Identification of markers linked with stem rust resistance genes *Sr33* and *Sr45*

Sambasivam PK[1] , Bansal UK[1], Hayden MJ [2], Dvorak J[3], Lagudah ES[4], Bariana HS[1]

[1]Faculty of Agriculture, Food and Natural Resources, University of Sydney, Plant Breeding Institute-Cobbitty, PMB11, Camden, NSW 2570, Australia. [2]Plant Genomics Center, University of Adelaide, Hartley grove, Urrbrae, SA, 5064, Australia.[3]University of California Davis, USA [4]CSIRO Plant Industry, GPO Box 1600, Canberra, 2601 ACT, Australia.

INTRODUCTION

Stem rust caused by *Puccinia graminis* f. sp. *tritici* (Pgt) has been and still remains a major threat for wheat production world wide[1,2]. Following "Green Revolution", the disease was brought under control by the use of stem rust resistant semi dwarf spring wheats and least attention was given to stem rust improvement for several decades[3]. Recently, evolution of a new Pgt pathotype, Ug99, in Africa has threatened the global wheat industry. This pathotype was initially detected in Uganda during 1999 and later spread to neighbouring countries like Kenya and Ethiopia and now has reached Iran[2,4]. This pathotype is virulent on many of the current stem rust resistance genes used in different wheat growing regions[5]. Some of the effective stem rust resistance are derived from the wild relatives of wheat. The genes *Sr33* and *Sr45*, derived from *Aegilops tauschii*, were among the genes effective against Ug99[2]. The present study was planned to identify molecular markers closely linked with stem rust resistance genes *Sr33* and *Sr45*.

MATERIALS AND METHODS

Plant materials

Substitutions lines for chromosome 1D in Chinese Spring carrying *Sr33* (CS1D5405) and *Sr45* (CS1D5406) were crossed with the susceptible parent Chinese Spring (CS) to generate single chromosome recombinant inbred line populations.

Stem rust evaluation

The CS1D5405/CS and CS1D5406/CS RIL populations were screened for stem rust response segregation at the seedling stage under greenhouse conditions using Pgt pathotype 34-1,2,3,4,5,6,7,11 (culture no. 171), following the procedures described in Bariana and McIntosh (1993)[6]. These populations were sown at two locations, Karalee and Breakwell, at the University of Sydney, Plant Breeding Institute, Cobbitty. Each 50 row experimental block was surrounded by susceptible wheat lines serving as infector rows to create rust epidemic. Adult plant stem rust response assessments were made according to a 1-9 scale described by Bariana et al. (2007)[7].

Molecular mapping

Genomic DNA was extracted from the leaf tissue of CS1D5405/CS and CS1D5406/CS RIL populations using modified CTAB method of Kleinhoffs et al. (1993)[8]. Bulk segregant analysis (BSA) was performed to identify marker-trait associations. BSA was performed using multiplex ready PCR method[9] with 80 markers located on chromosome 1D. Rust response-linked markers were mapped on RIL populations.

Data analyses and genetic mapping

Chi-squared tests were used to determine the goodness of fit of observed RIL segregation to that of the expected genetic ratio. Recombination fractions were calculated with MAP MANAGER Version QTXb20[10] using the Kosambi (1944)[11] map function.

RESULTS

Genetic analysis

The CS1D5405 (*Sr33*) and CS1D5406 (*Sr45*) produced low infection types (IT) IT2= and IT;;1-, respectively, when tested against Pgt pathotype 34-1,2,3,4,5,6,7,11 at the seedling stage. The susceptible parent Chinese spring displayed a susceptible response (IT3+). At the adult plant stage resistant parents produced rust response of 4 (CS1D5405) and 5 to 6 (CS1D5406), whereas susceptible parent produced high field response of 8. Both RIL populations showed 1:1 segregation both at the seedling and adult stages indicating the presence of single gene for resistance in each population (Table 1).

Genetic mapping:

Sr33

Fifteen chromosome 1D markers showed associations with resistant and susceptible bulks in CS1D5405/CS RIL population. These linked markers were mapped on entire RIL population consisting of 87 lines. *Sr33* was flanked by *Xbarc152* and *Xcfd15* at a distance of 1.8 cM on either side. The LOD value for flanking markers was 20.5. The locus order and genetic distances between *Sr33* and markers are presented in Fig 1a.

Table 1: Segregation for stem rust response in CS1D 5405/CS and CS1D 5406/CS RIL populations

Plant stage	RIL Population	Rust response		$\chi^2_{1:1}$
		Resistant	Susceptible	
Seedling stage (75L9=171)	CS1D5405/CS	47	38	0.953
	CS1D5406/CS	20	26	0.783
Adult plant stage (Karalee)	CS1D5405/CS	47	38	0.953
	CS1D5406/CS	20	26	0.783
Adult plant stage (Breakwell)	CS1D5405/CS	47	38	0.953
	CS1D5406/CS	20	26	0.783

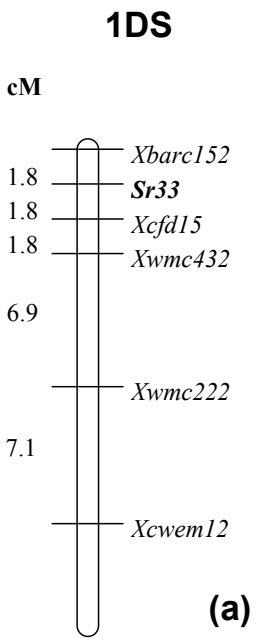
The table values of χ^2 at P=0.05 and P=0.01 and 1d.f. are 3.84 and 6.64, respectively.

Sr45

For *Sr45*, 30 markers showed linkage with bulks. The linked markers were genotyped on CS1D5406/CS RIL population consisting of 48 lines. The cluster of markers *Xwmc222, Xgwm106, Xstm694tgag, Xcfa2158* and *Xcfd21* mapped 3.3 cM distal to *Sr45* and *Xbarc229* was 3.7 cM proximal (LOD=8.5) to the gene (Fig 1b).

DISCUSSION

The D genome of hexaploid wheat, derived from *Aegilops tauschii*, contributed many important rust resistance genes[12,13]. Two stem rust resistance genes *Sr33* and *Sr45* genes were also transferred from chromosome 1D of *Aegilops tauschii*[14,15]. The substitution lines CS1D5405 and CS1D5406 showed low stem rust responses at the seedling and adult plant stages against Australian Pgt pathotypes. These genes were also effective against the Pgt pathotype Ug99[5]. The seedling stem rust response segregation among CS1D5405/CS and CS1D5406/CS RIL populations conformed to monogenic inheritance of resistance in each population. The resistance gene *Sr33* was flanked by markers barc152 (1.8cM) and cfd15 (1.8cM). The marker cluster wmc222/gwm106/stm694tgag/ cfa2158/cfd21 and the marker barc229 flanked *Sr45* distally (3.3 cM) and proximally (3.7 cM). Marker order was consistent with the consensus map of Somers et al. 2004[16]. *Sr33* was previously reported to be tightly linked with *Gli-D1*[17]. These additional *Sr33* and *Sr45* linked markers will be validated on backcross-derived germplasm in several different genetic backgrounds. The low recombination values suggest that these markers can be used in marker assisted selection of *Sr33* and *Sr45*, where parental polymorphism exists. However the cluster of markers distal to *Sr45* may indicate low resolution based on the small population size. Both these genes provide protection under field conditions and their pyramiding with other effective stem rust resistance would enable achievement of durability.



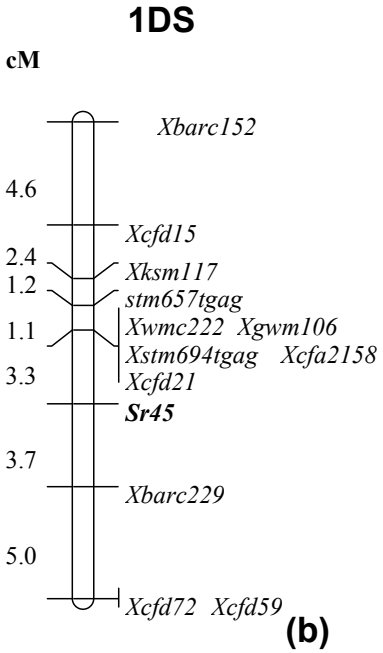


Figure 1: Genetic map of 1DS with respect to the location of Sr33 (a) and Sr45 (b).

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