Activity 9
Canola and Microbial Signals
Annual Report – March 31, 2016

Overall Objective

The objective of this activity is to improve the understanding of the ability of plant-to-microbe signals to enhance the growth and productivity of canola. To achieve this, five specific objectives were identified:

1. Better understand the interactions between stimulation of plant growth by microbial produced signals and plant stress;
2. Determine the effects of chronic versus acute exposure of canola plants to plant-to-microbe signals on canola plant growth and productivity;
3. Investigate the effects of canola genotype differences on responses to microbe-produced signal compounds;
4. Investigate some of the mechanisms underlying canola responses to microbial produced signals; and
5. Conduct field trials with best formulations of signal compounds.

Audience

The audience is crop producers, specifically those who grow or who have an interest in growing canola.

Performance Measures

New/Improved Products

Three new/improved products were generated. Novozymes is marketing LCO Products commercially and these products are:

1. JumpStart LCO for Corn and Wheat
2. Tag Team LCO for Pea , Lentil and Soybean
3. Optimize for Pea, Lentil, Alfalfa, soybean and Peanut.

New/Improved Practices

Two new/improved practices were developed:

1. Foliar fertilization of PGPR(LCO (lipo-chitooligosaccharide) and Thuricin 17) for canola
2. Seed treatment of PGPR (LCO and Thuricin17) for canola

New/Improved Knowledge

This project identified that the PGPR (Thuricin 17) reduced the canola yield.

Highlights

The general objective of the study is to determine the effect of PGPR (LCO & Th17) on plant growth and yield in canola.

The experiment was conducted in two soil types (sandy and loam). Fertilizer was broadcast onto the experimental site at the rate of 50 kg N ha^{-1} (27.5-0-0), 30 Kg P ha^{-1} (11-50-0), 40 kg K ha^{-1} (0-0-60) for clay soil, 50:50:60 kg NPK ha^{-1} for sandy soil along with sulphur, as ammonium sulphate (21-0-0 with 24% S) at 20 kg ha^{-1} and boron as Granubor (10% boron) at 2 kg ha^{-1}. The signal compounds (LCO 10^{-6}M and Thuricin 10^{-9}M) were applied in two levels: seed treatment (seeds (InVigor 5440) were surface wetted and allowed for drying) and foliar application at 20% flowering.

The herbicide Liberty 200 (2.5L ha^{-1}) (post emergence) was applied on canola seedlings at the 2-3 leaf growth stage.

The experiments were organized following a completely randomized block design with four blocks for each experiment and the plot size was 4 x 2.6 m.

Data were collected and recorded according to the established protocol, on the following variables for canola: stand count (3-4 weeks after seeding and at harvest m^{-2}); yield components (10 plants per plot); branches per plant; pods per plant; seeds per pod; 1000-seed weight; and seed yield (at 10% moisture). Canola plots were regularly observed for the presence of insects and diseases.

The results are:

1. The number of pods increased in seed treated with Thuricin 10^{-11} in clay soil.
2. Thuricin reduced the yield of canola in both soil types.

<u>Outcomes</u>

Introduction

Plant growth promoting rhizobacteria (PGPR), a term first use by Kloepper and coworkers (Kloepper and Schroth, 1978), are the free-living bacteria which exist in the rhizosphere and have beneficial importance in agriculture. The rhizosphere is the very thin area around the plant roots, as opposed to bulk soil further from the roots, which is very rich in plant exudates and micro-organisms. PGPR are found in the rhizosphere, either in the soil near plant roots, on the surface of plant roots or inside the cells of root nodules (Gray and Smith 2005) and can stimulate plants growth through a wide array of mechanisms.

Direct modes of action include biological nitrogen fixation, increased availability of soil nutrients to plant roots, production of phytohormones, production of siderophores and induced systemic resistance, while

indirectly they can suppress disease through antibiosis there by lessening the deleterious effect of phyto-pathogens (Glick, 1995). Some PGPR can exploit more than one mechanism to enhance plant growth (Ahmad et al., 2008).

PGPR have been regularly exploited to enhance the emergence, growth and overall yield of agriculture crop production systems. There are various examples of PGPR stimualting plant growth and development: by increasing leaf area, chlorophyll content and total biomass of shoots or roots (Dobbelaere et al., 2001; Esitken et al., 2003; Mia et al., 2010).

The LCOs produced by *Bradyrhizobium japonicum* (eg. Nod Bj-V C18:1, MeFuc) have a pentameric backbone with C18:1, C16:0 and C16:1 fatty acid chains at the non-reducing end and 2-0-methylfucose at the reducing end of the chitin backbone (Carlson et al., 1993). The initial findings with the LCOs have been widely repeated. Several LCO technologies from Dr. Don Smith lab are now commercially available. Novozymes is now marketing products based on these findings.[1]

The PGPR *Bacillus thuringiensis* NEB17 was isolated from soybean nodules (Bai et al., 2002) and was shown to increase growth and nodulation when applied as a co-inoculant with *B. japonicum* 532C (Bai et al., 2003). This bacterium produces the bacteriocin Th17. Th17 has a molecular weight 3.1 kDa and which is not toxic to *B. japonicum* 532C (Gray et al., 2006b). Bacteriocins are bacteria-produced peptides which generally kill bacteria that are closely related to the producer strain (Jack et al., 1995), which provides a competitive advantage for the producer strain (Wilson et al., 1998). It had been already demonstrated that the application of Th17, to either leaves or roots can enhance plant growth, early seedling growth, photosynthetic rate, soybean nodule number and total fixed N (Lee et al., 2008). However, a great deal of research remains to be done regarding matters such as the range of crops affected, interaction with crop stress and their specific effects on crop physiology and development. The general objective of the study is to determine the effect of PGPR (LCO & Th17) on plant growth and yield in canola.

Methodology

The experiment was conducted in two soil types (sandy and loam). Fertilizer was broadcast onto the experimental site at the rate of 50 kg N ha^{-1} (27.5-0-0), 30 Kg P ha^{-1} (11-50-0), 40 kg K ha^{-1} (0-0-60) for clay soil, 50:50:60 kg NPK ha^{-1} for sandy soil along with sulphur, as ammonium sulphate (21-0-0 with 24% S) at 20 kg ha^{-1} and boron as Granubor (10% boron) at 2 kg ha^{-1}. The signal compounds (LCO 10^{-6}M and Thuricin

[1] http://bioag.novozymes.com/en/products/unitedstates/biofertility/Pages/default.aspx

10^{-9}M) were applied in two levels: seed treatment (seeds (InVigor 5440) were surface wetted and allowed for drying) and foliar application at 20% flowering.

The herbicide Liberty 200 (2.5L ha^{-1}) (post emergence) was applied on canola seedlings at the 2-3 leaf growth stage.

The experiments were organized following a completely randomized block design with four blocks for each experiment and the plot size was 4 x 2.6 m.

Data collection

Data were collected and recorded according to the established protocol, on the following variables for canola: stand count (3-4 weeks after seeding and at harvest m^{-2}); yield components (10 plants per plot); branches per plant; pods per plant; seeds per pod; 1000-seed weight; and seed yield (at 10% moisture). Canola plots were regularly observed for the presence of insects and diseases.

Statistical analyses

Data were subjected to statistical analyses using the ANOVA procedure of SAS (9.3) to detect differences among the treatments. Means were compared using the LSD test ($P < 0.05$).

Results

Clay soil:

Table 1. Effect of LCO 10^{-6} M and Thuricin 10^{-9} M on canola emergence (no), no of plants at harvest, no of branches and pods

Treatments	Emergence m^2	No of plants at harvest m^2	No of branches $Plant^{-1}$	No of Pods $plant^{-1}$
1) Control	74	73	4	73
2) Control (seed treatment with water)	82	84	3	61
3) LCO 10^{-6} M (seed treatment)	73	71	4	75
4) LCO 10^{-6} M (foliar spray 20% flowering stage)	73	65	3	66
5) Thuricin 10^{-9}M (seed treatment)	66	70	4	84
6) Thuricin 10^{-9} M (foliar spray 20% flowering stage)	70	70	4	73
7) Thuricin 10^{-11} M (foliar spray 20% flowering stage)	61	68	5	93
($P \leq 0.05$)	0.0618	0.2548	0.2986	0.7549

The results showed that, there were no significant differences in emergence, no of plants at harvest, no of branches and no of pods due to treatments (Table 1). The maximum no of pods was recorded in seed treated with Thuricin 10^{-11} M (93).

Table 2. Effect of LCO 10^{-6} M and Thuricin 10^{-9} M on no of seeds, yield, 1000 seed weight and harvest index in canola

Treatments	No of Seeds Pod^{-1}	Yield kg ha^{-1}	1000 seed weight (g)	Harvest Index
1) Control	24	4129	1.5	0.19
2) Control (seed treatment with water)	23	4298	1.5	0.16
3) LCO 10^{-6} M (seed treatment)	24	4150	1.5	0.19
4) LCO 10^{-6} M (foliar spray 20% flowering stage)	22	3802	1.5	0.15
5) Thuricin 10^{-9}M (seed treatment)	24	3776	1.5	0.19
6) Thuricin 10^{-9} M (foliar spray 20% flowering stage)	24	3671	1.5	0.16
7) Thuricin 10^{-11} M (foliar spray 20% flowering stage)	24	3752	1.5	0.18
($P \leq 0.05$)	0.3774	0.5151	0.6000	0.6537

There were no significant differences in no of seeds, yield, 1000 seed weight and harvest index due to treatments. The highest yield was occurred in LCO 10-6 (seed treatment) (4150 kg ha^{-1}) (Table 2).

Sandy loam:

Table 3. Effect of LCO 10^{-6} M and Thuricin 10^{-9} M on canola emergence (no), no of plants at harvest, no of branches and pods

Treatments	Emergence m^2	No of plants at harvest m^2	No of branches Plant^{-1}	No of Pods plant^{-1}
1) Control	85	89	2	34
2) Control (seed treatment with water)	102	97	3	81
3) LCO 10^{-6} M (seed treatment)	92	89	3	52
4) LCO 10^{-6} M (foliar spray 20% flowering stage)	102	91	2	46
5) Thuricin 10^{-9}M (seed treatment)	90	90	3	68
6) Thuricin 10^{-9} M (foliar spray 20% flowering stage)	88	78	3	56
7) Thuricin 10^{-11} M (foliar spray 20% flowering stage)	95	82	3	50

(P≤ 0.05)	0.7751	0.7153	0.2843	0.5705

There were no significant differences in emergence, no of plants at harvest, no of branches and no of pods due to treatments (Table 3).

Table 4. Effect of LCO 10^{-6} M and Thuricin 10^{-9} M on no of seeds, yield, 1000 seed weight and harvest index in canola

Treatments	No of Seeds Pod^{-1}	Yield kg ha^{-1}	1000 seed weight (g)	Harvest Index
1) Control	22	2343^{ab}	1.5	0.16
2) Control (seed treatment with water)	23	2569^{a}	1.4	0.16
3) LCO 10^{-6} M (seed treatment)	23	2154^{ab}	1.5	0.18
4) LCO 10^{-6} M (foliar spray 20% flowering stage)	21	2281^{ab}	1.5	0.17
5) Thuricin 10^{-9}M (seed treatment)	20	2027^{b}	1.5	0.17
6) Thuricin 10^{-9} M (foliar spray 20% flowering stage)	22	2116^{ab}	1.4	0.20
7) Thuricin 10^{-11} M (foliar spray 20% flowering stage)	20	2194^{ab}	1.4	0.14
(P≤ 0.05)	0.1316	0.0035	0.4280	0.4185

Statistical significance was observed in yield due to treatments. The highest yield occurred in the Control (seed treatment with water) (2569 kg ha^{-1}) (Table 4).

Summary

- There were no significant differences in yield parameters due to signal compounds ((LCO 10^{-6}M and Thuricin 17 10^{-9}M).
- Thuricin reduced the yield in canola.

Future Work

This will be the last year for the experiment. The PGPR (LCO 10^{-6}M) increased the yield for canola more in 2013 than 2014. More data is required for confirmation of results, which could lead to commercialization.