Closely Linked Markers for Stem Rust Resistance Gene *Sr48* in Wheat

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INTRODUCTION

Stem rust was assumed to be successfully controlled through the deployment of stem rust resistance genes. The widespread cultivation of wheat varieties carrying *Sr31* led to the detection of *Sr31-virulent* race Ug99 by Pretorius et al. (2000)

Ug99 and its variants caused huge losses in several African nations and reminded the global wheat research community of deadly nature of stem rust fungus.

Several stem rust resistance genes were observed to be effective against Ug99 group of races. The efficient deployment of such genes in combination can be achieved through marker assisted selection.

While markers linked with stem rust resistance genes (e.g. *Sr9h*, *Sr13*, *Sr22*, *Sr33*, *Sr45*, *Sr55*, *Sr56*, *Sr57*, *Sr58*) are available, some Ug99-effective stem rust resistance gene still remain untagged.

This study was planned to identify and validate markers linked to the Ug99-effective stem rust resistance gene *Sr48*.

MATERIALS AND METHODS

Plant materials

Stem rust resistant cultivar Arina was crossed to susceptible cultivar Cezanne and advanced to generate RIL population (178 lines)

Phenotyping in greenhouse

The parents were phenotyped at the 2-leaf stage in greenhouse against Pgt pathotype 98-1,2,3,5,6,7 (PBI culture no. 580) at two temperatures (17˚C and 27˚C) to identify if temperature plays any role in the expression of *Sr48*. RIL population was phenotyped at 17˚C.

Genotyping and mapping of Sr48

Total genomic DNA was extracted from RIL population and parents using the modified CTAB protocol (Bansal et al. 2014)

DNA was sent to Diversity Arrays Technology Pty Ltd (Canberra, Australia) for genotyping by sequencing (GBS) with the wheat DArTseq® platform (http://www.diversityarrays.com).

Linked DArTseq markers were converted into PCR markers and genotyped on the entire RIL population using established protocols.

Chi-squared tests were performed to test the goodness of fit of observed segregation to the expected genetic ratios. Trait-marker associations were detected using MapManager version QTXb20 (Manly et al. 2001). The final linkage map was generated using MapChart software Version 2.2 (Voorrips 2002).

Genomic in-situ *hybridization (GISH) to determine translocations in parental genotypes*

Parental cultivars Arina, Forno and Cezanne were characterized by Genomic *in-situ* hybridization (GISH) following the procedure of Zhang et al. (2001).

Validation of markers closely linked to Sr48

The markers closely linked to *Sr48* were tested on a panel of 82 Australian and 92 Nordic wheat cultivars to determine their robustness in marker-assisted selection (MAS.)

RESULTS

Rust response assessment

Arina displayed IT22- at 17˚C and IT23C at 27˚C, while Cezanne displayed IT3+ at both temperatures.

Consequently, the RIL population was evaluated at 17˚C against Pgt pathotype 98-1,2,3,5,6,7. Chi-squared analysis ($\chi^2 = 0.29$, $df = 1$, $P > 0.50$) confirmed segregation at a single locus *Sr48*.

Molecular mapping

A total of 32,679 DArTseq markers were received and following quality check 1391 markers constituted 49 linkage groups.

The rust response data were converted into genotypes and incorporated in the DArTSeq linkage map.

Sr48 mapped in chromosome 2D (Fig 1b).

BLASTN search of the DArTseq marker sequences flanking *Sr48* identified five CSS contigs with good hits on 2DS.

Two (*sun590* and *sun592*) out of the 14 SSR/STS markers developed were polymorphic between parents.

We also tested 2DS-specific markers (Cao et al. 2012; Yu et al. 2015) and polymorphic markers were genotyped on the entire Arina/Cezanne RIL population.

A partial linkage map of *Sr48* comprising five markers was constructed and covered 24.3 cM (Fig 1c).

GISH analysis

GISH on Arina, Forno and Cezanne showed an interstitial translocation from '2A' to '2D' genome in Forno in addition to the 4AL-5AL-7BS translocation present in all hexaploid wheat genotypes (Fig 2).

These results confirmed the DArTseq-based results. The initial location of *Sr48* on chromosome 2A by Bansal et al. (2009) based on its association with *Yr1* (Fig 1a) may have resulted from pseudo-linkage.

Although meiotic analysis was not performed, we believe that formation of quadri-valent involving chromosome 2A and 2D would have led to the pseudo-linkage between *Sr48* and *Yr1* in Arina/Forno RIL population.

Despite the relocation of *Sr48* on chromosome 2D, comparison of resistance gene (*Sr6*, *Sr46*)-marker associations demonstrated its uniqueness.

Validation of markers closely linked to Sr48

Markers *sun590* (1.6 cM distal) and *sun592* (1.6 cM proximal) flanked *Sr48*.

The amplification of PCR products different to that produced by *Sr48*-carrying cultivars Arina for marker *sun590* in 84 Australian and 92 Nordic genotypes demonstrated its robustness for MAS.

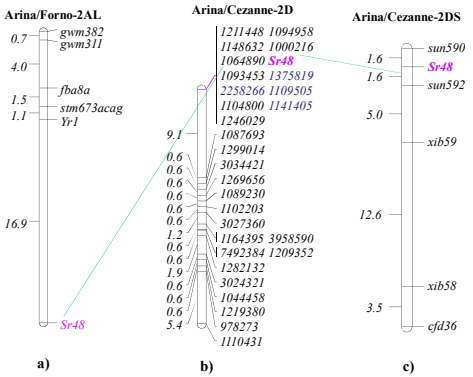

Fig 1. Genetic linkage map of *Sr48* a) 2AL: Arina/Forno-Bansal et al. (2009) b) The DArTSeq-based map c) PCR based marker of 2DS: Arina/Cezanne RIL population

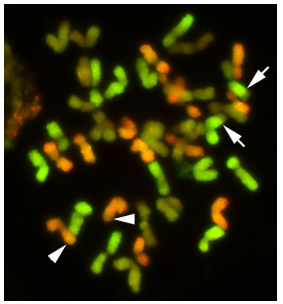

Fig 2 . A karyogram of wheat cultivar Forno stained with 6-diamidino-2-phenylindole. Arrow heads point to a short segment of chromosome 2A (green) interstitially translocated to chromosome 2D (red)

Conclusions

1. A chromosome 2A/2D translocation in cultivar Fono led to the initial location of *Sr48* in the long arm of chromosome due to its pseudo-linkage with *Yr1* in Arina/Forno RIL population
2. The lack of identification of markers linked to *Sr48* using Arina/Forno RIL population prompted us to develop Arina/Cezanne RIL population in which *Sr48* was mapped on chromosome 2D.
3. Based on the genomic locations of linked markers and different pathogenic specificities, *Sr48* appears to be unique locus.
4. The robustness of *Sr48-sun590* association was demonstrated through the amplification of PCR product different to that observed in Arina tests in Australian and Nordic wheat genotypes.

References

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