



# A new strategy to map *scs* genes: combining radiation hybrid mapping with high resolution genetic mapping



Farhad Ghavami<sup>1</sup>, Monika K. Michalak<sup>1</sup>, Oscar Riera-Lizarazu<sup>2</sup>, Yong Q. Gu<sup>3</sup>, Roger Thilmoney<sup>3</sup>, Schivcharan S. Maan<sup>1</sup>, and Shahryar F. Kianian<sup>1</sup>

<sup>1</sup> Department of Plant Sciences, North Dakota State University, Fargo, ND 58105

<sup>2</sup> Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331

<sup>3</sup> USDA-ARS, Western Regional Research Center, Albany, CA 94710

## Introduction:

Wheat is unique among plants in having a large collection of alloplasmic lines (lines with alien cytoplasm) developed over 50 years of intensive research effort<sup>1</sup>. Hexaploid (*T. aestivum*) wheat is usually less sensitive to cytoplasm substitution as compared with tetraploid wheat (*T. turgidum*)<sup>1,2,3</sup>. *Triticum aestivum* with *T. longissimum* (2n=2x=14;S<sup>1</sup>S<sup>1</sup>) cytoplasm [represented by (lo)] have normal fertility and plant vigor. However, *T. turgidum* nucleus is incompatible with the (lo) cytoplasm, producing non-viable progeny. This incompatibility is relieved by the introduction of the *scs* (species cytoplasm specific) gene, *scs<sup>ti</sup>*, a gene from long arm of chromosome 1A (1AL) derived from *T. timopheevii* or its homoeologue on chromosome 1D (1DL) from *T. aestivum*, *scs<sup>ae</sup>*. The addition of a *scs* gene lessens the incompatibility between nucleus and cytoplasm but the progeny is male sterile. Addition of the *Vi* gene, located on the short arm of chromosome 1B (1BS), restores male fertility.

## Material and Methods:

### Radiation Hybrid Mapping Panels:

Three radiation hybrid mapping panels were prepared. Two alloplasmic radiation hybrid mapping panels, RH<sub>2</sub> with 72 and RH<sub>1</sub> with 71 individuals, were developed by treating the seeds of (lo) *scs<sup>ae</sup>* - (with 350 Gy gamma ( $\gamma$ ) rays. Additionally, durum 1D(1A) substitution line was treated with 150 Gy  $\gamma$  rays to develop RH<sub>1</sub> panel of 94 radiation hybrids.

### High Resolution Genetic Mapping Population:

110 F<sub>2</sub> plants were genotyped for molecular markers and also *scs<sup>ti</sup>* by test crossing to (lo) *scs<sup>ae</sup>* - to generate an initial genetic map (Fig 2D). After identification of the flanking markers an additional 2,316 F<sub>2</sub> plants were analyzed and recombinant lines genotyped for *scs<sup>ti</sup>* to generate a more saturated map of the region (Fig 2B).

## Results:

The analysis of two alloplasmic radiation hybrid (RH) panels with 45 and 28 molecular markers specific for chromosome 1D revealed 68 and 28 chromosome breaks, respectively. A total of 23 markers with 100% co-retention (i.e., co-segregating) with the *scs<sup>ae</sup>* gene were identified. Table 1 shows eighteen of these gene-based markers and their putative functions. Interestingly, five of these genes were mitochondrial-related nuclear genes. An RH panel derived from an euplasmic 1D(1A) durum substitution line was used to create a physical map of chromosome 1D (Fig 2A) to better define the physical distance of markers with 100% co-retention in the alloplasmic RH panels. An RH map was generated using Carthagene 0.999 with a LOD score of 7.0 and 2-point distance of 40 cR. This radiation hybrid panel was screened with 54 molecular markers and revealed 116 chromosomal breakages.

Previous genetic mapping studies localized *scs<sup>ti</sup>* on 1AL with respect to a number of molecular markers<sup>4,5</sup>. The flanking marker loci found for *scs<sup>ti</sup>* were *Xbcd12* and *Xbcd1449b* with a distance of 2.4 and 0.6 cM, respectively<sup>5</sup>. There is co-linearity between the region surrounding the *scs<sup>ti</sup>* gene and chromosome 10 of rice (Fig. 3D) and also super contig 8 from *Brachypodium*. This information helped identify additional markers based on rice genes in this region and their corresponding Tentative Consensus sequences (TCs) from wheat.

Polymorphic markers based on rice sequence information from AC074232 and the next 3 or 4 BAC clones toward AC069300 could not be identified. Thus the focus was shifted toward genome walking from the Wmc120 (Receptor kinase) or finding a gene-based marker close to it (Mag 834) through available markers on Grain Genes.

Table 1. Gene-based markers, which have shown 100% co-retention with the *scs<sup>ae</sup>* gene, and their putative function as found by BLAST against nr nucleotide collection in NCBI. Mitochondria-related nuclear genes are in red.

Gene-Based Marker ID	Putative function	Species	
1	TC238759	Electron transfer flavoprotein; mitochondrial precursor	<i>O. sativa</i>
2	BE403956	Expansin EXPB2, Beta-expansin EXPB4 (EXPB4)	<i>T. aestivum</i> , <i>O. sativa</i>
3	TC232355	Putative gamma lyase amino acid metabolic process	<i>O. sativa</i> , <i>T. aestivum</i>
4	TC252572	Putative Ste20-related protein kinase	<i>T. aestivum</i>
5	BF475149	Conserved hypothetical protein	<i>O. sativa</i>
6	BE518358	Protein kinase-like protein	<i>O. sativa</i>
7	BG314205	Conserved hypothetical protein	<i>O. sativa</i>
8	TC241839	NA	<i>O. sativa</i>
9	TC280803	UBX domain containing	<i>O. sativa</i>
10	TC278524	Putative gibberellin oxidase	<i>O. sativa</i>
11	TC232332	Cytochrome b5, putative	<i>O. sativa</i>
12	BM138654	Protein kinase family protein	<i>A. thaliana</i>
13	BF291549	GSK-like kinase (GSK1)	<i>T. speltoides</i> , <i>T. Aestivum</i>
14	BG607230	Mitochondrial origin, ARM repeat fold domain containing protein	<i>O. sativa</i>
15	TC247223	Mitogen-activated protein kinase homolog MMK2	<i>O. sativa</i>
16	TC290833	Inner membrane protein OXA1-like, mitochondrial precursor	<i>O. sativa</i>
17	TC268917	Fertility restorer	<i>O. sativa</i>
18	BE500104	Hypothetical protein, nodulin MtN3 family protein	<i>O. sativa</i> , <i>A. thaliana</i>

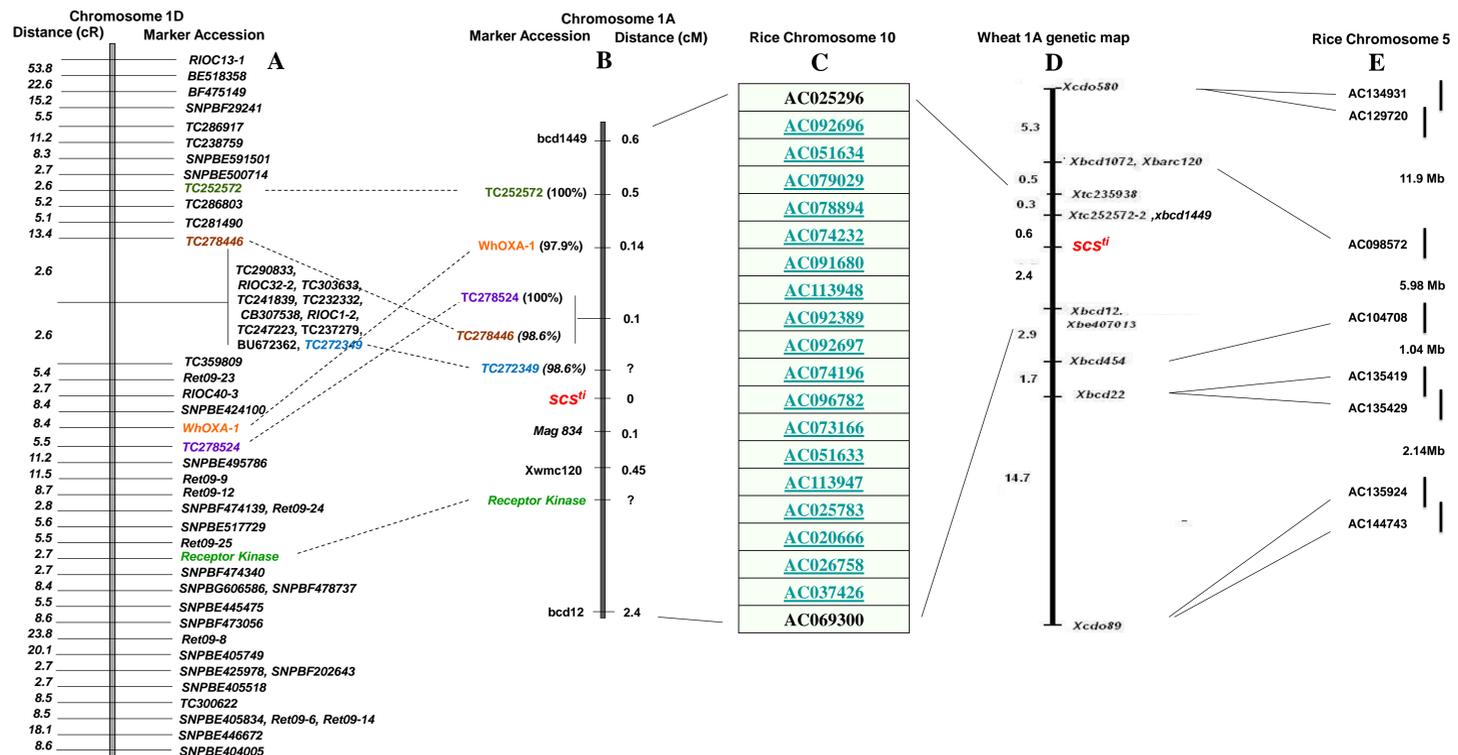


Figure 2. A) RH map of chromosome 1D is 347.3 cR in size and spans 54 molecular markers. This map is derived from mapping LDN 1D(1A) population treated with 150 Gy  $\gamma$  rays. B) High resolution mapping of the wheat 1AL region containing *scs<sup>ti</sup>* gene, a homoeologue of *scs<sup>ae</sup>*. Marker accession and marker co-retentions with *scs* gene, based on both alloplasmic RH populations, are specified on the left side. Numbers on the right are cM distances of a particular marker from *scs<sup>ti</sup>* gene. C) BAC clones of rice chromosome 10 showing conserved synteny with chromosome 1AL surrounding the *scs<sup>ti</sup>* gene. D) Genetic map (28 cM) of the chromosome 1AL which was a starting point for high resolution mapping of the region. E) BAC clones of rice corresponding to the markers mapped on wheat 1AL shows the synteny breaks between wheat Chr1AL and rice Chr5 with a part from rice Chr10.

## Conclusion:

Results from our work indicated that the map-based cloning of the *scs<sup>ti</sup>* gene is possible due to availability of many recombinants in the region, despite its close proximity to the centromere. Co-linearity with rice chromosome 10, *Brachypodium* super contig 8 and available BAC libraries of durum wheat is aiding our identification of the open reading frames and candidate genes. The radiation hybrid map of the Chr1DL has helped identify markers co-retained with *scs<sup>ae</sup>* which can be used to increase the saturation of the genetic map. Additionally, we have observed a higher probability of having breakages in a smaller RH population relative to recombination events in the genetic mapping population.

Close proximity of markers to the *scs* gene defined by RH mapping has been confirmed with the genetic map of a homoeologous region. There are also some re-ordering of the markers or flipping of the segments which can be due to the structural differences between the homoeologues. The conserved synteny between chromosome 10 of rice and wheat 1AL is interrupted very close to the *scs<sup>ti</sup>* gene by segments of chromosome 5 from rice where a receptor kinase and mag834 are located.

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