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DEVELOPMENT OF THE FIRST SPRING CANOLA LINES WITH RESISTANCE TO SCLEROTINIA STEM ROT

IGOR FALAK, DWIPAK SEN, JAYANTILAL PATEL, WINNIE MCNABB¹

SUMMARY: Spring canola is susceptible to Sclerotinia stem rot (SSR) and fungicides are used to control disease in the field. S1 per se recurrent selection and greenhouse and field screening were utilized in order to improve resistance of spring canola to SSR. Due to an apparent lack of trait improvement in Cycles 1 to 4, Population T was opened to convergent introgression of new resistance sources and subjected to selection utilizing Field Limited-Term Incubation (FLTI) method. These changes resulted in trait improvement beginning in Cycle 5, gradually leading to a near complete shift towards resistant field reaction by Cycle 10. On a scale of 1 (highly susceptible) to 9 (completely resistant) for SSDIS (Sclerotinia sclerotiorum Disease Incidence Severity), the Population T mean increased significantly from 2.4 in Cycle 5 to 6.7 in Cycle 10. The improved lines have potential for use in developing hybrids with field resistance to Sclerotinia.

Key words: Sclerotinia, canola, screening, FLTI, Population, resistance.

INTRODUCTION

Sclerotinia sclerotiorum infects over 400 species of plants, including numerous economically important crops such as *Brassica* species, sunflowers, dry beans, soybeans, field peas, lentils, lettuce, and potatoes (Boland and Hall, 1994). The fungus causes Sclerotinia stem rot of canola (SSR). Canola is a *Brassica napus* oilseed type having a low level of glucosinolates and erucic acid in the seed (Daun, 1984).

Fungal sclerotia germinate carpogenically producing apothecia that release windborne ascospores. Ascospores cannot infect leaves and stems of canola directly but use dropped flower petals as a nutrient source for germination and infection of leaves (Heran et al., 1999). The disease is favoured by moist soil conditions and temperatures of 15-25 °C, prior to and during canola flowering. On average, yield losses equal 0.5 times the percentage of infection (Del Rio et al., 2007). Canola quality spring and winter products in North America and Europe are considered susceptible to SSR and no

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significant improvements have been achieved through breeding. Therefore, fungicide applications are used to control disease (Morall et al., 1985).

Differences in field resistance to SSR in crops like white beans and soybeans can be attributed to canopy differences and/or partial resistance. Hunter et al. (1981) and Cline and Jacobsen (1983) described the use of a Limited-Term Inoculation (LTI) indoor method on white beans and soybeans. LTI was developed after observations that Sclerotinia kills all plants under favorable conditions irrespective of their level of resistance. Limited exposure to the fungus under favorable conditions was used to determine if partial resistance was involved in reduction of field symptoms.

Some semi-winter Japanese cultivars of rapeseed have partial stem resistance to SSR. Partial stem resistance was detected with stem testing in comparison with winter canola variaties (Brun et al., 1987). Some Chinese semi-winter varieties of rapeseed are partially resistant to SSR as well. The partial field resistance in Chinese varieties originated mostly from the rapeseed variety Zhong you 821. Despite improvements in partial resistance in Zhong you 821, its reaction to disease is variable under environmental conditions favorable for development of SSR (Li et al., 1999). Fungicide application is recomended to protect rapeseed crops against SSR in China.

Patel et al. (1991) demonstrated that recurrent selection can successfully improve yield and % oil in populations of canola. The introduction of strict quality and blackleg resistance standards in the Canadian registration system after 1991 prompted the use of S1 *per se* as a strategy for trait improvement (Patel et al., 1999). S1 *per se* recurrent selection is suitable for trait improvements and was used successfully to improve a number of traits including blackleg resistance and seed oil and protein content.

Between 1986 and 1988, Pioneer Hi-Bred acquired a collection of semi-winter rapeseed Germplasm from Plant Genetic Resources (PGR) Canada, The Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan and USDA North Central Regional Plant Introduction Station at Iowa State University in the USA. Initial screening work and some of the sources of resistance were based on the research of Brun et al. (1987). The goal of the efforts was to develop spring canola lines with partial stem resistance to SSR.

Lack of available resistance to SSR in elite spring canola varieties and the apparent success of S1 *per se* in trait improvement triggered initiation of this trait development project in 1993.

MATERIAL AND METHOD OF THE STUDY

Population T development

Lines originating from two partially stem resistant Japanese sources as well as elite lines with low field susceptibility were used to assemble Population T. The synthesis of cycle zero (C_0) was as described by Patel *et al.* (1991). Each cycle was completed in one year following three steps: (a) S0 seed production via intercrossing in the greenhouse of S1 field selections during fall; (b) S1 seed production in the greenhouse during winter and (c) S_1 plant evaluation in the field during summer. SSR pressure was applied at all three steps in each Cycle through indoor stem screening (a, b) and field selection (c).

The first three cycles of Population T were tested under moderate SSR pressure in the field using a relatively small number of lines (90-150). The fourth cycle was evaluated under high pressure for lodging which increased disease severity in the field. The results of the first three cycles did not show a significant improvement in resistance in the Population T compared with the control varieties. The number of lines in the fourth cycle was increased to 1100 in order to confirm the trends of the first three cycles, and prepare for an opening of Population T to new introgressions. Material in Cycle 4 was selected for better resistance to SSR and stem lodging as well as other agronomic and quality characteristics.

Cycles 0 to 4 were closed Cycles. Selections from Cycle 4 were used for the production of Cycle 5 with the opening of Population T and BC1 introgression of a third Japanese source of partial stem resistance. New sources were incorporated into Cycles 5 to 8 via BC1 convergent introgression.

Lines for intercrossing were selected based on *Sclerotinia sclerotiorum* Disease Incidence Severity value (SSDIS), agronomic performance of plants and quality characteristics. Selections were further reduced indoors before intercrossing on the basis of individual stem reaction to SSR. Plants originating from intercrossed seed were also subjected to SSR testing and selection indoors, thus producing S₁ seed for field evaluation.

In each cycle, the S₁ plant evaluation was carried out in replicated nursery rows planted at two locations in Ontario. One location was used for SSR screening while the other was utilized for visual agronomic score (1=poor, 9=excellent). At harvest, 15 g of S2 seed was harvested from each selected entry and analyzed for oil % and protein % using Near Infrared Spectroscopy.

During each year, two check varieties were included in field Sclerotinia trial as running checks (augmented RCBD design) while evaluating the S_1 s. 46A65 susceptible (S) check was used from Cycle 1 to Cycle 10. 46A76 moderately susceptible (MS) check was used from Cycle 5 to Cycle 10. 46A65 (S), 46A76 (MS) and 44A89 highly susceptible (HS) were used as check entries covering the range of existing variation (Table 1). As such, they were randomized within experiment and their mean (X_C) was calculated (Table 2).

Analysis of variance (ANOVA) was performed for the SSDIS trait after adjusting for alternating running check deviations (running check every 6^{th} row in the field). Since SSDIS frequency values (Figure 1) were adjusted with running checks, no adjustment was done with X_C . Thus, deviation of X_C from expected mean shows actual variation in exerting extreme disease pressure in the field in a given season.

For SSDIS in each cycle, the Population T mean (μ) and phenotypic standard deviation (σ) were calculated as well as other standard deviation-based parameters. Heritability % was derived from ANOVA output based on genetic and phenotypic variance values. The heritability estimates are not available for Cycle 10 due to a loss of one replicate in the field.

Indoor disease stem screening

Indoor screening for stem reaction was conducted using the LTI principles of Hunter et al. (1981) and Cline and Jacobsen (1983). The basic premise was development of 20mm long lesions on stems of susceptible plants with a removal of inoculum and cessation of high humidity after that. This target was adequate for a differentiation of partial stem resistance (based on unreported data). Over the span of 11 years (1995-2005) the methodology has evolved from the use of mycelial PDA agar plugs attached to stems via parafilm into the usage of low nutrient PDA mycelial agar plugs attached to the stem with an entomological needle.

Field disease screening

Field screening based on natural inoculum commenced with Cycle 1 in 1996 and finished with Cycle 4 in 1999. This type of screening was conducted under moderate SSR pressure in an irrigated field with a heavy load of sclerotia in the soil. Cycle 5 testing in 2000, as well as the opening of Population T for convergent introgressions, was a starting point for standardized field LTI (FLTI) testing until Cycle 10 evaluation in 2005.

FLTI method

A mycelial seed carrier was produced as per Kim et al. (1999). Instead of oat seeds, Niger seed (*Guizotia abissinica*) was utilized as a carrier. The application of *Sclerotinia*-colonized Niger seeds was done by fertilizer spreader at the rate of 20kg/ha during late flowering, to mimic petal distribution in the leaf canopy. A sprinkler system with nozzles that produce misting droplets of water was used to maintain moisture in the field. The system was controlled with leaf moisture sensors (Campbell Scientific (Canada) Corp, Edmonton, Alberta, Canada). Ground water was used for irrigation of fields. Ground water was found to be inhibitory to *Sclerotinia* in years without natural rainfall. Deionized water (DI) treatment (Siemens Canada Limited, Brampton, Ontario, Canada) enabled *Sclerotinia* development under such conditions. Finally, the field was covered with fine netting to modify the environment under unfavourable conditions for the development of SSR.

Rating Field Reactions

The FLTI method is based on monitoring the reactions of running checks 46A65/46A76 planted at regular frequency within testing material with five rows of tested material and the sixth row being an alternating check. When these lines reached disease incidence thresholds achieved under natural conditions of extreme pressure (Table 1), rating was performed. Each plant was individually assessed for disease severity (*Sclerotinia sclerotiorum* disease severity-SSDS) according to the scale 1 to 9 (1=dead plant, 8=small lesion, 9=no symptoms). Such data allow calculation of % of diseased plants (*Sclerotinia sclerotiorum* disease incidence % - SSDI %). Parameters SSDI% and SSDS were used to derive an index parameter SSDIS (*Sclerotinia sclerotiorum* disease incidence severity) relative to location adjusted performance of 46A65 and 46A76 (expected SSDI%=70% and 60% respectively).

Table 1. Measuring field performance under extreme disease pressure *Tabela 1. Ocene intenziteta oboljenja pod ekstremnim pritiskom parazita*

Rating SSDIS	Category	Disease incidence SSDI%	Checks
Ocena SSDIS	Kategorija	% obolelih biljaka SSDI%	Kontrolne sorte
1.0	Highly susceptible Visoko osetljiva	≥80	44A89=1
1.1 - 2.0	Susceptible <i>Osetljiva</i>	79 – 70	46A65=2
2.1 - 3.0	Moderately susceptible	69 - 60	46A76=3
3.1 - 4.0	Umereno osetljiva	59 – 50	
4.1 - 5.0	Moderately resistant	49 - 40	
5.1 - 6.0	Umereno otporna	39 - 30	Single fungicide
6.1 – 7.0	Resistant Otporna	29 – 20	application Jedno prskanje fungicidom
7.1 - 8.0		19 - 10	Two fungicide
8.1 - 9.0	Highly resistant Visoko otporna	9 – 0	applications Dva prskanja fungicidom

RESULTS

Table 2 shows an increase in the number of field-tested S1 progenies from the lowest number of 176 in Cycle 5 to the highest number of 600 in Cycle 7. The number of lines in Cycle 10 was lowered to 390 as it became obvious that trait improvemen, was progressing. The number of intercrossed selections was determined by the number of available S1 lines meeting upward moving thresholds of SSDIS without compromising other traits. The lowest number of intercrossed lines was recorded in the synthesis of Cycle 8 (16) and the highest in the synthesis of Cycle 6 (38). Cycles opened for convergent introgressions (5-8) had either one (5 and 6) or two sources introgressed (Cycle 7) while Cycle 8 had six sources introgressed within its synthesis. The analysis of variance for each cycle showed mean squares due to genotypes to be significant for SSDIS. The population mean (μ) , standard deviation (σ) , mean of the selected lines (X_s) , and mean of the checks (X_c) are presented in Table 2. Standard deviation range was 1.0 to 1.1 with the exception of Cycle 7 (1.5). SSDIS improvement trends, based on $(\mu - X_c)$ and $(\mu - X_c)/\sigma$ comparisons between the cycles, showed that the recurrent selection was gradually improving SSDIS values over Cycles 5 to 10. Broad sense heritability estimates ranged from as low as 21.5 for Cycle 7 to as high as 50.8 for Cycle 8.

Cycles 5 to 8 were opened for significant introgressions. Population T was closed for Cycles 9 and 10. SSDIS Population T-based results are shown in Table 2. The SSDIS frequency distribution is presented within Figure 1. In contrast to previous cycles, where there was no significant increase in resistance, Cycle 5 resulted in the first S1 lines with SSDIS values of 5 and higher. The trend of increasing resistance continued until the 10th cycle where almost the entire Population T exceeded the best lines of Cycle 5. On the scale 1 (highly susceptible) to 9 (completely resistant) for the SSDIS parameter, the Population T mean increased significantly from 2.4 in Cycle 5 to 6.7 in Cycle 10. The SSDIS population mean increased gradually across Cycles with the exception of Cycle 7 where it was 4.3 vs. 4.5 for Cycle 6. The SSDIS mean of the selected lines (X_8) was increased steadily from 3.6 for Cycle 5 to 7.9 for Cycle 10.

Table 2. SSDIS parameter for Population T – Cycles 5 (2000) to Cycle 10 (2005)

Tabela 2. Srednje vrednosti parametra SSDIS Populacije T tokom ciklusa od 5 (2000.god) do 10 (2005.god)

Pop T Cycles Ciklusi populacije T		6	7	8	9	10
Number of S1 lines Broj S1 linija		238	600	585	585	390
Mean of $S_1(\mu)$ Srednja vrednost SSDIS S_1 linija(μ)	2.4	4.5	4.3	5.6	5.9	6.7
Range of SSDIS values Raspon SSDIS vrednosti	1.0- 6.0	1.5-7.0	1.0- 8.2	1.9- 8.1	1.7-8.1	1.1- 8.4
Std. Dev. (σ) Standardna devijacija (σ)	1.1	1.1	1.5	1.1	1.0	1.1
Mean of Checks (X _c) Sred. vrednost SSDIS kontrolnih linija(X _c)	2.0	3.2	2.8	2.9	2.6	1.7
Number of intercrossed selections Broj ukrštenih selekcija		24	16	26	32	23
Mean of intercrossed selections $S_1(X_s)$ Sred. vrednost ukrštenih selekcija $S_1(X_s)$		5.6	6.9	7.1	7.4	7.9

Number of new sources introgressed Broj unešenih novih izvora otpornosti		1	2	6	N/A	N/A
Heritability % / Heritabilnost		38.2	25.1	50.8	46.4	N/A
$(X_s-\mu)/\sigma$	1.07	1.07	1.73	1.37	1.51	1.12
$(X_s-X_c)/\sigma$	1.4	2.3	2.7	3.9	5.0	5.6
$(\mu$ - $X_{c})/\sigma$	0.4	1.2	1.0	2.6	3.5	4.5

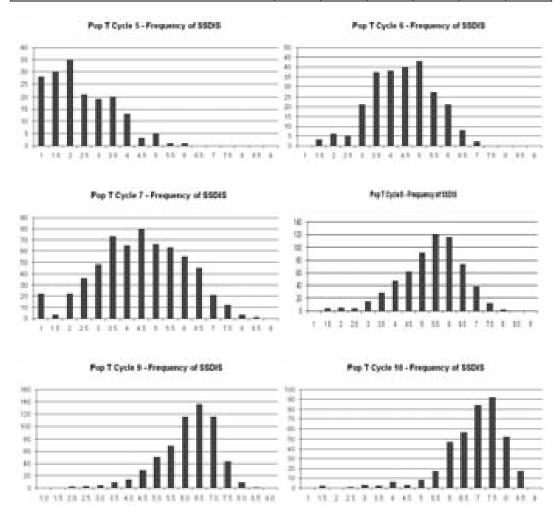


Figure 1. Distribution of SSDIS parameter through Population T Cycles 5 to 10 (2000-2005) Y axes - Frequency of SIlines; X axes – SSDIS (1-9)

Grafikon 1. Frekvencije vrednosti SSDIS Populacije T i njihovih selekcija u Ciklusima 5,6,7,8,9 i 10 Kordinatna osa Y – Frekvencija S1 linija;Kordinatna osa X – SSDIS (1-9)

DISCUSSION

Patel et al. (1999) used *SI per se* recurrent selection to improve canola traits successfully. Spring canola is susceptible to SSR. Population T work begun in 1993 with the specific goal of increasing field resistance to SSR. The initial Population T was based on a combination of less susceptible elite lines and lines carrying partial stem resistance from two Japanese sources. Despite the selection and testing in Cycles 1 to 4,

there was no obvious improvement in the level of field resistance compared to the control lines. A limited level of genetic resistance in Population T and an inadequate selection pressure/methodology were identified as the main reasons for the lack of progress. Access to further sources of resistance, their convergent introgression and use of the FLTI methodology elevated the SSDIS performance in Cycles 5 to 10.

The FLTI method with its running check thresholds enabled the establishment of the extreme disease pressure while avoiding excessive SSR incidence and severity in the field. Hunter et al. (1983) pointed out that Sclerotinia kills all plants under continuous exposure and favourable conditions. On the other hand, lower pressure in the field has a potential to enable escapes, diluting selection quality and ability to make sustainable phenotypic/genetic gains. Table 1 describes the extreme pressure of disease in the field. One application of fungicide protects the crop for two weeks as per label. Extreme disease pressure occurs in years where flowering is extended due to wet/cooler conditions and two fungicide applications are needed. On top of that disease pressure, the FLTI method standardizes data collection so comparable data is generated across years. Other aspects of the FLTI method, like sensor-based irrigation, water treatment and netting enclosure were critical in years with unfavourable weather conditions. The methodology of infection is important for success in developing canola lines with resistance to SSR. By mimicking the natural infection pathway of Sclerotinia, FLTI enables selection of material with field resistance, ultimately the most important parameter in farmers' fields.

Population T was opened for convergent introgressions after Cycle 4 and exposed to a combination of extreme pressure and the FLTI method. This led to significant improvements in the resistance of Population T and selection of improved S1 lines in Cycle 5 (SSDIS highest value 5.5) to cycle 10, where 348 lines out of total of 390 had SSDIS score greater than 5.5. Artificial selection for high levels of horizontal resistance should provide almost complete control of disease, provided there is adequate genetic variability (Robinson, 1996). Considering that FLTI detects performance under highest possible field pressure, near-complete resistance in the best progenies of cycle 10 is evident. Population T yielded canola lines with field resistance significantly reducing disease when compared with the mean of checks in Cycle 10 (8.4 vs. 2.6). The purpose of indoor screening was important in preparation of sources with partial stem resistance as well as for elevating frequency of the progenies with better stem resistance before intercrossing of selections (S1to S0) and before field testing (S0 to S1).

Population T (Figure 1.) shows a relatively small shift in resistance in Cycle 7, and Cycle 8 to a lower extent. This may have been partly due to significant introgressions of new sources of resistance as well as their dissimilarity to existing sources within Population T. It is also possible that opening of Population T to convergent introgressions may have introduced some less desirable alleles, as it may be seen from Cycles 7 and 8. Table 2 as well as Figure 1 show that Cycle 7 is an 'outlier' relative to other Cycles. The SSDIS standard deviation in Cycle 7 was higher than for other Cycles (1.5 vs. 1.0-1.1), demonstrating a dispersed population as can be seen from Figure 1. Although the population mean value was reduced from 4.5 (Cycle 6) to 4.3 (Cycle 7), selection improvement was uninterrupted as it increased from 5.6 (Cycle 6) to 6.9 (Cycle 7). The broad sense heritability estimates were lower (25.1) than other Cycles as well (38.2-50.8).

After the closing of Population T in cycle 8, level of resistance was elevated in Cycles 9 and 10. S1 *per se* enables the accumulation of positive alleles over time within

the closed Population T. Once adequate genetic variability was introgressed, trait improvement results were achieved similar to non-modified S1 *per se* (Patel et al., 1999). Population T's aim was not only to increase the resistance, but also generate lines that could be used to produce hybrids with resistance to SSR which resulted in release of the first canola hybrid with improved field resistance to SSR in 2008. Dickson and Petzddt (1996) found that the resistance in cabbage *Brassica oleracea* was based on a single recessive gene. Selection of the S0 and S1 plant generations generally leads to lines that show resistance in the heterozygous state. Resistance based on such lines should be better suited for the production of hybrids.

CONCLUSION

The first spring canola lines with resistance to Sclerotinia stem rot were generated using modified S1 *per se* recurrent selection and Field Limited-Term Inoculation method. These lines will be used in the development of canola products with a 'built in' level of genetic resistance, such that fungicide applications may be reduced or eliminated entirely.

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STVARANJE PRVIH LINIJA JARE ULJANE REPICE OTPORNIH NA BELU TRULEŽ STABLJIKE

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Izvod

Jara uljana repica je osetljiva prema beloj truleži stabljike pa se fungicidi koriste za zaštitu od obolenja u polju. S1 per se rekurentna selekcija, u kombinaciji sa testiranjem otpornosti u staklari i polju, je korištena za poboljšanje otpornosti prema obolenju. Pošto ciklusi 1 do 4 nisu doneli značajno poboljšanje otpornosti, populacija je otvorena za konvergentno unošenje novih izvora otpornosti i podložena selekciji korištenjem poljske metode ograničene inkubacije bolesti (FLTI). Ove promene su rezultirale poboljšanjem otpornosti u ciklusu 5. Postepena poboljšanja otpornosti su pomerila gotovo celu populaciju u ciklusu 10 u otpornu kategoriju. SSDIS parameter (Sclerotinia sclerotiorum Disease Incidence Severity) je korišten za praćenje nivoa otpornosti gde 1 predstavlja jako osetljivu reakciju a 9 kompletnu otpornost. Srednja SSDIS vrednost populacije u ciklusu 5 (2.4) je značajno poboljšana u odnosu na srednju vrednost populacije u ciklusu 10 (6.7). Pošto se FLTI metod zasniva na ekstremonom pritisku obolenja u polju, koji traži primenu dva prskanja fungicidom, otporne linije imaju potencijal za razvoj proizvoda uljane repice sa otpornošću na belu trulež stabljike.

Ključne reči: Sclerotinia, uljana repica, testiranje, FLTI, populacija, otpornost.

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