Rust resistance in *Aegilops speltoides* var. *ligustica*

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ABSTRACT

Five accessions of *Aegilops speltoides* were screened for resistance to stem, leaf rust and stripe rust. Resistance to all three diseases were observed after inoculation with pathotypes predominant in Australia. All accessions were crossed with the cultivar 'Angas' using wheat as the female parent and viable F_1 seeds produced from the cross with accession *Ae. speltoides* AEG357-4. F_1 plants were treated with colchicine and crossed to BC_4 with cv. Angas and then to BC_2 with cv. Westonia. RFLP probes were used to select for addition lines carrying either single or multiple *Ae. speltoides* chromosomes. A plant carrying a segment of the 2S chromosome from AEG357-4 (named 2S#3) was found to be resistant to stem rust. The stem rust resistance gene was tentatively named *Sr2S#3*.

INTRODUCTION

Distantly related or uncultivated relatives of wheat have been a reservoir of sources of new resistance genes. Several stem rust (*Puccinia graminis* Pers. f. sp. *tritici*) (*Pgt*), leaf rust (*P. triticina*) (=*P. recondita* Rob. ex Desm. f. sp. *tritici*) (*Pt*) and stripe rust (*Puccinia striiformis* Westend f. sp. *tritici*) (*Pst*) resistance genes have been deployed in commercial cultivars, namely: *Sr22* (*Triticum monococcum* L.); *Sr24/Lr24* and *Sr26* (decaploid *Agropyron elongatum* (Host) Beauv. [*Thinopyrum ponticum* (Podp.) Barkw. & D.R. Dewey]); *Sr31, Lr26* and *Yr9* (*Secale cereale* L.); *Sr36* (*T. timopheevii* Zhuk.) and *Sr38*, *Lr37* and *Yr17* (*Triticum ventricosum* Ces) [1]. Some of these genes are now out of favour for agriculture when deployed singly because of the emergence and widespread distribution of virulent pathotypes in Australia (*eg. Lr24*, *Lr26, Lr37, Sr31*, *Sr36, Sr38*, *Yr9* and *Yr17*). New sources of rust resistance are being sought to achieve sustained wheat production[2].

Aegilops speltoides (=*Triticum speltoides*) has been a donor of two stem rust resistance genes, namely, *Sr32*[1] and *Sr39*[3]. *Sr32* is located on the 2S#1 chromosome[4] and has never been used in agriculture. *Sr39* is located on the 2S#2 chromosome[4] and lines with this gene have been undergoing backcrossing for several years in Australian[2] and Canadian (Dr Taing Aung, personal communication) wheat breeding programs. Leaf rust resistance genes which have been derived from *Ae. speltoides* are *Lr28*, *Lr35*, *Lr36*[1] and several un-named genes transferred by Dr J. Dvorak[5]. *Lr28* has been used in one Australian cultivar and *Lr35* is linked with *Sr39* in the original translocation line[3].

The purpose of this study was to screen new sources of *Aegilops speltoides* Tausch var. *ligustica* (Savign.) Fiori. (=*T. speltoides* subsp. *ligustica* (Savign.) Chennav.)[6] for resistance to pathotypes of stem rust, leaf rust and stripe rust prevalent in Australia and to transfer the resistance genes to bread wheat. We report the chromosomal location of a stem rust resistance gene from *Ae. speltoides* accession AEG357-4.

MATERIALS AND METHODS

Five accessions of *Aegilops speltoides*, namely, AEG357-4, AEG363-5, AEG818-4, AEG874-60 and AEG2106-38, were imported to Australia by courtesy of The Harold and Adele Lieberman Germplasm Bank, Tel Aviv University, Israel and grown in the quarantine greenhouse at the Waite Campus, Urrbrae, South Australia. All accessions were crossed with cv. Angas (provided by Dr Hugh Wallwork, SARDI) using wheat as the female parent. F_1 seeds were germinated, 4-week old seedlings treated with 0.07% colchicine in 3% dimethyl sulphoxide for 5 hrs (aerated), washed in running water and re-potted. Plants were crossed as females with cv. Angas and later with cv. Westonia.

Seedlings of the five *Ae. speltoides* accessions were tested for resistance to three rust diseases at the University of Sydney, Plant Breeding Institute, Cobbitty, N.S.W., using different pathotypes (Tables 1 and 2) and standard rust screening protocols[7]. Later, backcrossed generations were tested for rust resistance at Cobbitty (*Pgt* 34-1,2,3,4,5,6,7) and at the Waite Campus, Urrbrae (*Pgt* 343-1,2,3,5,6; *Pt* 104-1,2,3,(6),(7),11+*Lr24*).

Chromosome counts of *Ae. speltoides* and derived lines were performed using the Feulgen staining procedure[8]. Alien chromosome addition lines were selected which carried either single or multiple *Ae. speltoides* chromosomes using representative RFLP probes previously mapped by others to each of the seven Triticeae homoeologous chromosome groups. Small scale genomic DNA extraction and RFLP analysis (using the random primer method to incorporate ^{32}P-labelled dCTP) was according to a modified procedure[9,10]. Prior to hybridization with the labelled probes, DNA from the control wheat lines was digested

with each of three restriction enzymes, *Bam*HI, *Dra*I, or *Eco*RV. RFLP probes were chosen to be representative of each of the seven homoeologous groups[11], namely, BCD22 (group 1), BCD111 (group 2), BCD134 (group 3), ABG394 (group 4), PSR128 (group 5), BCD276 (group 6) and CDO595 (group 7).

RESULTS AND DISCUSSION

All of the five accessions of *Ae. speltoides* showed high levels of resistance to the three rust diseases (Tables 1 and 2) and all accessions of *Ae. speltoides* had 2n=14 chromosomes (Fig 1a). After crossing *T. speltoides* accessions as pollen parents with cv. Angas, two F_1 seedlings were produced from the cross involving AEG357-4. F_1 plants exhibited morphological characteristics of the *Ae. speltoides* parent, for example, the expression of maroon-red pigmented auricles. Spike features of F_1 and F_2 plants were between those of the parents (Figure 1b). The progeny of a colchicine-treated plant from the Angas x *Ae. speltoides* AEG357-4 cross (plant 510/01) showed chromosome numbers from 53 to 56 (Fig 1c).

All RFLP probes representing the seven homoeologous groups showed polymorphisms using *Bam*H1, *Dra*I and *Eco*RV for each of the *Ae. speltoides* accessions, which enabled the tentative identification of the S-genome chromosomes in an Angas wheat background. A BC_2F_2 wheat plant (plt 1001b/04) was identified which showed the group 2 *Ae. speltoides* AEG357-4 BCD111 marker genotype, assumed to represent the presence of chromosome 2S. No other S-genome RFLP marker was present in this plant. BC_2F_3 progeny and subsequent generations showed segregation of the AEG357-4 BCD111 marker. Of 210 seedlings across 8 segregating families, 166 plants were positive for the *Ae. speltoides* BCD111 marker and 44 were negative. A Chi-squared test conformed to a 3:1 ratio (P=0.337).

To confirm the identity of the *Ae. speltoides* chromosome, a range of RFLP markers previously mapped to Triticeae group 2 chromosomes[11] was hybridized to DNA extracted from the diploid *Ae. speltoides* AEG357-4 line and progeny of plant 1001b/04. Accession AEG357-4 showed S-genome specific bands for the group 2 short arm probes ABG58, BCD221, ABG2, ABG358, ABC454 and long arm probes BCD111, ABG72, ABC153 and WG645. In contrast, plants derived from the BC_2F_2 plant 1001b/04 were positive only for ABG358, ABC454 and BCD111. The *Xbcd111-2B* locus was absent in plants homozygous for the AEG357-4 BCD111 marker. The high transmission rates of this chromosome and the absence of distal segments of both arms suggests that a wheat-2S recombinant chromosome was produced spontaneously during the backcrossing process. This chromosome from *Ae. speltoides* AEG357-4 was named "2S#3".

There was insufficient seed produced on the colchicine-treated F_1 plants and BC_1F_2 and BC_1F_3 generations for rust testing. A stem rust test was conducted on BC_2F_3 plants derived from plant 1001b/04 which carried the *Ae. speltoides* 2S#3 chromosome and high level of resistance to stem rust was observed: (Infection Type [IT] ; to 1) (Angas; IT, 3 to 4) (*Pgt* 343-1,2,3,5,6) and (IT, 2-, 2=) (*Pgt* 34-1,2,3,4,5,6,7). This indicated the presence of a stem rust resistance gene, located on the 2S#3 chromosome, which had been derived from *Ae. speltoides* accession AEG357-4.

The line carrying the 2S#3 chromosome was backcrossed twice with cv. Westonia (wheat as male) and selection for the *Xbcd111-2S#3* band made at each generation. Progeny of these plants showed an IT of 1 to 2 to stem rust (*Pgt* 343-1,2,3,5,6). Later, a population segregating for the *Xbcd111-2S#3* marker was screened for stem rust resistance (*Pgt* 343-1,2,3,5,6). Twelve seedlings were resistant (IT, 0 to 2) and five susceptible (IT, 4), fitting a 3:1 segregation model (P = 0.67). A leaf rust resistance test (104-1,2,3,(6),(7),11+*Lr24*) on a line carrying the 2S#3 chromosome showed nine plants with an IT of 4 and one plant with an IT of 3 (Angas/Westonia; IT, 4) indicating that the leaf rust resistance in the diploid *Ae. speltoides* accession was absent from this line. We have not yet tested for stripe rust resistance in this line.

The BCD111 marker for the 2S#3 chromosome showed a distinct polymorphism compared to that of chromosome 2S#1 (*Sr32*) and chromosome 2S#2 (*Sr39*) which suggests that the 2S#3 chromosome may carry a different stem rust resistance gene. The resistance gene from *Ae. speltoides* AEG357-4 has been named *Sr2S#3*.

ACKNOWLEDGEMENT

This research was funded by the Grains Research and Development Corporation (GRDC) of Australia.

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Table 1. Reactions of *Ae. speltoides* accessions to stem rust. Infection type scores after McIntosh *et al.*[1]

	Stem Rust Pathotype		
Line	34-2,12,13 =427	74-L-1 =103	98-1,2,3,5,6 =279
AEG357-4		0;-	
AEG363-5	0;;	0;;	0;
AEG818-4	;CN	;	;CN
AEG874-60	;12-C	1P3+, 8P0;	;CN
AEG2106-38	;CN	0;;CN	;N

Table 2. Reactions of *Ae. speltoides* accessions to leaf and stripe rust pathotypes. Infection type scores after McIntosh *et al.*[1]

	Leaf Rust		**Stripe Rust**
Line	53-1,(6),(7),10,11 =365	104-1,2,3,(6),(7),9,11 =521	110E143 A+ =444
AEG357-4		0;-	0;=
AEG363-5	0;	;CN	1P3+, 6P;
AEG818-4	0;	0;;	0;;
AEG874-60	;;+C	;CN	2P2, 5P;
AEG2106-38	0;;	;-	1P3, 5P;

Figure 1. (a) mitotic chromosomes of *Ae. speltoides* AEG357-4 (2n=14), (b) spikes of (L to R) *Ae. speltoides* AEG357-4, progeny plant of colchicine-treated F_1 Angas x *Ae. speltoides* AEG357-4 with 54 chromosomes, and cv. Angas, and (c) mitotic chromosomes of progeny plant of colchicine-treated F_1 Angas x *Ae. speltoides* AEG357-4 (2n=56). (Bar=10 µm)