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Marker assisted breeding for improvement of Fusarium head blight resistance in spring wheat

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Introduction

White wheat is of interest to milling companies due to its higher milling yield and generally lighter and brighter flour, which results in aesthetically more appealing end products (Ambalamaatil et al. 2002). Because of the desire to increase the intake of fibre through whole-grain consumption, white wheat is being used to produce whole-wheat bread with much of the same appearance and taste as traditional white bread made from bleached red wheat flour (Ransom et al. 2006). Fusarium head blight (FHB) is an important fungal disease of wheat affecting wheat production around the world and most varieties of hard white spring wheat are not resistant to FHB. Here we report the white-seeded spring wheat line, WS175, which exhibits a high level of resistance to Fusarium head blight similar to the international resistance check, Sumai3.

Materials & methods

WS175 is derived from the cross Snowbird/Sumai3 made in January, 2005 at the Ottawa Research and Development Centre of AAFC in Ottawa. Sumai3 is a red seeded Chinese spring wheat variety, which has been used as a source of resistance to FHB around the globe. Three QTLs for FHB resistance in Sumai3 have been identified and mapped on chromosomes 3BS, 5A and 6BS, respectively (Anderson et al. 2001). Snowbird is a hard, white-seeded, spring wheat developed at the Cereal Research Centre of Agriculture and Agri-Food Canada that is moderately susceptible to FHB (Humphreys et al. 2007).

F_1 seeds from Snowbird / Sumai3 were increased in the greenhouse. Subsequently, approximately 20,000 F_2 plants were grown in the greenhouse and approximately 1,500 white seeded genotypes were visually selected from the F_2 population. This population was advanced to F_5 using single seed descent in the greenhouse. In winter 2008, 1300 F_5 lines were genotyped for their haplotypes at the loci associated with FHB resistance QTLs on chromosomes 3B, 5A and 6B. Nine molecular markers gwm533, umn10, gwm493, gwm293, gwm415, wmc96, barc180, wmc397 and wmc398 closely linked to the three QTLs were used for marker assisted selection (MAS) (Table 1). DNA extraction and PCR procedure were carried out as previously described by Somers et al. (2003). Two hundred and fifty F_5 lines with two or three FHB resistance haplotypes (i.e. haplotypes similar to Sumai3) were selected and grown in a FHB inoculated field nursery in Ottawa, in 2008. Fifteen F_6 lines were visually selected in the FHB nursery based on FHB resistance and agronomic performance. The 15 F_7 lines were increased in the greenhouse to provide seed for field evaluation of FHB reaction and agronomical traits.

Fifteen hard white wheat lines, including WS175 and the two parents Snowbird and Sumai3, plus three checks Snowstar, AC Vista and AC Foremost were evaluated for FHB reaction in three replicates in inoculated FHB nurseries at the Ottawa Research and Development Center in Ottawa, Ontario (2009-2011). Each experimental plot consisted of two one meter long rows. The nurseries were inoculated twice during growing season with infected 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 metre. The first inoculation was at about 5-6 leaf stage and the second inoculation was made 10 days later. The FHB nurseries were irrigated twice each day (morning and afternoon). FHB incidence (% plants in plot infected with FHB) and severity (% of 10 random spikes/plot showing FHB infection symptoms) were visually rated at 21 days after flowering (50% anthesis). FHB index was calculated as Incidence x Severity/100. FHB nursery plots were hand harvested to provide samples for dioxynivalenol (DON) determinations (Sinha and Savard, 1997). Evaluation of FHB severity was also conducted in a growth cabinet with point inoculation. The point inoculation procedure used was previously described (Cao et al. 2009).

In addition to FHB evaluations, the same 15 lines and the two parents were yield tested using a randomized complete blocks design with two replications at Central Experimental Farm in Ottawa, Ontario (2009-2011). The least significant differences (LSD) test was used to identify significant differences in the means compared with the check cultivars.

Results & discussion

WS175 was selected based on one round of marker assisted selection with 9 molecular markers and haplotypes of WS175 and its parents Sumai3 and Snowbird are shown in Table 1. Over the three years of evaluation (2009 -2011) for FHB reaction in the fields, the FHB index and DON content of WS175 was significantly lower than those of check cultivars Snowstar, AC Vista and AC Foremost (Table 2).

Table 1. Haplotypes of the line WS175 and its parents Snowbird and Sumai3 revealed by 9 molecular markers

	Amplicon sizes of selected microsatellite markers								
	Chromosome 3B			Chromosome 5A				Chromosome 6B	
Entry	gwm533 (bp)	umn10 (bp)	gwm493 (bp)	gwm293 (bp)	gwm415 (bp)	wmc96 (bp)	barc180 (bp)	wmc397 (bp)	wmc398 (bp)
Sumai3	160	256	213	217	146	298	218	175	172
Snowbird	131	253	158	208	150	302	213	181	198
WS175	160	256	213	217	146	298	218	175	172

Table 2. FHB index and DON content of the line WS175, three checks and the parents in 2009, 2010 and 2011 field test, plus FHB severity evaluated with point inoculation in the growth cabinet in 2013

Entry	Kernel[a] colour	2009 Ottawa		2010 Ottawa		2011 Ottawa		2013 Ottawa[c]
		FHB index[b] (%)	DON (ppm)	FHB index (%)	DON (ppm)	FHB index (%)	DON (ppm)	FHB severity (%)
Sumai3	Red	3	0.67	3	1.50	2	2.93	7
Snowbird	White	37	14.30	37	31.23	42	7.20	69
AC Vista	White	90	35.93	92	28.10	98	19.57	-
Snowstar	White	-	-	43	18.47	57	3.93	-
AC Foremost	Red	-	-	98	22.80	100	13.60	98
WS175	White	8	2.20	3	3.03	2	1.20	7
$LSD_{0.05}$		8	5.60	7	4.68	7	3.11	7

[a]Kernel color determined at 1.25-M NaOH (Lamkin and Miller, 1980); [b]Index = % incidence x % severity / 100;
[c]Point inoculation in a growth cabinet.

Table 3. Comparison of agronomic and grain quality traits of WS175 and their parents Snowbird and Sumai3, plus the check Snowstar evaluated at Ottawa (2009-2011).

Entry	Ottawa 2009					Ottawa 2010					Ottawa 2011				
	Yield kg/ha	DH (d)	TKW (g)	PH (cm)	PROT (%)	Yield kg/ha	DH (d)	TKW (g)	PH (cm)	PROT (%)	Yield kg/ha	DH (d)	TKW (g)	PH (cm)	PROT (%)
Snowstar	-	-	-	-	-	1173	45	27	89	15.8	3922	45	30.7	85	14.5
Snowbird	2163	48	30	95	14.7	2000	46	30	97	17.0	3938	48	33.8	95	15.4
Sumai3	2053	55	34	108	14.2	1420	52	33	84	17.2	2140	54	33.2	93	15.4
WS175	2148	45	35	108	14.8	1645	49	32	99	17.0	2677	48	34.8	104	15.4
$LSD_{0.05}$	220	1.7	2.1	7.7	0.5	257	1.3	1.2	6.7	0.7	580	2.8	3.4	8.1	0.7

DH = days to 50% spike emergence; TKW = thousand kernel weight; PH = plant height; PROT = grain protein

Point inoculation in the growth chamber also showed that the level of type II FHB resistance of WS175 was significantly higher than that of Snowbird, AC Foremost and Roblin. The results indicated that WS175 had improved FHB resistance, compared to the check cultivars and was similar to the FHB resistant parent, Sumai3.

In general, WS175 had lower grain yield than Snowbird and the hard white check, Snowstar was similar to Sumai3 for grain yield (Table 3). Days to heading for WS175 were similar to Snowbird, significantly earlier than Sumai3 and later than Snowstar. WS175 was taller than Snowstar in all environments. The 1000-kernel weight of WS175 was generally similar to Sumai3 and Snowbird but higher than Snowstar. WS175 had grain protein content similar to Snowbird and Sumai3 but was significantly higher than Snowstar (Table 3). WS175 had significantly higher Hagberg falling number. Thus, WS175 is a white seeded spring wheat line with pre-harvest sprouting resistance, which is a highly desirable trait particularly in hard white wheat breeding.

Conclusions

The hard white spring wheat line, WS175 with high level of FHB resistance, was developed through marker assisted selection. WS175 has FHB resistance similar to the international resistant check Sumai3 in field, greenhouse and had DON content similar to Sumai3. The release of WS175 should facilitate development of hard white spring wheat cultivars with high levels of FHB resistance.

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