Development of a new source of resistance to Fusarium head blight and Stagonospora nodorum blotch in spring wheat

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ABSTRACT

The objective of *this study was to transfer FHB resistance from Triticum timopheevii to spring* wheat *Triticum aestivum*. Crocus spring wheat was crossed to *T. timopheevii*, PI343447, in the greenhouse in 1999. A segregating population of 1,500 BC_1F_2 plants was established and advanced to F_7, using single seed descent (SSD). One hundred lines were selected from 535 BC_1F_7 lines, based on plant fertility and agronomic traits, and evaluated for reaction to FHB and *Stagonospora nodorum* blotch in the greenhouse for two seasons. One line, TC 67, had high levels of resistance to FHB, comparable to that of Sumai 3, the most FHB resistant wheat available, based on point inoculation. TC 67 also showed a high level of resistance to *Stagonospora nodorum* blotch at the seedling stage in inoculated greenhouse trials. The resistance of TC 67 to FHB was further evaluated in replicated field trials, in comparison with two resistant wheat lines Sumai 3 and HY 644, in a FHB disease nursery in 2003 and 2004. The results showed that TC 67 was significantly better than HY 644 in FHB incidence and severity and it was comparable with Sumai 3 in deoxynivalenol (DON) content in the grain.

INTRODUCTION

Fusarium head blight (FHB), caused by *Fusarium graminearum,* is a very destructive disease of spring wheat. Epidemics of the disease can result in significant economic losses in terms of reducing yield and degrading quality. Development of cultivars is an effective means of controlling the disease. However, sources of resistance to FHB are limited to a few wheat cultivars, such as Sumai 3 and Frontana, which hinders the development of improved cultivars with resistance to FHB. Therefore, new sources of resistance must be identified and exploited. Several wild species were identified with a high level of resistance to FHB, such as *Leymus racemosus* (*Elymus gigantus* L.), *Roegneria kamoji* and *Roegneria ciliaris* (Chen et al, 2004). Several translocation lines that are resistant to FHB have been developed (Chen et al, 2004). However, these lines need to be improved for agronomic traits before they are used in a wheat breeding program because of linkage drag. *Triticum timopheevii*, a relative of bread wheat with genome AAGG, is known for its resistance to many diseases, such as common root rot (Bailey, 1993), stem rust (Allard and Shands 1954), leaf rust (Leonov et al, 2007), power mildew (Peusha 1995) and Septoria nodorum blotch (Ma and Hughes 1995). An accession of *T.timopheevii*, PI 343447, has been found to have a high level of resistance to Fusarium head blight. The objective of this study was to transfer FHB resistance from *Triticum timopheevii* to spring wheat *Triticum aestivum* through wide crosses.

MATERIALS AND METHODS

Plant materials:

Crocus, a spring wheat, was crossed to *T. timopheevii*, PI 343447, in the greenhouse in 1999. Crocus, provided by G. Scoles, University of Saskatchewan, is a line with the genetic background of cv. Columbus plus three crossability genes *kr1*, *kr2* and *kr3*. The F_1 plants were backcrossed with Crocus. F_1 plants of both the cross and the backcross were sprayed with 2, 4 –D (100 ppm) after pollination and embryo rescue techniques were used for establishing the hybrid. Following a backcross with Crocus, a population of 1500 F_2 plants was established and 535 BC_1F_7 lines were developed in the greenouse, using single seed descent (SSD). One hundred lines were selected, based on plant fertility and agronomic traits, and evaluated for reaction to FHB in the greenhouse and field FHB nursery with four replications for two growth seasons.

Point inoculation:

Wheat plants were grown in the greenhouse. During the flowering stage, a floret in the middle spike was injected with 10 uL of inoculum. The inoculum was a suspension of 50,000 spores per mL of three isolates of *Fusarium graminearum*. The inoculated plants were misted for 48 hours in a misting room at 25 ^{o}C. Symptoms were rated at 21 days after inoculation.

Field evaluation:

One FHB resistant line, TC 67, was further evaluated for FHB reaction in a 4-replcate FHB nursery in 2003 and 2004. Each experimental plot consisted of two rows; one meter long and 30 cm apart. The nurseries were inoculated twice each year with corn and barley kernels that were autoclaved and inoculated with three isolates of *Fusarium graminearum*. The infected kernels were spread in the FHB nurseries at 100 grams per square meter. The first inoculation was at the tillering stage and the second inoculation was made 10 days later. The FHB nurseries were irrigated twice each day (morning and afternoon) for the interval between the first inoculation and the soft dough stage. Cultivars Sumai 3, Roblin and HY 644 were included as checks.

Deoxynivalenol (DON) analysis:
Samples of wheat were analysed by ELISA using a monoclonal antibody. A 1.0 g sample of ground wheat was extracted with 5 mL of 9:1 water/methanol for one hour. The samples were then centrifuged and a portion of the extract withdrawn. The extracts were mixed with a solution of DON-horseradish peroxidase conjugate and the resulting solutions were added to a 96-well plate pre-coated with DON antibodies. After 30 minutes, the plate was washed 8 times with PBST and the amount of bound peroxidase was determined by reaction with o-phenylenediamine in the presence of hydrogen peroxide. The intensity of the resulting colour was measured at 490 nm and DON concentration was estimate from a standardised curve.

RESULTS AND DISCUSSION

The greenhouse evaluation showed that TC 67 had a high level of resistance to FHB, and only one spikelet was infected at 21 days after point inoculation. In the field nursery the FHB incidence and severity of TC 67 were much lower than those of the partially resistant check HY 644 and its DON level was comparable to that of Sumai 3, a world-wide known resistant source. Kernel appearance showed that TC 67 displayed a lower frequency of fusarium damaged kernels (FDK) compared to the partially resistant check HY 644. TC 67 is also resistant to *Septoria nodorum* of wheat at the seedling stage (data not shown).

Table 1. Means of FHB incidence, FHB severity, FDK and DON for three spring wheat checks and line TC 67 in the field FHB nursery at Ottawa in 2003 and 2004.

Line	Incidence (%)	Severity (%)	FDK (%)	DON (ppm)
Sumai 3	14.4	6.3	9.5	6.6
HY 644	42.6	20	35.1	25.3
Roblin	90.7	81.9	66.05	35.0
TC 67	29.4	16.9	14.5	7.7

Sumai 3 is a well-known source for resistance to FHB. The major resistant gene is located on chromosomes 3BS (Anderson et al, 2001). TC 67 is comparable to Sumai 3 for FHB resistance based on symptoms and DON levels. After the resistant gene(s) for resistance to FHB of TC 67 (derived from *T. timopheevii*) is identified and located on a specific chromosome, we can pyramid the genes for FHB resistance from Sumai 3 and TC 67 into a well-adapted wheat cultivar, using marker assisted-selection.

TC 67 is fertile and has an awned, erect, mid-loose spike, which is white-chaffed at maturity. TC 67 has a spring growth habit with a plant height of 110 cm in irrigated fields. Seed of TC 67 is hard, red and ovate. This line has been crossed with AC Brio and an F_6 population of 235 lines have been developed through SSD. Currently, these lines are being phenotyped for FHB reaction in the greenhouse and the genes for resistance to FHB will be mapped.

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