Reference genome-assisted identification of stem rust resistance gene *Sr62* encoding a tandem kinase

Guotai Yu[1,2], Oadi Matny[3], Nicolas Champouret[4,15], Burkhard Steuernagel[1], Matthew J. Moscou[4], Inmaculada Hernández-Pinzón[4], Phon Green[4], Sadiye Hayta[1], Mark Smedley[1], Wendy Harwood[1], Ngonidzashe Kangara[1], Yajuan Yue[1], Catherine Gardener[1], Mark J. Banfield[1], Pablo D. Olivera[3], Cole Welchin[3], Jamie Simmons[3], Eitan Millet[5], Anna Minz-Dub[5], Moshe Ronen[5], Raz Avni[5,15], Amir Sharon[5], Mehran Patpour[6], Annemarie F. Justesen[6], Murukarthick Jayakodi[7], Axel Himmelbach[7], Nils Stein[7,8], Shuangye Wu[9], Jesse Poland[9], Curtis Pozniak[10], Miroslava Karafiátová[11], István Molnár[11,16], Jaroslav Doležel[11], Eric R. Ward[4,12,17], T Lynne Reuber[12,18], Jonathan D. G. Jones[4], Martin Mascher[7,13], Brian J. Steffenson[3] & Brande B. H. Wulff[1,2]

[1]John Innes Centre, UK; [2]King Abdullah University of Science and Technology, SA; [3]University of Minnesota, USA; [4]The Sainsbury Laboratory, UK; [5]Tel Aviv University, Israel. [6]Aarhus University, Denmark; [7]Leibniz Institute of Plant Genetics and Crop Plant Research, Germany; [8]Georg-August-University, Germany; [9]Kansas State University, USA; [10]University of Saskatchewan, Canada; [11]Institute of Experimental Botany of the Czech Academy of Sciences, Czech Republic;[12]2Blades Foundation, USA; [13]German Centre for Integrative Biodiversity Research, Germany; [14]Present address: Ball Horticultural Company, USA;[15]Present address: Leibniz Institute of Plant Genetics and Crop Plant Research, Germany; [16]Present address: Agricultural Institute, Centre for Agricultural Research, Hungary; [17]Present address: AgBiome, Inc., USA; [18]Present address: Alliance Management at Enko Chem, USA.

Abstract

The wild relatives and progenitors of wheat have been widely used as sources of disease resistance (*R*) genes. Molecular identification and characterization of these *R* genes facilitates their manipulation and tracking in breeding programmes. We developed a reference-quality genome assembly of the wild diploid wheat relative *Aegilops sharonensis* and used positional mapping, mutagenesis, RNA-Seq and transgenesis to identify the stem rust resistance gene *Sr62*, which was also transferred to common wheat. This gene encodes a tandem kinase, homologues of which exist across the plant kingdom, suggesting an ancient origin. Stable *Sr62* transgenic wheat lines showed high levels of resistance against diverse isolates of the stem rust pathogen, highlighting the utility of *Sr62* for deployment as part of a polygenic stack to maximize the durability of stem rust resistance.

Results

We used whole genome shotgun, mate pair, Hi-C, 10X and chromosome flow sorting sequence data to assembled a reference quality genome (N50 12.3 Mb) of *Aegilops sharonensis* accession 1644 (**Table 1**).

We genetically isolated *Sr62* in the progeny from a cross between accession 2189 (susceptible) and 1644 (resistant) and mapped the gene to a 480 kb interval (**Figure 1**). We obtained 14 susceptible EMS-derived mutants from the wheat-*Ae. sharonensis* 1644 introgression line and RNA mapping to identify *Sr62*. Transformation of the *Sr62* wheat tandem kinase gene into wheat cv. Fielder conferred wheat stem rust resistance (**Figure 3**). The phylogenetic trees on both whole gene and individual domains indicates that *Sr62* is close to *Pm24* (**Figure** 4).

Table 1 *Aegilops sharonensis* AS_1644 v 1.0 genome assembly statistics.

Assembly characteristics	**Values**
Assembly size	6.7 Gb
*Scaffold N50	12.3 Mb
*Scaffold N90	1.1 Mb
Pseudomolecule size	6.3 Gb
Unfilled gaps	886 Mb
Chromosome 1S^{sh}	783 Mb
Chromosome 2S^{sh}	1022 Mb
Chromosome 3S^{sh}	972 Mb
Chromosome 4S^{sh}	827 Mb
Chromosome 5S^{sh}	868 Mb
Chromosome 6S^{sh}	807 Mb
Chromosome 7S^{sh}	1016 Mb
Unassigned to a chromosome	420 Mb
Complete BUSCOs	0.965
Fragmented BUSCOs	0.013
Missed BUSCOs	0.022

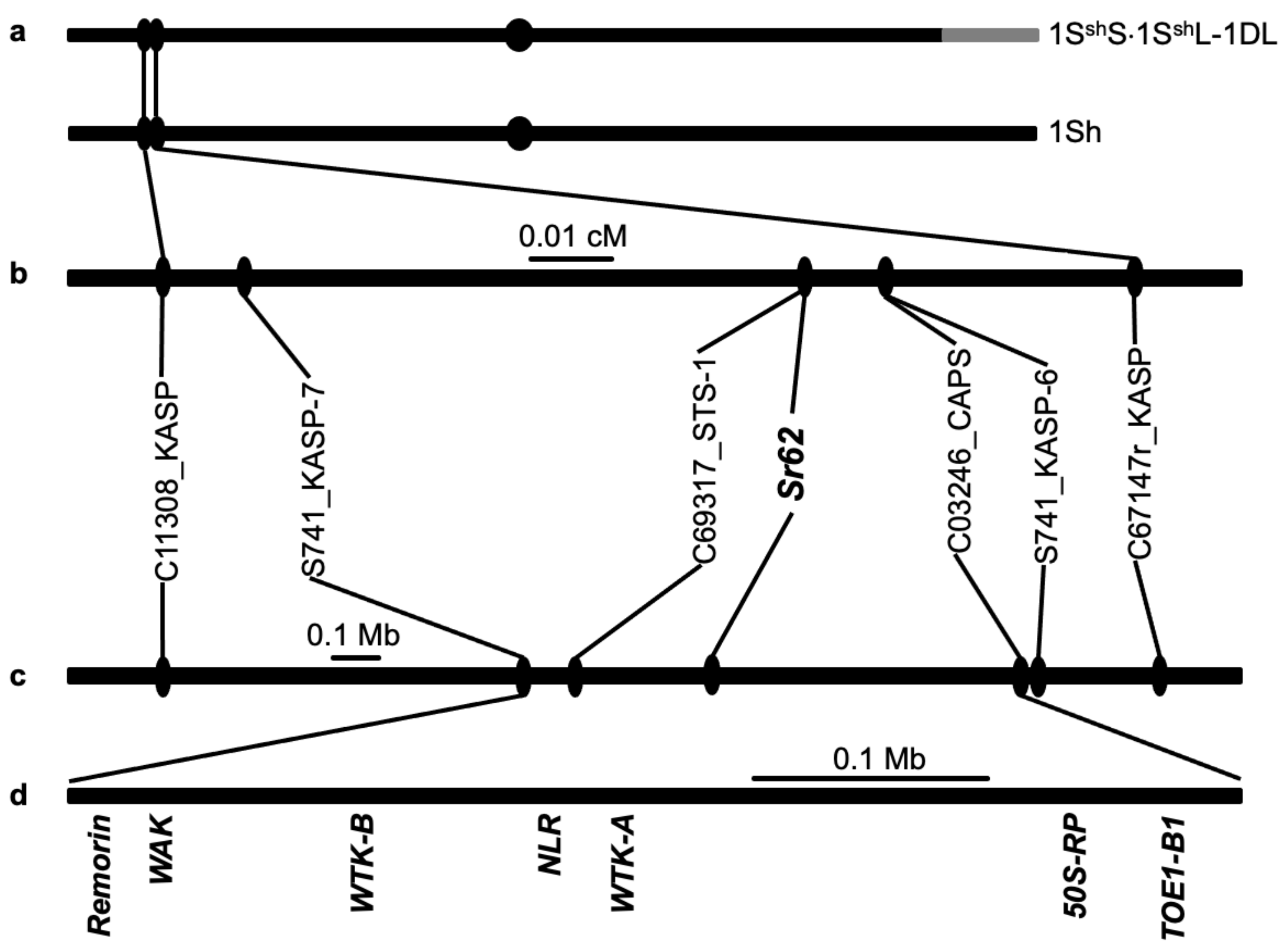

Fig. 1 Positional mapping restricts *Sr62* to a 480 kb interval on chromosome 1S^{sh}.

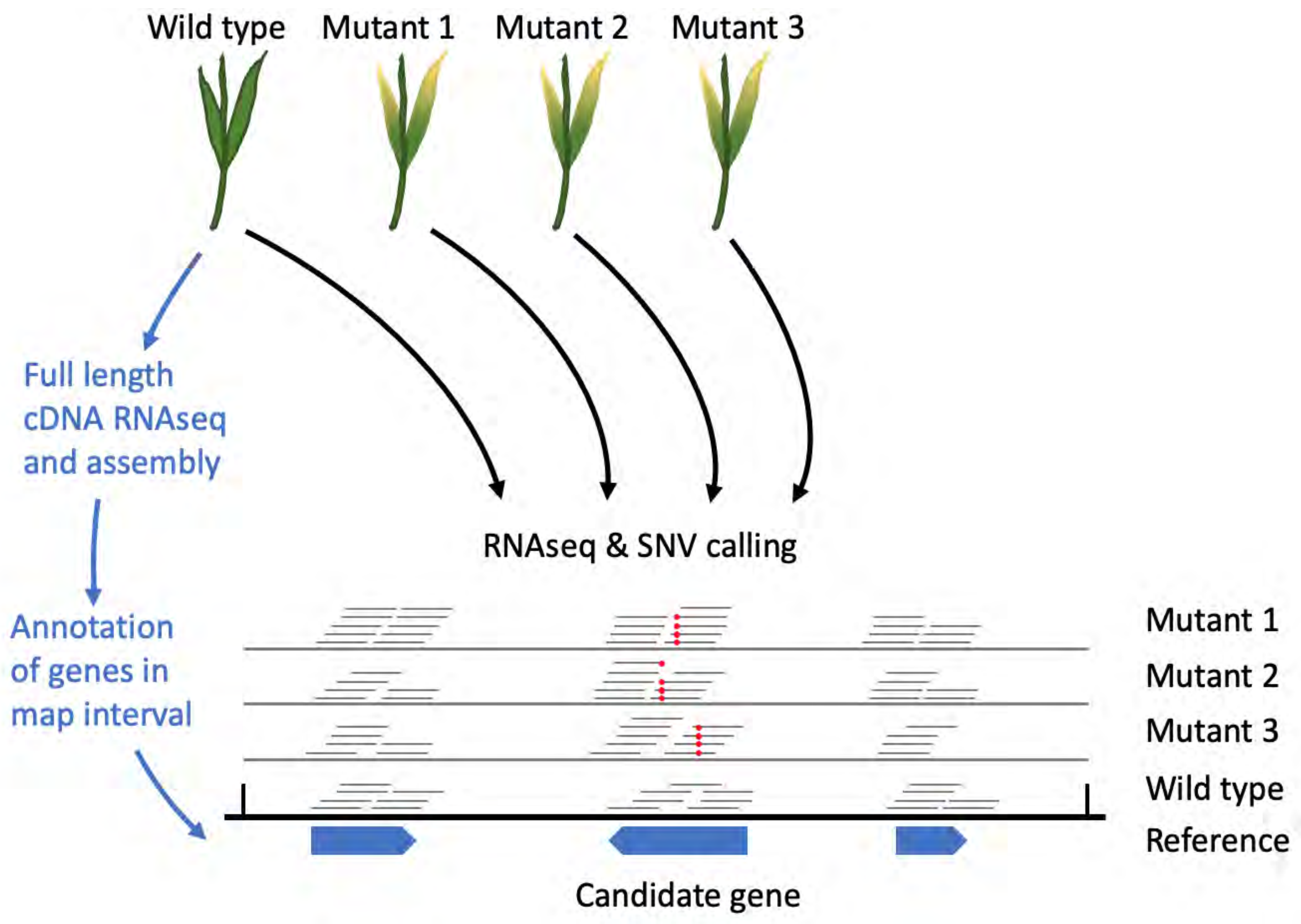

Fig. 2 Candidate gene identification by mutagenesis and transcriptome sequencing.

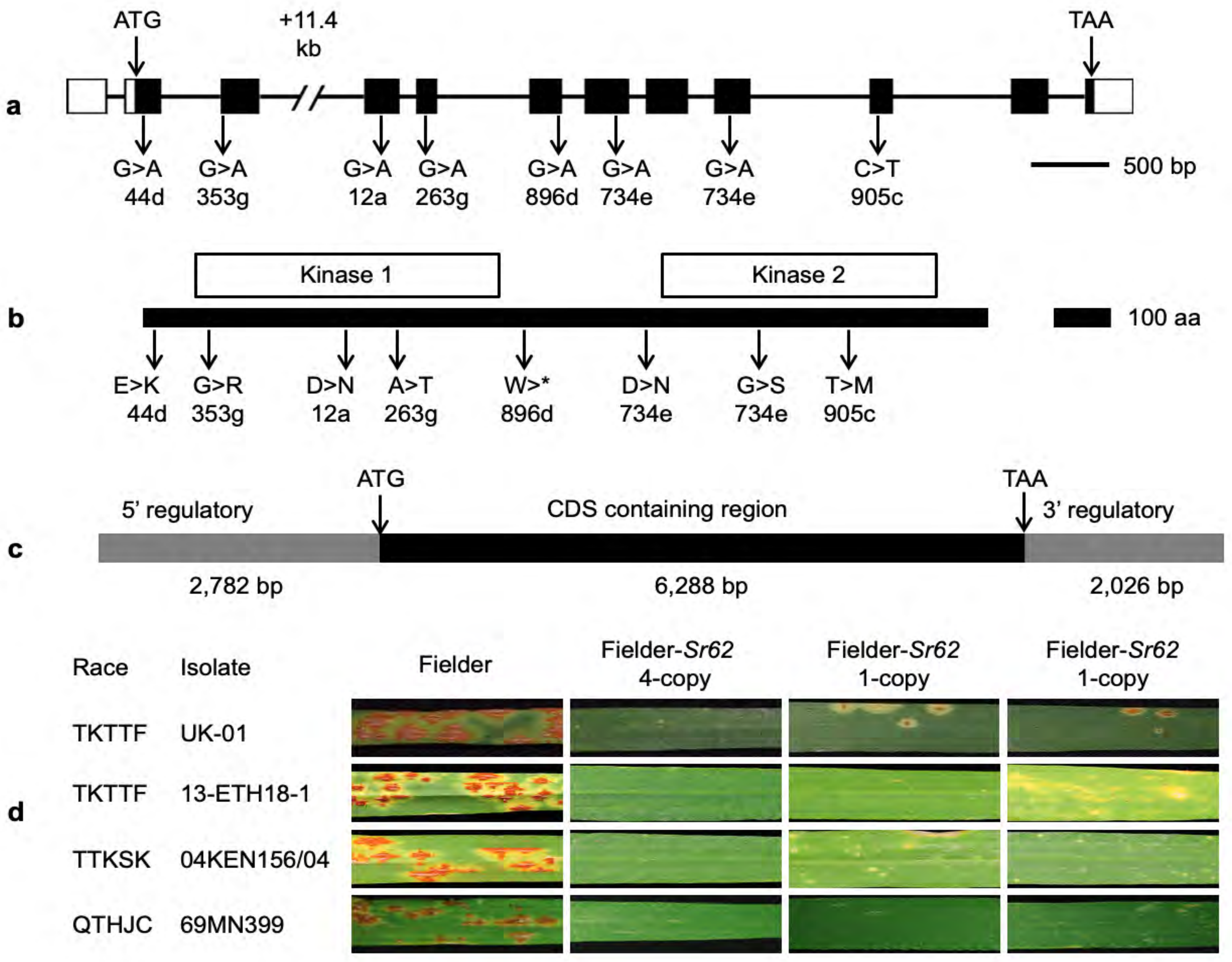

Fig. 3 Functional validation of *Sr62* by EMS mutagenesis and transformation into wheat.

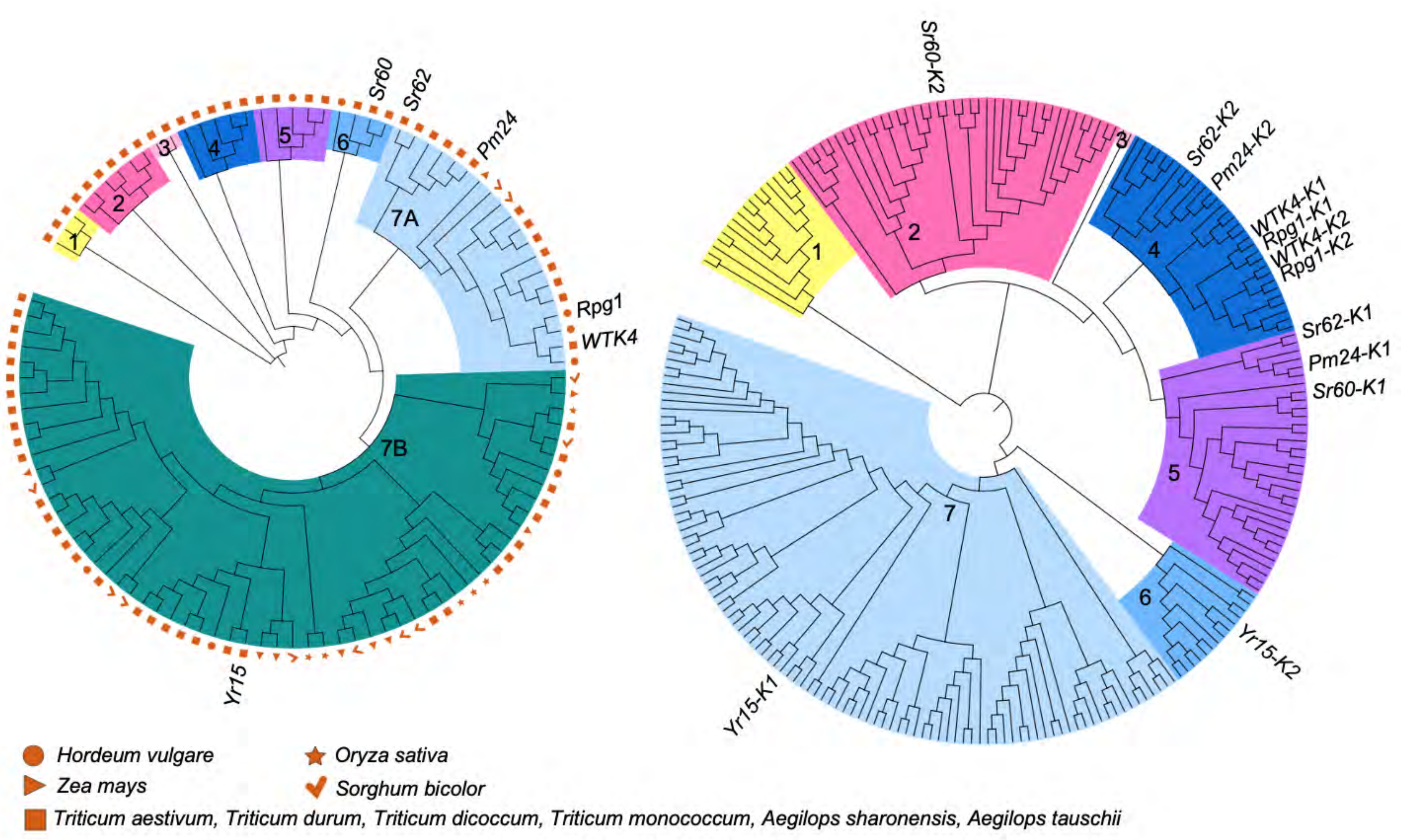

Fig. 4 Phylogenetic relationship between tandem kinases from cereal crops and wild grasses.

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