O.47 - Consequences of phytoplasma infection on canola crop production in the Canadian prairies.

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Abstract

Canola plants (*Brassica napus* and *B. rapa*) showing typical Aster Yellows Diseases (AY) symptoms and canola plants showing no AY symptoms were collected in commercial fields of canola in Saskatchewan, Canada. DNA of "Ca. Phytoplasma asteris" (AY phytoplasma) was detected by PCR technology in every canola plant showing typical AY symptoms and in 10-30% of asymptomatic canola plants. AY phytoplasma DNA was found in leaf, stem and root tissues as well as in normal-looking and misshapen seeds of infected plants. In canola plants showing AY symptoms, 25-80% of the misshapen seeds and 20-60% of normal-looking seeds contained AY phytoplasma DNA, while in asymptomatic infected plants, 9-20% of the misshapen seeds and 2-10% of the normal-looking seeds contained AY phytoplasma DNA. Misshapen seeds never germinated. Normal looking seeds sampled in plants showing AY symptoms or in asymptomatic infected plants germinated at 50-90%. AY phytoplasma DNA was detected in seedcoats from AY infected seeds, as well as in cotyledons, roots and stems of seedlings grown from AY infected seeds. Progeny plants grown from infected seedlings showed malformations such as an increased number of trichomes, lack of growing point, stocky stem, shrivelled leaves and a general growth delay. PCR performed on plants grown out of infected seedlings became negative after the 4 leaf stage. These results showed that AY phytoplasma are present in a large proportion of asymptomatic plants meaning that canola production losses will be higher than estimated from visual assessment of AY incidence. Moreover, the presence of phytoplasma DNA in canola seeds and in seedlings growing out of infected seeds raised the concern of possible seed transmission.

Introduction

In Canada, Aster Yellows Disease (AY), caused by '*Candidatus* Phytoplasma asteris' (Firrao et al., 2005), affects several economically important crops including canola (*Brassica napus* and *B. rapa*). Canola plants infected with AY phytoplasma show symptoms such as stunting, leaf yellowing or purpling, phyllody and formation of bladder-like siliques as well as normal-looking pods containing small misshapen seeds (Bailey et al., 2003). Until recently, the disease has been considered to be of little importance, with overall incidence less than 1% in most fields. AY incidence in canola is estimated by visually assessing the percentage of plants that show symptoms. In 2007, the percentage of canola plants showing symptoms ranged from traces to 12% depending on the location, with a provincial average of 2%. However, in 2007 crop production losses were higher than estimated with the visual AY assessment. Seed productions were below average and high numbers of misshapen seeds were observed in harvested field seeds. Also, the recent detection of AY phytoplasma DNA in seeds sampled from AY-infected canola plants (Olivier et al., 2008) raised concerns of possible seed transmission.

The objective of the research was to use PCR tests 1) to determine an accurate incidence of AY disease in canola crop, 2) to estimate the percentage of seeds infected with AY phytoplasma and study their viability and 3) to determine if phytoplasma are present in seedlings grown from AY infected seeds.

Material and methods

Canola plants were collected at seed maturity in August 2007 in two commercial fields, located in Saskatchewan, Canada. One commercial field was seeded with *Brassica napus* and the other with *Brassica rapa*. AY incidences, performed by visually estimating the percentage of canola plants with AY symptoms, were 2% for the fields of *B. napus* and 7% for the field of *B. rapa*. Twenty plants showing typical AY symptoms and 100 asymptomatic plants were randomly collected in every field. For every plant, leaf tissues were sampled, freeze-dried and stored at -20C, and all seeds were collected and stored in two individual paper bags, one containing normal-looking seeds and the other containing misshapen seeds. For each field, 500 mg of harvested seeds were collected from the producer before and after spiral cleaning. Controls consisted of seeds from the 2006 seddlots used to seed both fields in 2007 as well as plants grown in the greenhouse from both seedlots (10 plants each).

The presence of phytoplasma DNA was examined in leaf and seed sampled from every field-collected and greenhouse grown plants as well as in seeds from both seedlots and field seeds. Leaf and seed tissues were assayed using a nested PCR, incorporating phytoplasma universal 16S rRNA primer pairs P1/P6, followed by R16R2/R16F2 (Tanne et al. 2001). Seed samples consisted of 10 well-shaped seeds or 10 misshapen seeds and leaf tissue samples consisted of 2g of leaf tissue. Two samples of leaf tissues were examined per field-collected plant to determine the presence of phytoplasma in the plants. Ten samples of normal-looking seed and 10 samples of misshapen seed were examined per field-collected plant that tested positive for phytoplasma DNA while 1 sample of normal-looking seeds and 1 sample of misshapen seeds were examined per field-collected plants that tested negative for phytoplasma DNA. PCR tests were also performed on 20 samples of normal-looking seeds and 20 samples of misshapen seeds from field seeds collected before and after spiral cleaning for both fields.

The presence of phytoplasma DNA was investigated in seedlings grown from seed harvested on field-collected plants that tested positive for the presence of phytoplasma DNA. Ten misshapen and 10 normal-looking seeds were collected from every plant that tested positive for phytoplasma. Seeds were placed on a moist paper in a Petri dish (20 seeds per Petri dish), and left at room temperature for 5 days. After 5 days, percent of germination were recorded. Half of the seedlings and their corresponding seedcoats were analyzed individually with PCR tests to determine the exact percentage of seedlings containing phytoplasma DNA and the other half was transferred into pots in the greenhouse and their leaf tissues tested for the presence of phytoplasma every 2 weeks. AY phytoplasma subgroup identification was performed using DNA sequencing on PCR products. PCR products were sent to Plant Biotechnology Institute (Saskatoon, SK, Canada) for sequencing. DNA sequences were then compared with sequences recorded in GenBank using BLAST.

Results

Controls: Seed samples from the seed lots and plant tissues sampled from the greenhouse-grown plants tested negative for phytoplasma DNA. Seedlings grown from seedlot seeds, from seeds harvested on non-AY infected field-collected plants and from seeds harvested on greenhouse grown plants, tested negative for phytoplasma DNA. Seeds from the seedlots showed 98% germination.

Phytoplasma DNA was detected in leaf tissue of all field-collected plants exhibiting AY symptoms and in 25 asymptomatic plants of *B. napus* and 32 asymptomatic plants of *B. rapa*. On average, pods of plants of *B. napus* or *B. rapa* showing AY symptoms contained 0-50% well-formed seeds while siliques of asymtomatic AY-infected plants contained 30-70% well-formed seeds. Pods of non-infected canola plants contained 95-100% well-shaped seeds. DNA sequencing revealed that plant tissues were mostly infected with phytoplasma belonging to subgroup 16SrI-A, a few plants being infected with strain 16SrI-B.

Seeds showing the presence of phytoplasma DNA all belonged to plants that tested positive for phytoplasma DNA. According to Table 1, symptomatic canola plants showed a higher % of infected seeds as compared to asymptomatic plants and *B. rapa* plants contained more infected seeds than

B. napus plants. Misshapen seeds were more infected than well-shaped seeds. Field seeds collected before spiral cleaning were infected at 3% for *B. napus* and 5.6% for *B. rapa*. No phytoplasma DNA was detected in seeds collected after spiral cleaning.

Table 1: Percentages of seeds infected with AY phytoplasma

	Seeds of *B. napus*		Seeds of *B. rapa*	
	Normal	Misshapen	Normal	Misshapen
AY symptoms	22	70	57	85
No AY symptoms	0	5	12	18
Field-collected seeds				
Before spiral cleaning	5	25	15	55
After spiral cleaning	0	(-)	0	(-)

Germination rate of seeds with normal appearance was 52 to 95% for AY-infected canola plants. None of the small, misshapen seeds collected on AY-infected plants germinated. According to table 2, DNA phytoplasma were found in seedlings grown from seeds harvested on AY-infected *B. napus* and *B. rapa*. A higher percentage of infection was observed with *B. rapa* seedlings. DNA sequencing revealed that phytoplasma found in the seedlings belong to the same subgroup that the plant parent.

Table 2: Percentage of infection in seedlings growing from field collected seeds and from seeds harvested on AY-infected canola plants.

	B. napus	*B. rapa*
AY symptoms	1.3	5.2
No AY symptoms	0	2.7
Field-collected seeds		
Before cleaning	1	3
After cleaning	0	0

Among the seedlings transplanted in the greenhouse, two AY-infected seedlings of *B. napus* and 5 AY-infected seedlings of *B. rapa* were positive with PCR test and remained positive until the 4 leaf stage. Twenty-two percent of *B. napus* seedlings and 36% of *B. rapa* seedlings, showed slow and abnormal growth, the remaining of the plants either died at the 3 leaves stage or showed no malformation. All malformed progenies had large, thick and shrivelled leaves showing high number of trichomes, associated often with stocky stems and sometimes with condensed flowers. Reduced fertility was a characteristic of all progeny plants, as self-seeds were difficult to obtain. Two plants of *B. rapa* showed no growing point.

Discussion- conclusion

The study revealed the presence of phytoplasma DNA in symptomless AY-infected plants of *B. napus* and *B. rapa*, meaning that the % of infected plant is greater than the % of plant showing AY symptoms. Based on the results presented in this work, an estimated 25% and 32% of plants were infected by AY in fields showing 2% and 7% of plants with symptoms, respectively. Considering that AY infected plants produced 30-70% of misshapen seeds, seed production losses could be estimated at 7.5-17.5% for the field with a 2% AY incidence and 9.6-22% for the field with a 7% AY incidence.

The study also revealed that phytoplasma DNA was detected in seeds and seedlings growing out of seeds harvested on AY-infected plants of *B. napus* and *B. rapa*, suggesting that phytoplasma are present in the seeds. Whether they remained viable after their passage in the seeds is not known. Their absence after the 4 leaf stage suggested that the phytoplasma do not remain viable as the plant grow. A significant percentage of seedlings grown out of seeds harvested from AY-infected plants showed malformations, regardless of the presence of phytoplasma DNA. Genetic modification due to phytoplasma infection was proposed as a cause for those malformations (Starzycki and Starzycka, 2000).

References

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