Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

Development of aneuploids in wheat by means of doubled haploid technology- corn pollen method

Wenguang Cao[1], Gavin Humphreys[1], George Fedak[1], Danielle Wolfe[1], Brent McCallum[2], Tom Fetch[2], Allen Xue[1], Lianquan Zhang[3] and Dawn Chi[1].

[1]Ottawa Research and Development Centre, AAFC, 960 Carling Ave. Ottawa, ON K1A 0C6;[2]Morden Research and Development Centre, AAFC, 101 Route 100, Unit 100, Morden Manitoba R6M 1Y5. [3]Triticeae Research Institute, Sichuan Agricultural University, Wenjiang of Chengdu city, 611130, Sichuan, China

Introduction

Wheatgrass [*Thinopyrum intermedium* (EEEstEst StSt)] is an invaluable source of resistance to wheat fungal diseases, such as leaf rust (Friebe et al. 1996) and stem rust (Li et al. 2013). Wheat amphiploids are an effective 'bridge' to transfer resistance genes from wild species into cultivated wheat. A partial amphiploid line 08-47-50 between wheat and *Th. intermedium* was previously developed using traditional methods (Zeng et al. 2013). The partial amphiploid was shown to have resistance to rusts and Fusarium head blight (FHB) and can be used as a donor for gene transfer. The objective of this study was to develop wheat aneuploids using the partial amphiploid 08-47-50 and wheat-maize pollen doubled haploid (DH) method.

Materials & methods

Plant materials:
The cross 08-47-50/Crocus was made in 2011. Crocus is a wheat line (AABBDD) with three crossability genes (kr1kr2kr3), while 08-47-50 is a partial amphiploid (AABBEE) with resistance to multiple fungal pathogens (Zeng et al. 2013). F_1 plants were used to produce DH progeny using the wheat-maize pollination based-method (Humphreys and Knox, 2016).

Disease evaluation:
Seedlings at the 1-2 leaf stage were inoculated with leaf rust and stem rust, incubated for 16 h in the dark in a dew chamber, then moved to a growth cabinet set at 18C with 14 h photoperiod (McCallum and Seto-Goh 2005; Fetch et al. 2015). For leaf rust testing, DH lines were screened with *P. triticina* race BBBD; whereas, for stem rust testing, *P. graminis* races TTKSK and TTKST (Ug99) were used. Infection types (ITs) were scored 14 d post-inoculation (Zeng et al. 2013). DH lines were evaluated for adult plant reaction to stripe rust in the field using a mixture of seven *P. striiformis* races: CYR32, CY33, Gui229, Gui22-14, Gui22-8, Shui4 and Shui5. Powdery mildew evaluation was conducted in the greenhouse using natural infection and disease symptoms were scored as that of stripe rust from 0 to 9 (Line and Qayoum, 1992). FHB evaluation was conducted in the growth cabinet using the point inoculation method (Cao et al., 2009). The inoculum used was a mixture of three *F. graminearum* isolates: DAOM178148, DAQOM232369 and DAOM212678 (Canadian Collection of Fungal Cultures, AAFC, Canada).

Cytological analysis:
Procedures of chromosome preparation and genomic *in situ* hybridization analysis were described (Zeng et al., 2013). Genomic DNA of *Th. intermedium* was used as probing DNA, while Chinese Spring DNA was used as blocking DNA.

Results & discussion

Table 1. Chromosome numbers and disease reactions of eight DH lines and their parents.

Line	# of Chrom.	FHB (Severity, %)	Leaf rust [a]	Stem rust [a]		Stripe rust [b]	Powdery mildew[b]
				TTKSK	TTKST		
08-47-50	42	17	;	;1+	;1	-	1.7
Crocus	42	100	1-	33+	33+	-	5.7
DH Cro50-1	42	78	3-,3	0;	0;	0	3.6
DH Cro50-2	42	100	3+	12-	1-2-	9	2.4
DH Cro50-3	44	100	0, 2	1-1	;2-	-	4.6
DH Cro50-5	48	23	1-,2	;1-	;2-	6	1.9
DH Cro50-6	48 + 2t ?	10	1,2	;1-	;1-	2	8.0
DH Cro50-7	-	89	0,;	;1-	;1-	1	1.1
DH Cro50-8	42	89	3	01	1	2	2.3
DH Cro50-9	46	33	2	0	;	-	3.6
LSD[c] (0.05)	-	28.7	-	-	-	-	1.1

a: Seedling stage test, ITs based on scale 0 to 4 (Zeng et al., 2013);
b: Adult plant stage test, ITs based on scale 0 to 9 (Line and Quyoum, 1992),
c. LSD of means was calculated using SAS Version 9.3.

Two hundred and eighty-eight embryos were recovered from 15,186 florets pollinated with corn pollen. Thirty-two haploid plantlets were obtained from these embryos. After colchicine treatment, eight stable doubled haploid lines were obtained with chromosome numbers ranging from 42 to 48 (Table 1). Results of rust disease evaluation revealed that all eight DH lines were resistant to Ug99 stem rust races TTKSK and TTKST, five lines were resistant to leaf rust race BBBD and four lines were resistant to stripe rust (Table 1).

Fig.1 Reactions to FHB at 21 days after point inoculation with *F. graminearum* in the growth cabinet.

In the FHB evaluation, three lines had FHB severity score similar to the resistant parent, 08-47-50. DH Cro50-5 with a high level of resistance to FHB is shown in Fig.1. Infection types ranged from 1 to 9 for reaction to powdery mildew (Table1) and four lines were as resistant as the resistant parent. Powdery mildew resistance of DH Cro50-7 is shown in Figure 2). Chromosome characterization of these DH lines is being conducted using genomic in situ hybridization (GISH). Preliminary GISH results revealed that the line DH Cro50-2 had 42 chromosomes with a pair of terminal translocated chromosomes (Fig.3A). The line DH Cro50-3 has 44 chromosomes with eight *Th. intermedium* chromosomes (Fig. 3B) and the line DH Cro50 -5 has 48 chromosomes with six *Th. intermedium* chromosomes (Fig. 3C). Development of aneuploids in wheat is traditionally conducted through crossing and backcrossing, followed by selfing for several generations to produce genetically stable progeny.

Fig. 2 Reactions to powdery mildew in the greenhouse: A, resistant parent 08-47-50; B, susceptible parent Crocus; C, resistant line DH Cro50-7 and D, susceptible line DH Cro50-6.

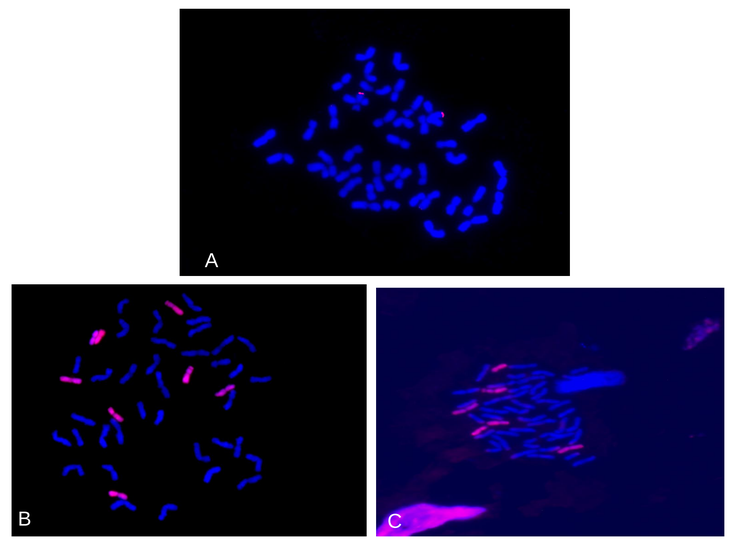

Fig. 3 The GISH analysis conducted with *Th. intermedium* genomic DNA as a probe and 'Chinese Spring' DNA as a blocker: A, the line DH Cro50-2 with a pair of terminal translocated chromosomes; B, the line DH Cro50-3 with eight *Th. intermedium* chromosomes; C, the line DH 50 -5 with six *Th. intermedium* chromosomes.

The results of this study indicate that application of DH technology can greatly accelerate wheat aneuploid development compared to traditional methods. Because genes for resistance in DH lines are fixed, the DH aneuploids may be used directly as resistance sources in wheat breeding programs for improvement of FHB, powdery mildew, leaf rust, stem rust and stripe rust resistance.

Conclusions

- Wheat-maize pollen method can be used to accelerate the development of wheat aneuploids;
- DH aneuploid lines with multiple disease resistance have been developed and those with 42 chromosomes are putative pure lines that can be used directly as parents in wheat breeding programs.

References

Cao W, Fedak G, Armstrong K *et al*. (2009) J Plant Reg 3:104–106
Fetch T, Fetch JM, Xue A (2015) Canadian J Plant Pathology 37:331-341
Friebe B, Jiang J, Raupp WJ *et al.* (1996) Euphytica 91:59 – 87
Humphreys DG, Knox R (2016) Double haploid breeding in cereals. In J.M. Al-Khayri et al. (eds.), Advances in Plant Breeding Strategies, Volume 1: Breeding, Biotechnology and Molecular Tools, Springer, Heidelberg (In press)
Li G-R, Liu C, Li C-H *et al* (2013) Mol Breed 31:843-853
Line RF, Qayoum A (1992) U.S. Department of Agriculture Technical Bullletin No. 1788, 44 p.
McCallum, B. and Seto-Goh, P. (2005) Can. J. Plant Pathol. 27: 90-95
Zeng, J., W. Cao, G. Fedak *et al*. (2013) Hereditas 150:10-16

Acknowledgements

We would like to thank Taye Zegeye-Gebrehiwot for stem rust testing, Elsa Reimer for leaf rust testing and Linda Langille for data analysis.

Canada