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DEVELOPMENT OF BLUE WHEAT DERIVED FROM WILD SPECIES *THINOPYRUM INTERMEDIUM*

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Introduction

Consumers are demanding healthier food choices, and there is an increasing interest from industry for new opportunities to provide innovative, value-added products to consumers (Gorden, 2012). Anthocyanins are the phytochemicals responsible for blue, purple or red pigments in grains, fruits and vegetables, some of which can have strong antioxidant activity levels (Tyl and Bunzel, 2012). Anthocyanins have been recognized as health-enhancing substances due to their antioxidant activity as well as their anti-inflammatory, anti-cancer, and hypoglycemic effects (Tyl and Bunzel, 2012). Antioxidants are substances, such as vitamin E or vitamin C, that protect cells from the damaging effects of oxidation and reduce the risk of chronic diseases such as cancer, heart disease and diabetes (Shipp and Abdel-Aal, 2010). It has been shown that blue wheat contains high levels of anthocyanins in its bran (Abdel-Aal and Hucl, 19 which can be as much as twice the amount of anthocyanins compared to colourful fruits and vegetables such as red cabbage or plums (Zilic et al., 2012). The objective of this study was evaluate lines of blue coloured wheat that were developed through the use of wide crosses between *T. aestivum* L. and a partial amphiploid wheat line derived from *Th. intermedium*.

Materials & methods

Plant materials:

The cross Crocus / 08-47-50 was made at the Ottawa Research and Development Centre, AAFC in 2010. Crocus is a hexaploid wheat with genome AABBDD, while 08-47-50 is a partial amphiploid with genome AABBEE, generated by crossing durum wheat (AABB) with *Th. intermedium* (Zeng et al. 2013). The F_1 hybrid was grown in the greenhouse. F_2 progeny were advanced to F_4 generation using a single-seed descent and 184 lines were obtained. At the F_4 generation, a wheat plant with a mixture of blue and red colour kernels was found. Sixty blue color kernels were selected from the plant and advanced to the F_7 generation. Twenty-one F_7 lines with blue colour kernels were selected based on fertility and evaluated for leaf, stem, and stripe rust resistance.

Disease evaluation:

Tests for stem and leaf rust resistance were done at the seedling stage. Seeds were planted in clumps of approximately 10 seeds evenly spaced in fibre flats (25 X 15cm). Approximately 14d after seeding, the seedlings at the two leaf stage were inoculated with urediniospores of *P. graminis* race TTKSK (Ug99) for stem rust and *P. triticina* isolates 12-3 MBDS 128-1 MBRJ, 74-2 MGBJ, 06-11 TDBG,77-2 TJBJ, 11-180-1 TDBG mixed with a light mineral oil (Bayol, Esso Canada, Toronto, ON) sprayed onto the leaves using a compressed air sprayer. The plants were allowed to dry, to evaporate the mineral oil, for at least 1 h then moved to a 100% humidity cabinet for approximately 17 h incubation. The plants were then moved to a greenhouse at 20C + 4C with supplemental lighting. For stripe rust test, the method was the same for inoculation with *P. striiformis* isolate YR6 except that the plants were kept at 12C during incubation and then grown in a growth chamber at 15C + 3C with a 12hr photoperiod. After approximately 14 d the plants were rated for symptoms using a '0' to '4' infection scale where '0' (no symptoms), ';' (hypersensitive flecks), '1' (small uredinia with necrosis), and '2' (small to medium-sized uredinia with chlorosis) were considered resistant responses and '3' (medium-sized uredinia without necrosis or chlorosis) and '4' (large-sized uredinia without necrosis or chlorosis) were considered susceptible responses.

Methods of cytological analysis:
Seeds were germinated on moistened filter paper in Petri dishes. Roots were collected at lengths of 1 – 1.5 cm. The root tips were immersed in ice water for about 24 h and fixed in ethanol-acetic acid (3:1) for about 3 days at room temperature and then stored in 70% (v/v) ethanol. After staining with 2% (w/v) acetocarmine for at least 2 h, the root tips were squashed in 45% (v/v) acetic acid. For meiotic chromosome preparation, young spikes at the meiotic metaphase I stage were fixed in 95% ethanol – chloroform – glacial acetic acid (6:3:1) at room temperature for 48 h, then maintained in 70% ethanol at 4 °C. Anthers were stained and squashed in 1% acetocarmine.

Results & discussion

All of the 21 blue wheat lines were fertile, with plump kernels at greenhouse conditions. The contrasting blue coloration of Line Blue-2B with its red seeded parents and the white seeded WS175 are shown in Figure 1. Cytological analyses indicated that the line Blue-2B had 42 chromosomes at the mitotic metaphase (Fig, 2A) and 21 pairs of chromosomes at the metaphase I of meiosis (Fig. 2B).

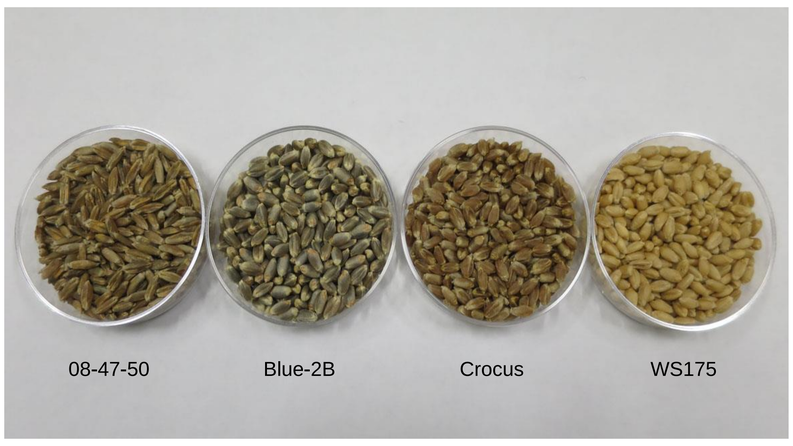

Fig.1 Grain colors of the line Blue-2B, its two parents 08-47-50 and Crocus, and the hard white wheat line WS175

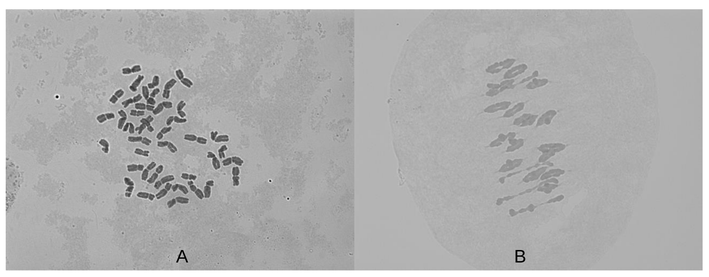

Fig.2 The line Blue-2B with 42 chromosomes at the mitotic metaphase (A) and 21 pairs of chromosomes at the metaphase I of meiosis (B).

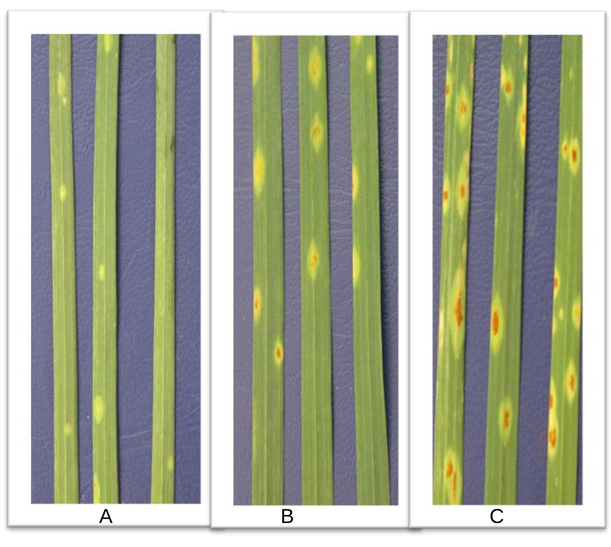

Fig. 2 Stem rust symptoms at seedling stage inoculated with race TTKSK (Ug99) A: 08-47-50; B: Blue-5 and C: Crocus

The results of disease evaluation indicated that five out of the 21 lines were resistant to stem rust race TTKSK (Ug99) and five lines were resistant to stripe rust race YR6. Seven lines showed resistance to leaf rust isolate 12-3 MBDS (Table 1). None of lines was resistant to isolates 128-1 MBRJ, 74-2 MGBJ, 06-11 TDBG, 77-2 TJBJ and 11-180-1 TDBG

Table 1. Disease evaluation of 21 blue wheat lines with their parents for stem rust, leaf rust and stripe rust at seedling stage

Entry	Stem rust	Stripe rust	Leaf rust					
	TTKSK	YR6	12-3 MBDS	128-1 MBRJ	74-2 MGBJ	06-11 TDBG	77-2 TJBJ	11-180-1 TDBG
Blue -2A	;1	3	23	3	3	3+	3	3
Blue -2B	13	2+	22+	23	3-	3+	3	3+
Blue-3	41	3+	23	33+	3	3+	3+	3
Blue-5	;1-	2	2	3	3-	3	3+	3
Blue-6	31	3	2+	3+	3	3+	3+	3+
Blue-7	31	3	2+	3	3-	3+	3+	3+
Blue-9	31	3	2+	3+	3-	3+	3	3+
Blue-10	12-	2+	2+	3+	3+	3+	3+	3+
Blue-12	41	3	3	3+	3+	3+	3+	3+
Blue-16	33+	2	3	3+	3	3+	3+	3+
Blue-20	2-;	2	2+	3	3+	3+	3+	3+
Blue-22	32	3	3+	3	3+	3+	3+	3+
Blue-23	;1+	2+	3	3	3	3+	3	3+
Blue-25	41	2	2	3	3	3+	3	3+
Blue-35	31	2	2+	3+	3+	3+	3+	3+
Blue-37	34	4	2	3	3	3+	3+	3+
Blue-39	41	2+	3+	3	3+	3+	3+	3+
Blue-40	3-3	3+	2-	3	3-	3+	3	3+
Blue-43	23	3	2-	3	3	3+	3+	3+
Blue-46	34	3	2-	3	3-	3	3	3
Blue-51	34	4	2-	3	3-	3+	3+	3
Crocus	41	2+	1+	1+	1+	1+	1+	1+
08-47-50	;1-	2	;	;	;	;	;	;

IT 1 and 2 considered as resistant response; 3 and 4 considered as susceptible response.

The line Blue-5 was resistant to stem rust race TTKSK and stripe rust race YR6 and race 12-3 MBDS of leaf rust. Most of the lines were not resistant to leaf rust. However, both parents were consistently resistant to all races of leaf rust, suggesting the parental leaf rust resistance may involved more than one gene. In future studies, these lines will be evaluated for FHB resistance and agronomic traits performance. Chromosome compositions of these lines will also be analysed using in situ hybridization to identify alien chromosomes and/or chromosome fragments. The long-term goal is to release blue coloured wheat germplasm with disease resistance for use in wheat breeding programs.

Conclusions

- Blue seed colour wheat lines with 42 chromosomes are genetically stable and fertile;
- Line Bule-5 was found to be resistant to stem rust and stripe rust and leaf rust. It could be used as a parental line in wheat breeding programs.

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