases of agricultural crops has emerged as a promising option. Biological control by fluorescent Pseudomonads has been studied for a number of years (Reddy, 1991). Moreover, biological control is a desirable strategy for control of damping-off diseases since breeding for resistance for many of the damping-off diseases has been unsuccessful (Reddy, 1991). However, the effect of biological control with the introduced microbials in the field has been inconsistent and therefore not yet applicable for commercialization. The object of this study was to test the efficacy of select Pseudomonads under extensive field trials infested with *R. solani* in order to bring the microbial towards commercialization as a seed treatment.

Materials and methods

Rhizobacterial strains used in this study are listed in Table 1. Bacterial strains were isolated from diverse soils and root regions as part of another study and were previously reported (Kloepper *et al.* 1986, 1988). Indentification of the strains was accomplished by determining their growth on various types of media, ability to form pigments, and by their responses to the biochemical tests included in API-20 E strips. Details on this were reported earlier (Kloepper *et al.* 1986, 1988). The strains used were selected for this study because they possessed one or several of the following characteristics: i) *in vitro* antibiosis against various fungal pathogens including *R. solani*, ii) promotion of rhizobial root nodulation, iii) enhanced root and shoot growth of various crops and vegetables and iv) ability to produce plant growth regulators (Kloepper *et al.* 1986, 1988, Reddy *et al.* 1990, 1991; Young *et al.* 1991 and unpublished results).

Field trials were conducted in 1990, 1991 and 1992 on canola CV. Westar in Saskatchewan. Experimental sites maintained by Agriculture Canada in Saskatoon, Regina and Melfort were chosen for this study. In 1990, experiments were conducted at one site (Saskatoon) and in the other two years experiments were set up at the three locations. Basically, there were four treatments in each experiment included: i) canola seed treated with select bacterial strain and planted in *R. solani* infested plot; ii) canola seed not treated with bacteria and planted in *R. solani* infested; iii) canola seed treated with Vitavax RS and planted in *R. solani* infested plot and iv) untreated canola planted in a plot without *R. solani* infestation. There were 5 bacterial strains tested in 1990 and 1991, and 6 bacterial strains in 1992 were tested as seed treatments for their efficacy to enhance stand establishment. In 1990 bacteria formulated in sterile peat and 1991 and 1992 bacterial formulated in a liquid inoculum were used to treat the canola seed before planting. This procedure yielded Log 5-6 cfu/ seed. Treatments at each site were replicated 8

times. Each replicate plot was 6 m x 3 m, 5 experimental rows, and were separated by 2 guard rows planted with winter canola. There were 300 seeds planted per each 6 m row. Experimental field plots were artificially infested with *R. solani* grown on barley grain. Treatment plots were arranged in a randomized complete block design. Urea N was deep banded at 40 Kg N/ha before seeding. Phosphorous was applied at the time of seeding in the seed rows at a rate of 50 Kg/ha as 12-51-0-2. All the plating was done mechanically. Final healthy stand was taken from 3 centre rows 1 m length per replication at 30 days after plating to asses the efficacy of the bacteria in all the sites. Harvest was done mechanically at maturity and grain yield were summed and converted to estimates of Kg/ha. The data was analyzed by ANOVA. LSD was used to determine the significance between bacterized and control treatments.

Results

Influence of seed bacterization on canola stand establishment grown in *R. solani* infested site at Saskatoon in 1990 was shown in Fig. 1. As shown, only one rhizobacterial strain G1-3 and Vitavax RS significantly enhanced the healthy stand of canola compared to nonbacterized control. These increases ranged a 28% (G1-3) to 44% (Vitavax RS) increase in stand above untreated control. Other strains showed that stand increase ranged from 5-12%. The results obtained from the field trial conducted during 1991 at three locations was shown in Fig. 2. Among the five bacterial strains tested only 2 strains significantly enhanced the stand establishment in 2 out of 3 sites tested. Strain U-14, significantly enhanced the stand at Saskatoon (65% increase over control) and Melfort (62% increase over control) sites, where as strain L-114, significantly increased the stand at Regina (78% increase) and Melfort (56% increase) sites. Canola seed treated with Vitavax significantly enhanced the stand at 3 sites tested. Other 2 strains also increased stand but is not significant. Canola stand enhancement influenced by the rhizobacterial strains tested during 1992 field trails was shown in Fig. 3. As shown, strains 63-49 and U-14 and Vitavax significantly increased the stand establishment at 3 locations tested. Where as strain G1-3, at Regina site, strain L-114 at Saskatoon and Regina sites and strain 63-28 at Regina and Melfort sites significantly enhanced the stand compared to nonbacterized control. Overall, strains U-14 and 63-49 consistently increased the stand irrespective of the sites tested during 1992.

Table 1. Bacterial strains used in this study.

Strains	Identification	Source of isolation
63-49	Pseudomonas fluorescens	Canola rhizosphere-Winnipeg
G1-3	Pseudomonas fluorescens	Soil-Arctic
U-14	Pseudomonas fluorescens	Cotton field soil-Mississipi
44-9	Coryneform group	Canola roots
17-114	Pseudomonas fluorescens	Corn field soil-Yellowknife
63-28	Pseudomonas fluorescens	Canola field-Winnipeg
L-114	Enterobacter agglomerans	Labrador

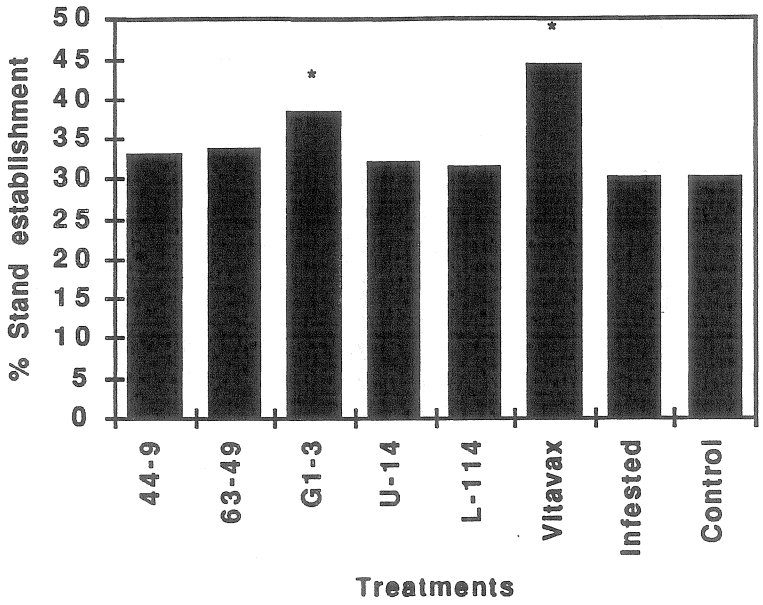

Fig. 1. Influence of rhizobacteria on canola (CV. Westar) stand establishment grown in Rhizoctonia solani infested field (Saskatoon) in 1990. Percent stand was measured 30 days after planting. Percent stand is the mean of 8 replications per treatment. * Significantly different from control (pathogen infested, nonbacterized) at P = 0.05.

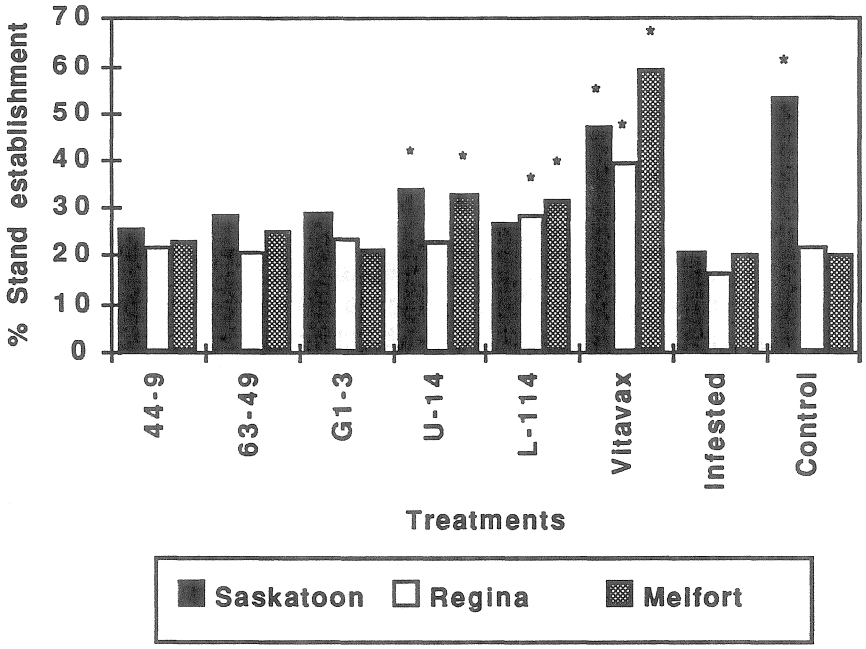

Fig. 2. Influence of rhizobacteria on canola (CV. Westar) stand establishment grown in Rhizoctonia solani infested fields of Saskatchewan in 1991. Percent stand was measured 30 days after planting. Percent stand is the mean of 8 replications per treatment. * Significantly different from control (pathogen infested, nonbacterized) at P = 0.05.

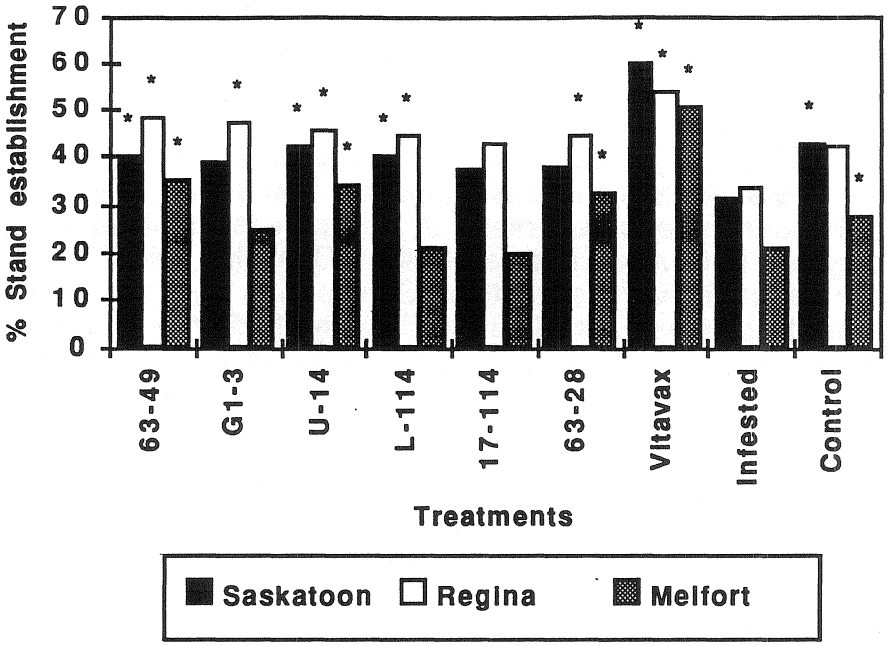

Fig. 3. Influence of rhizobacteria on canola (CV. Westar) stand establishment grown in Rhizoctonia solani infested fields in 1992. Percent stand was measured 30 days after planting. Percent stand is the mean of 8 replications per treatment. * Significantly different from control (pathogen infested, nonbacterized) at P = 0.05.

Discussion

Earlier studies in the greenhouse over the years we found several of the strains significantly increased canola root and shoot dry weights and also suppressed the preemergence and postemergence damping-off of canola caused by *R. solani.* Also many of these strains able to colonize the canola rhizosphere at significant levels. Some of these strains has been shown to enhance emergence of canola and soybeans (Kloepper *et al.* 1986) and to improve yield of canola grown in low or no disease field sites (Kloepper *et al.* 1988). Current results showed that the canola seed treated with rhizobacteria resulted in effective stand establishment when tested under commercial growing conditions with a very high disease pressure. Strains U-14 and 63-49 were the most effective and also consistent in their efficacy irrespective of the field site or year tested. These strains were also increased canola seed yield at an average of 5% over control (results not shown here). Concurrent studies on root colonization by strains marked with antibiotic resistance indicate Strain 63-49 persist in the canola rhizosphere throughout the growing season. Majority of strains tested here were antagonistic to *R. solani.*

All the bacterial strains evaluated in this study with the exception of 44-9, grew in agar media amended with high concentrations of Vitavax RS and (or) captan (Zablotowicz *et al.* 1992). Strains that survived on Vitavax treated-treated seed also exhibited good root colonization on canola. Our studies also showed that these bacteria when introduced onto Vitavax treated seed significantly increased the canola stand grown

in *R. solani* infested field site (results are not shown here). The ability of these strains to enhance stand establishment of canola treated with Vitavax RS under disease pressure by *R. solani* suggests that the effects of disease control by fungicides can be combined and are additive. However, the final assessment of potential for combining bacteria with fungicides on seeds requires extensive field testing. This is in progress. The mechanism (s) by which these bacteria achieve stand establishment has not been clearly demonstrated. Further studies is now planned to determine the mechanism. It is concluded that seed bacterization with select Pseudomonads can result in a suppression of damping-off of canola grown in *R. solani* infested western Canadian soils. The observed yield increase is very attractive. Considering the effectiveness of treatment to increase the yield consistently, further research on other agronomic characters is of great importance. Fundamental research is also necessary to improve the bacterization effect and to make it more consistent on commercial scale.

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